MATERIAL AND METHOD
4. MATERIAL AND METHODS

4.1. GENERAL DESCRIPTIONS

4.1.1. General settings of the study: The present study was a community based field study, undertaken in the general settings of the army population in a large cantonment.

4.1.2. Place of the study: The work was undertaken in Pune and Khadki cantonments, in the general settings as described in para 4.1.1. above.

4.1.3. Time schedule: The work was carried out over a two year period, from October 1997 to October 1999. Prior to this, the initial planning and pilot study was completed over a 6 months period, from April 1997 to September 1997, after which the final protocol was submitted to the University of Pune in beginning of October 1997. Actual data collection spanned over one and a half year period, from October 1997 to March 1999. Subsequently, data entry, analysis and final write-up took another six months, till September/October 1999.

4.1.4. Study design: The epidemiological design used in this research work was that of “cross-sectioned analytical design” with the postulated exposure and outcome variables being measured at a given point of time (308).

4.2. DESCRIPTION OF STUDY POPULATION

4.2.1. Reference population: The reference population (synonymous with total population; universe) which was kept in mind for maintaining “external validity” and on which study results were proposed to be generalised (309) was defined as “all serving male personnel of the Indian army, aged 35 years and above.

4.2.2. Actual (study) population: Against the background of the reference population defined in para 4.2.1. above, the actual (study) population from which sample was
drawn (309) was defined as “all serving male persons of Indian army, aged 35 years and above serving in Pune or Khadki cantonment.” A detailed list of army units / establishments in Pune and Khadki, and a list of army persons serving in these units formed the sampling frame.

### 4.3. METHODOLOGY OF SAMPLING

4.3.1. While working out the issues of sampling for the study, the two important requirements for any sample, as described by standard text books in medical research (310), which were kept in mind were, firstly, that the sample should be of adequate size, and, secondly, that it should be representative of the reference population. These details pertaining to the sample size and sample collection are described in the succeeding paragraphs :-

4.3.2. **Sample size** - One of the major objectives of analysis, with a view to meet the proposed objectives, was to determine the correlation between the various components of syndrome X. With this background, the study envisage to detect a correlation coefficient of even as small as 0.15 in a statistically significant manner. The two tailed Type I (alpha) error and Type-II (Beta) errors were kept at the conventional levels of 0.05 and 0.20 respectively (311). The equation used for calculation of sample size was as mentioned in standard text books on clinical research (10), as follows :-
\[ N = \left\{ \left( \frac{Z_{1-a/2} + Z_{1-b}}{C} \right) \right\}^2 + 3 \]

Where, \( C = 0.5 \times \ln \left( \frac{1 + r}{1 - r} \right) \); \( r = \) correlation coefficient envisaged to be detected in a statistically significant manner; \( \ln = \) log normal value;

\( Z_{1-a/2} = \) Standard normal deviate value of two-tailed alpha error at the proposed level of significance (i.e., 0.05); \( Z_{1-b} = \) value of beta error; \( N = \) minimum sample size required.

4.3.3. With the above equation, the minimum sample size worked out to 347 subjects. Another method of calculating the minimum sample size, as described by WHO (312) and in other standard text book on Biostatistics (313) was also evaluated as follows:-

Keeping another important objective of the study, which was to estimate the prevalence of syndrome X, sample size calculation for estimating a proportion was undertaken. Based on the review of literature, it was felt that a rough estimate of the prevalence parameter was likely to be about 5%. Keeping the acceptable alpha (Type-I) and beta (Type-II) errors at conventional level of 0.05 (two tailed) and 0.20 respectively, and with a view to estimate the parameter within an acceptable 95% confidence interval (95% CI) of 2% to 8% (d=0.03, i.e. 3% on each side), the minimum sample size (N) was calculated using the following equation:-

\[ N = \frac{(Z_{1-a/2} + Z_{1-b})^2 \times p \times q}{d^2} \]

4.3.4. With the parameters specified above, the minimum sample size worked out to 414, while it had worked out at slightly lesser, i.e. 347, by the earlier equation. Since these figures indicated the minimum sample size, it was decided to study an even larger sample with a view to improve the precision of statistical estimates. After consultation with the statisticians, it was decided to further increase the sample by approximately one
and a half times, i.e. to study at least 600 subjects. Finally, a total of 614 subjects were studied by random selection process as described in the next paragraph.

4.3.5. **Sampling method**: The required sample size of 614, as calculated above, was selected from the actual study population (as described in para 4.2.1 above), using the standard random selection method of “multistage random sampling” as described by Yates in his text book on "sampling methods in surveys and censuses" (314). A complete list of all army units in Pune and Khadki was obtained from the military administrative authorities and formed the “sampling frame” for the first stage. In the first stage, a 1 in 4 sample of the military units was drawn randomly by giving them serial numbers and drawing a 1 in 4 random sample from random number tables. In the second stage, the detailed list of “sub-units” of the selected military units formed the sampling frame and a 1 in 4 sample of the sub-units was drawn using random number tables. In the third (last) stage, a complete and serially numbered list of all army persons in the randomly selected sub-units (from the second stage) formed the sampling frame from which the required sample was drawn, using random number tables. In this third stage, the “sampling ratio” was kept as 1 : 4, since it was worked out that, from the expected number of subjects in the study population from the third stage (sampling frame for third stage), a 1 in 4 sample would give the proposed sample of approximately 600 subjects. Finally, a sample of 614 was studied since the sampling frame in the third stage was finally 2456 (614 being one-fourth of 2456).

4.3.6. **Exclusion criteria**: The following were the exclusion criteria for the study: -

(a) Persons who were less than 35 years old, since the study population had been defined as “aged 35 years and above “.
(b) Female army personnel were excluded since their strength, at present, is too small to give an adequate sample. Similarly, since the reference population pertained to serving army personnel, the dependant family members were not included in this study.

(c) Persons who were already known to be suffering from ischaemic heart disease, hypertension or diabetes mellitus were excluded from analysis, but a separate mention has been made about them in the results. This is for the reason that such persons, being aware of their disease and possibly taking some form of pharmacological or non-pharmacological treatment for the diseases, were likely to have changed their risk profile (as cessation of smoking, initiation of weight control activities etc.); even their physiological and biochemical parameters (blood pressure, body weight, blood sugar etc) might have changed because of such treatment. Thus, this step was undertaken to prevent the "Neyson's incidence-prevalence bias" (315). This step has been undertaken in other large scale studies also (316). Any subject excluded from the sample on this criteria was replaced with the next subject on the list of sampling frame. In fact, out of the total sample studied, only two were known to have existent diseases – one had NIDDM and the other had hypertension. As said above, these two subjects were not studied but the next two subjects on the list of sampling frame were taken.

(d) Persons who were not permanently posted to units in Pune or Khadki but were only on temporary attachments were excluded.
(c) Subjects who had been selected in the sample but could not be contacted despite 3 repeated visits at weekly intervals were excluded. In such an exigency, the next serial number on the sampling frame was selected. This, again, was a minor problem with only 4 subjects being excluded and being replaced by the next subject in the sampling frame, on this ground.

4.4. MEASUREMENTS

4.4.1. Measurements made on subjects, in this study, were broadly of four types:

(a) History of various risk factors and other personal details.
(b) Clinical measurements including anthropometry.
(c) Body specimen (Fasting and 2 hours post oral glucose blood samples, and overnight urine samples).
(d) Resting ECG tracings.

4.4.2. Study proforma: For recording the data in respect of the above defined categories, a proforma was developed. It included details of personal particulars, details of physical activity, tobacco use, alcohol consumption, family history of diseases, as well as details of physical examination, anthropometry, results of biochemical investigations and ECG results. The proforma was developed in accordance with textbooks on survey methodology and epidemiologic research (317,318,319). The proforma was pre-tested and standardized during the pilot study (described later), on 25 subjects. The proforma was also pre-coded for computer entry to facilitate accurate data entry into the computer. A copy of the proforma is attached as Appendix 'A' in this thesis.

4.4.3. Methods of making measurements: The proforma was used for obtaining information from the subjects, using personal, face to face interview technique. The
subjects were informed of the scope of the study and were assured of full confidentiality. A verbal, informed consent was obtained. After building up rapport with the subjects, the various details of personal particulars and risk factors were obtained. The methods of physical examination and anthropometric measurements, methods of collection, dispatch and analysis of blood and urine samples, and recording of ECG are being described in detail, subsequently in para 4.5. The survey methodology is also being described in detail in para 4.6.

4.5 METHODOLOGY OF MEASUREMENTS AND RECORDING OF STUDY VARIABLES

4.5.1. **Age** : Age was recorded in terms of nearest completed year, as a numerical continuous variable. It was recorded as per reply given by the individual and verified from his official records (soldier's pay book).

4.5.2 **Socio-economic status** : In the army, the military rank is a direct indication of both, the economic as well as social status of an individual. For this reason, social status was recorded according to the military rank (officer / junior commissioned officer (JCO) / other rank (OR), as a polychotomous ordinal variable, as has been done in earlier published studies in armed forces (320).

4.5.3 **Military occupation** : In the army, there are two broad categories of occupations, viz, “arms” who are concerned with direct combat, and “services” who are concerned with logistic and support duties. Military occupations was recorded in terms “arms” and “services”.

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4.5.4 **Educational Status**: Educational status was recorded as the actual civil educational standard passed by the subject. The details were verified from his official records.

4.5.5 **Duration of army Service**: Duration of service was recorded to the nearest completed year, from the official records.

4.5.6 **Native place**: The native place was recorded according to the subject’s permanent residence, from his service records, on a nominal polytomous scale.

4.5.7 **Assessment of physical activity**:

4.5.7.1 Detailed assessment of physical activity levels of the subjects was undertaken in the present study. The protocol for such assessment was based on the large scale studies done by other workers and recommendations of experts. The overall consensus of available scientific evidence is that assessment of physical activity in large scale surveys should be restricted to “leisure time physical activity” (176, 191, 198, 321). Secondly, it has been recommended by authorities that moderate to severe intensity exercises are the ones that are more important for prevention of various chronic diseases, and also since these categories of physical activities are performed less frequently, they are easier to recall and describe, than hours of light activities that most of individuals would be performing (196). Thus, the subjects’ attention was directed in the present study towards the moderate and strenuous activities undertaken by them. A list of these activities was prepared and given in the questionnaire. This list was prepared based on the observations during pilot study and also discussions made with specialists in Sports Medicine in the Armed Forces Sports Medicine Center in Pune. This history was
recorded along the same lines as recommended by other workers in the field of physical exercise and cardiovascular diseases (115, 322).

4.5.7.2. Parameters considered while assessing physical activity:- As per the guidelines available in large scale research studies (191, 198, 270, 321, 323, 324), for each activity performed (during leisure time), information on following three parameters must be enquired with a view to adequately and correctly assess the leisure time physical activities:-

(a) The type of physical activity along with its intensity, so as to get an idea of the energy expenditure.

(b) The average duration (in hours / minutes) for performing each of these activities in each session.

(c) The average frequency, i.e. the number of sessions that were performed for each of the physical activities for a defined period of recall, i.e., during one week or during one month, etc.

4.5.7.3. In any army unit the working schedule revolves periodically around a "weekly cycle", with clearly demarcated allotments for physical training (PT), organized games, and drills, on specified days and times. A soldier gets used to this weekly periodic cycle. However, there are times when he is away from such schedules, like when on long leave, or temporary duty, or during sickness. Against the backdrop of the above mentioned considerations, observations made during pilot study, as well as published reports / studies of other workers in this field (191, 198, 201, 270, 321, 324), it was decided to assess the frequency and duration of each type of leisure time physical activity, on an average, during one week, keeping the overall period of recall as 1 year.
(12 months). In addition, the most valid answers, as ascertained during pilot study, were obtained when the frequency and duration was recorded, on an average, for a week, with the overall period of recall specified as 1 year.

4.5.7.4. **Unit of measurement of physical activity**: Most of the recent large scale epidemiological studies, which have assessed physical activity in the population based settings, recommend that physical activity should be measured in terms of Metabolic Equivalents (METs) (154,191,201,321). This metabolic rate is the ratio of metabolic rate during exercise to the metabolic rate at rest (196,201,321,322). MET multiples have the advantage that they are close to the earlier used BMR multiples, even when they need not take into account the subject's sex and body surface area (154,322).

\[
\text{MET} = \frac{\text{Metabolic rate during a particular exercise}}{\text{Metabolic rate at complete rest}}
\]

4.5.7.5. Apparently, when the person is at complete rest, the numerator in the above equation becomes equal to the denominator, and that is what 1 MET is equal to. In calorie equivalents, one MET is equal to 1 kcal/kg body weight/hour (196,201,321,322).

To put the same in the perspective of equations,

\[
\text{Caloric expenditure due to a particular exercise} = (\text{MET level of that particular exercise}) \times (\text{Body weight in kg}) \times (\text{duration in hours for which the exercise is undertaken}).
\]

4.5.7.6. The METs of various types of physical activities have been worked out and used by various workers or have appeared in reports of experts (175,196,201,321,323, 325). Based on these publications, the METs of various physical activities as used in the present study were:- walking (average intensity at 4.8 Km/hr : 4.2
MET; jogging 10.1 MET; cycling 5.8 MET; swimming 5.4 MET; ball games (football, hockey, basketball, volleyball) 6 - 7 MET; gymnastics, dancing or weight lifting 5.0 MET; crafts and domestic repairs 2.7 MET; gardening 4.3 MET; farming / grass cutting 4.3 MET; walking to work 3.5 MET; bicycling to work 5.1 MET; sweeping and mopping : 4 MET; orderly or peon's jobs 4 MET; golf while carrying own golf equipment : 4 MET; tennis or badminton doubles : 5 - 6 MET; singles tennis, badminton or squash : 8 – 9 MET; Armed forces – cleaning the kit 2.4 MET; drill : 3.2 MET; route marching 4.4 MET; assault course 5.1 MET; jungle march 5.7 MET; digging holes 5.0 MET; fast cycling at >16 Km per hour : 6.0 MET; mowing lawn with a hand mower : 6.0 MET.

4.5.7.7. **Calculations**: For each or the leisure time physical activity as mentioned by the subject, the total calories expenditure per week was calculated as:

\[(\text{METs for the particular physical exercise}) \times (\text{body weight in kg}) \times (\text{number of times that activity was undertaken in a week}) \times (\text{duration in hours for which that activity was undertaken on an average, on a given day})\]

4.5.7.12. Keeping in line with the recommendations of Blair et al made while developing the physical activity schedule (196), caloric expenditure for each and every leisure time physical activity was worked out separately. Finally they were all added up, to get the energy expenditure in leisure time physical activity in one week.

4.5.7. **Cut off point for physical activity**: Keeping in view the background of large scale studies on the relationship between physical exercise and cardiovascular health undertaken by other workers (175, 198), the following levels of caloric expenditure were considered for initial analysis in the present study: no expenditure; expenditure upto 1400 k cal per week; between 1401 to 2100 k cal; 2101 to 2800 k cal; and 2801 k cal and
more per week. Subsequently, as the analysis proceeded, more precise levels were developed.

4.5.8. Alcohol consumption:

4.5.8.1. Details of alcohol consumption were obtained, keeping the duration of recall for 1 year from the date of interview, as kept for physical exercise. This was in accordance with the criteria used by other workers like Pehm et al (326). Information was obtained as regards the type of alcoholic drink consumed, the “usual” frequency of consumption, i.e., the number of days in a week on which alcohol was usually consumed, and the usual amount consumed in a sitting, in terms of “small pegs” (each small peg being equal to 30 ml of Indian manufactured foreign liquor (IMFL) at 25 U.P. (under proof) and 42.8% v/v which works out to approximately 33.7 grams of ethanol per 100 ml of the liquor, or 10 grams per small peg of 30 ml. This conversion was worked out after personal discussion with the scientific officer in charge of the Public Health Chemistry laboratory in Armed Forces Medical College, Pune. Essentially the same conversion factors have been suggested by other workers (327). Beer consumption was recorded in terms of bottles, each bottle of 650 ml containing approximately 30 grams of alcohol (327). For subjects who had not consumed any alcohol during past one year, history of previous use, if any, was asked. Based on the above information, as well as on the basis of work done by earlier authors (326), average alcohol consumption in grams per week was worked out as:

\[
\text{Alcohol consumption in grams of ethanol per week} = (\text{Number of small pegs usually consumed in a day}) \times (\text{Number of days in a week when alcohol was usually consumed}) \times 10.
\]
4.5.8.2. For example, for a subject who said that he used to drink rum, on an average on two days in a week, and on a particular day on which he drank, he was usually consuming two large pegs (i.e. 4 small pegs), his alcohol consumption per week worked out to 2x4x10=80 grams / week. In addition, some workers have also suggested the importance of the overall consumption of alcohol over the lifetime (102), thus including not only the amount and frequency per week, but also the total duration for which the subject had been drinking. With this background, information was also obtained from the subjects about the total number of years for which they had been consuming alcohol, and the lifetime consumption of alcohol in Kilograms was calculated as:

\[
\frac{(52 \times \text{Average weekly consumption in grams}) \times \text{Total number of years for which the subject had been drinking}}{1000}
\]

4.5.9. **Tobacco use**:

4.5.9.1. Details of tobacco use were obtained during the personal interview and recorded on the proforma. The duration of recall was kept as one year, as for physical exercise and alcohol use. For subjects who had not used tobacco during the past one year, history of previous use, if any, was asked. The details of tobacco use included the form in which tobacco was being consumed (cigarette, beedi, guthka etc) and frequency of consumption in a week (i.e. the number of days in a week that the subject usually used tobacco, viz, daily, alternate days, two days a week, on one day in a week, or not even one day in a week). Thirdly, the usual amount on any day when the subject consumed tobacco (eg, usual number of cigarettes / beedis / guthka packets consumed in a particular day when he consumed tobacco) was asked. Recording was done as a numerical variable (i.e. number of cigarettes / beedis, or guthka packets consumed in a
day). For the purpose of computation, the average number of cigarettes consumed per day were calculated as:

\[
\frac{\text{(Usual number of days in a week when the subject smoked) \times (Number of cigarettes usually smoked on a day on which the subject smoked)}}{7}
\]

4.5.9.2. For the purpose of calculation, 2 beedis were taken to be equivalent to one cigarette. Computation of chewed tobacco (ghutka and other forms) was done separately.

4.5.10. **Further information regarding variability in the pattern of alcohol and tobacco consumption over the years:** It was revealed in the pilot study that a small proportion of subjects may vary their pattern of consumption of alcohol and tobacco over the lifetime, as regards frequency of consumption per week as well as usual amount consumed. For this reason, in each of the sections meant for collecting information about alcohol and tobacco usage, additional questions were kept, asking the subject whether the general pattern of consumption as regards frequency and amount was consistent over the duration of consumption, or else there were fluctuations. In case the subject replied in the affirmative, then further details of consumption as regards frequency, amount and duration were asked. Finally, for subjects who said that they had not used tobacco / alcohol during the past one year, details of earlier use were asked. However, the proportion of subjects who gave history of variable consumption pattern over the lifetime was quite low, with only 3 subjects giving such history of variable consumption as regards alcohol consumption and 4 subjects gave such history of variable consumption as regards tobacco use. Similarly, the proportion of “past users” was also quite low, being 2 for alcohol consumers and 10 for tobacco users. The information provided by all these
subjects was duly included while calculating the lifetime consumption of alcohol and tobacco.

4.5.11. **Family History**: Family history of ischaemic heart disease, diabetes mellitus and hypertension among first degree relatives (parents and siblings) was recorded from each subject. The subject was asked to give his answers as “definitely yes”, “probably yes” or “definitely no” for each of these diseases, for each of the first degree relatives. In case the answers were definitely or probably yes at any one or more of the places, the subject was asked to provide additional details in the form of an open ended question. Based on these details, a decision was taken whether the family history could be taken as positive or not.

4.5.12. **Medical History**: The subjects were asked if they were having any symptoms presently, and if so, the details were taken. Next, detailed past medical history as well as any history of downgrading of medical category, by the army medical authorities, was asked for.

4.5.13. **Physical examination and anthropometry**

4.5.13.1 **Blood pressure measurement**: Blood pressure was measured according to latest guidelines provided by the WHO (7) by auscultatory method, using a mercury sphygmomanometer and stethoscope. Measurements were made with the subject sitting comfortably, cuff tied on the right arm, the right arm being supported on a table kept in front, with the elbow at the level of fourth intercostal space. The first appearance of sound (phase I of Korotkoff’s sounds) was taken as systolic pressure, while complete disappearance of sound (phase V) was taken as diastolic pressure. Two readings were
recorded at an interval of 3 minutes, and the average of the two readings was recorded both for systolic, as well as for diastolic pressure.

4.5.13.2 **Height**: Height was measured using standard guidelines given by a recent WHO expert committee (150). A height measuring stand having a foot-board, vertical board with attached metric markings, and a mobile, horizontal headboard (that can be brought into contact with the uppermost point on the head) was used. The subject, in undergarments and barefooted, stood on the foot-board with weight distributed evenly on both feet, heels together, and the head positioned such that line of vision was perpendicular to the body. The arms were hanging freely by the sides, and the head, back, buttocks and heels were in contact with vertical board. The subject was asked to inhale deeply and maintain a fully erect position. The moveable horizontal headboard was brought down, onto the topmost point on the head with just sufficient pressure to compress the hair. The height was read off from the metric markings on the vertical stand, to the nearest 0.1cm.

4.5.13.3 **Weight**: Weight was measured using portable scales. These were standardised against beam balance type of machine, initially at the start of the study and subsequently on first working day of every week. Weight was measured using standard guidelines of Dowse and Zimmet as appearing in a WHO publication (328). The scale was kept on a firm, horizontal surface. The subject was weighed in undergarments, standing on the centre of the scale’s platform, and weight evenly distributed on both the feet. Weight was recorded to the nearest 0.5 Kg.
4.5.13.4 **Chest, Waist and Hip measurements**: These measurements were also made based on WHO guidelines (150,328). A dress-maker’s measuring tape, as recommended by Dowse and Zimmet was used (328). The details of measurements are as follows:

(a) **Chest**: Chest was measured with the subject standing and weight distributed evenly on both the feet. The subject was asked to abduct the arms slightly, to permit passage of the tape around the chest. When the tape was snugly in place, the arms were lowered to their natural position, at the sides of the trunk. Chest measurement was made at the level of the fourth costo-sternal junction, counting the number of ribs from above. The measurement was made, in a horizontal plane, at the end of a normal expiration. Measurements were recorded to the nearest 0.1 cms.

(b) **Waist**: Waist circumference was measured with the subject in undergarments, standing comfortably, weight evenly distributed on both feet, and the feet 25 to 30 cms apart. The measurement was taken at a level midway between the inferior margin of lowest rib, and the crest of ilium. Both these landmarks (inferior margin of lowest rib and crest of ilium) were palpated on both sides and marked; the points mid-way between these landmarks, on both sides, were also marked using a tape measure. Measurement was made in a horizontal plane, with tape passing around, over the midpoints marked on each side. The observer sat in front of the subject and passed the tape, at the marked level, horizontally, and snugly, but not so tightly as to compress the underlying soft tissues. Measurement was made at the end of a normal expiration, to the nearest 0.1 cm (150,328).

(c) **Hip**: Hip circumference was measured with the subject in undergarments, standing erect, arms at the sides and feet together. The observer sat at the side of the subject so
that the levels of maximum extension of the buttocks could be seen. Hip girth was measured at the maximum circumference around the buttocks, in a horizontal plane, indicated posteriorly by the maximum extension of the buttocks and anteriorly by the symphysis pubis (328,150). The tape was kept snug against the skin, but taking care that it does not compress the soft tissues. Measurements were recorded to the nearest 0.1 cms. As recommended by Dowse and Zimmet, measurements of waist and hip were repeated once more, following both the initial readings, and mean values of the two readings were recorded. If there was a variation of more than 2 cms between the two readings, then a third reading was also taken and recorded. In such cases, the 2 readings most consistent with each other out of the three were taken and their mean was recorded (328).

4.5.13.5. **Subcutaneous skin folds**: As recommended in the WHO monograph on cardiovascular survey methods (318) and a WHO expert report (150), subcutaneous skin folds (subcapular and triceps) were measured using “Lange type” calipers which exert standardised pressure at the jaw openings.

(a) **Triceps skin fold (TSF)**: This was measured in the midline of the posterior aspect of the right arm, over the triceps muscle, at a level midway between the lateral projection of the acromion process at the shoulder, and the olecranon process of the ulna (at the point of the elbow). With the elbow flexed to 90°, this midpoint was determined by measuring the distance between the two landmarks, using a tape measure; it was marked on the lateral side of the arm. The subject was then asked to stand comfortably, with the arm hanging loosely and comfortably by the side, and the caliper held in observer’s right hand, a vertical fold of skin and subcutaneous tissue was picked up gently, with the left
thumb and index finger, approximately 1 cm proximal to the marked level, and the tips of the calipers were applied perpendicular to the skin fold at the marked level.

Measurements were recorded to the nearest 0.5 mm (15,318).

(b) **Subscapular skin fold (SSF)**: Was measured on the unclothed back, with the subject standing in a relaxed position, arms hanging by the sides. To locate the site, the observer palpated the right scapula, running the fingers inferiorly and laterally, along its vertebral border, until its inferior angle was identified. The SSF was picked up just below the inferior angle of the right scapula, between the left thumb and index finger, gently, in a diagonal fold, inclined inferolaterally at about 45° to the horizontal plane, in the natural cleavage line of the skin. The fold thus lifted was seen to be running at an angle of about 45° downwards from the spine. The caliper jaws were applied 1 cm inferolateral to the left thumb and index finger which were raising this fold, and measurements made to the nearest 0.5 mm. (150,318).

4.5.13.6 **Computation of Ratios**

(a) **Body Mass Index**: The Body Mass Index (BMI) (also known as Quetlet’s index) has been recommended by the WHO as a standard measure of overweight and obesity (329). In addition, most of the large scale epidemiological studies have used this as the criteria of defining overweight / obese. In this study the BMI was calculated to the second decimal place and used as per the WHO guidelines, as

\[
\text{BMI} = \frac{\text{Weight in Kg}}{(\text{Weight in metres})^2}
\]

The levels of BMI for defining normal weight, overweight and obese were (329) :-
BMI less than 25 : Normal weight
BMI 25 to 29.9 : Overweight
BMI 30 to 39.9 : Obese
BMI 40 and above : Morbid obesity.

(c) **Waist : Hip ratio (WHR)**: Waist : Hip ratio is also used as a standard surrogate measure of central obesity. A large number of epidemiological, clinical and laboratory studies have given a clear demonstration of the importance of WHR as an important predictor of various non-communicable diseases, including syndrome X. The same has already been discussed at length, while reviewing the literature. WHR was calculated in this study, to the nearest second decimal place as

\[
\text{WHR} = \frac{\text{Waist circumference (cms)}}{\text{Hip circumference (cms)}}.
\]

Since the present study was on male subjects only, the various levels of WHR were decided as recommended by various studies and experts, for males. As far as developing countries are concerned, there are no standard guidelines for defining the cut off points for optimum WHR. However, as mentioned by Gupta et al, the United Status national cholesterol education program recommends that ideally the WHR should be less than 0.9 in males (and less than 0.8 in females) (160). This criteria has also been used in other studies (56, 76). On the other hand, some other studies (163, 164) have recommended a cut off level of 0.95 and above for men. In view of the same, in the present study, it was decided to use the following levels of WHR:

- WHR < 0.90 : Normal
- WHR 0.90 – 0.949 : Level I increase in central obesity
- WHR > 0.95 : Level II increase in central obesity.
In addition to WHR, since there have been some studies which have indicated the importance of waist circumference in predicting various diseases (164, 330), it was decided to study the role of waist circumference, in addition to the role of WHR as defined above.

(d) Subscapular skin fold : Triceps skin fold (SSF / TSF) ratio: In addition the SSF / TSF ratio was calculated as the ratio of SSF : TSF and was used as an additional measure of central obesity, as done by other workers (154, 47, 141).

4.5.14. Electrocardiographic (ECG) recordings: ECG was recorded using a standard, portable ECG machine, manufactured by BPL, India. The subject was asked to lie down supine, on a couch, in his undergarments, in a warm, comfortable room. The recording started after the subject had relaxed for 3 to 5 minutes. A resting, standard, 12-lead ECG was recorded (leads I, II, III, aVF, aVL, aVR, v1 to v6). Recording was done by trained paramedical worker who had been trained, tested and certified by a faculty member from Department of Medicine in recording of ECG.

4.5.15. Collection and analysis of Body specimen

4.5.15.1. Collection of blood: All subjects were adequately briefed, three days prior to the date of examination, to consume a normal diet for three days, and to have a light diet between 6 p.m. and 7 p.m. on the evening before the examination. Subsequently, they were asked to remain fasting till the time of physical examination and not consume anything, even tea. However, they were allowed to drink water after having dinner on the day prior to the examination. They were also instructed not to smoke on the morning of the examination.
12 ml of venous blood was drawn by well trained laboratory assistant and transferred into three separate vials as follows:

(a) 2 ml in sodium fluoride vial for fasting blood sugar.
(b) 2 ml in clear sterile vial for uric acid.
(c) 1 ml in a vial containing Wintrobe’s mixture, for total leukocyte count.
(d) 7 ml in clean sterile vial for serum lipids and insulin.

Specimens for blood sugar and T1.C examination were kept in fridge at 4°C. Specimen for uric acid and serum lipids were kept at room temperature for half an hour to allow for clotting, and thereafter kept in the fridge at 4°C.

2 ml blood sample for post prandial (PP) blood sugar examination was drawn two hours after giving 75 g of anhydrous glucose. Blood was drawn in the same manner as defined for fasting sample. Subsequently, at the end of day’s work, by about 9 a.m., all specimens were transported to the laboratory, on icepacks, in a thermos.

It was ensured that all specimens reach the laboratory by 10 a.m.

Urine sample: All the subjects were provided with a clean, wide mouthed bottle of 1 litre capacity and were instructed to pass urine into the bottle starting from 7 p.m. on the evening before the day of the examination till next day morning. This total overnight collection of urine was used for analysis of urinary sodium and potassium. Apparently, in clinical studies, analysis of 24-hours urine sample is the preferred method. However, in population based epidemiological studies on free living healthy subjects, collection of 24-hours urine sample is almost impossible: besides, in such free living healthy subjects, there is always a problem of “incompetence of collection”, if 24-hours samples are collected, thereby causing a bias. In this regards, various workers have
conclusively proved that overnight urine samples are quite practical and acceptable method of assessment of urinary sodium and potassium. These issues have been further elaborated, subsequently, while discussing the results of urinary sodium and potassium. With this background, overnight urine samples were analysed for sodium and potassium in the present research work.

4.5.16. **Procedure for testing of body specimen in laboratory**: Blood and urine samples collected as above were analysed at Armed Forces Medical College, Pune. The tests were done by trained laboratory personnel, under supervision of faculty members in Biochemistry and Public health chemistry. The procedures were:

4.5.16.1. **Biochemical analysis**: Biochemical analysis was carried out using autopack reagent. Blood glucose (venous plasma) was estimated using glucose oxidase–peroxidase method. Estimation of cholesterol was done using cholesterol ester, hydrolase, cholesterol oxidase and peroxidase. The chromogen used was 4-aminophenazone and phenol. HDL-cholesterol was also estimated in the supernatant using the same method, after precipitating serum with phosphotungstic acid and magnesium chloride. The triglycerides in serum were estimated enzymatically. The triglycerides were phosphorylated and oxidised and colour produced with 4-amino-antipyrine and peroxidase. LDL-cholesterol was calculated from triglycerides, total cholesterol and HDL-cholesterol (331,332).

4.5.16.2. **Urinary sodium and potassium**: The determination was done using a flame photometer. Suitably diluted urine sample was sprayed into the flame photometer and the readings were compared with a set of four mixed standards of sodium and potassium. The 4 standards had the concentration of sodium / potassium as 100/3, 120/4, 140/5, and
160/6 mEq/litre respectively. Final concentration of sodium/potassium in urine samples was obtained by multiplying with the dilution factor. Assessment of urinary sodium and potassium was done by the senior faculty member – in charge of Public health chemistry laboratory of department of Preventive and Social Medicine. Flame photometer of EEC, England make, which was available in the laboratory, was used for this purpose.

4.5.16.3. **Estimation of insulin** :- Serum insulin was assayed by Radio-Immuno-Assay (RIA), using “coat-a-count” insulin kit from Diagnostic Products Corporation, Los Angeles, USA. The tests were put in duplicate and the results were calculated according to the protocol given with the kits. The results were expressed in micro units per millilitre (mU/ml). The insulin antiserum used in this kit is highly specific for insulin with a very low cross-reactivity with other compounds that may be present in the serum samples.

4.5.16.4. **Serum uric acid** : Serum uric acid was measured using standardised autoanalyser in the Department of Biochemistry.

4.5.16.5. **Total Leucocyte Count (TLC)**: TLC estimation was done using the chamber count method by well trained and experienced laboratory technicians.

4.5.17. **Issues of validity and reliability of measurements and quality control** : All physical instruments used in the study (height measuring stand, weighing machine, ECG machine, skin calipers, measuring tape and sphygmomanometer) were validated against standard equipment in the Department of Internal Medicine at the start of the study and, subsequently, once a week during the conduct of the study. Quality control procedures for laboratory methods and test samples were run by experienced faculty members in
biochemistry/public health chemistry to cross-check the results obtained by the technicians, at periodic intervals. In addition, as far as possible, replicate measurements were made for variables like blood pressure and anthropometric measurements.

4.6. **SURVEY METHODOLOGY AND DATA COLLECTION**

4.6.1. The detailed methodology for carrying out the survey and collection of data from the subjects selected in the sample was as per the standard guidelines given by the World Health Organization (WHO) for survey methods in cardiovascular diseases (318) and for diabetes and other non communicable diseases (328). Details of the steps are described in the succeeding paragraphs.

4.6.2. **Contact with the administrators**: At the time of planning the study, the local military commanders of Pune and Khadki cantonments were contacted, and the scope of the research work was explained in detail. Their co-operation was solicited as regards permission to conduct the project, and for making available the selected subjects at the required days and time. The military commanders were also requested to provide logistic support in the form of proper accommodation at various sites for undertaking the survey, as well as provision of transport for bringing the selected subjects from their respective units to the site of the survey. All the military commanders were very co-operative as regards the assistance that was asked of them.

4.6.3 **Time, place and number of subjects**

4.6.3.1. An optimum time for obtaining the selected subjects in army set up is in the early morning, when all personnel of any army unit report for morning physical training (PT). This suited very well to the study objectives since one of the objectives of
the present study was to draw fasting blood samples for sugar, insulin and lipid estimation.

4.6.3.2. Normally, all the army units conduct morning PT on at least 4 days a week, viz., Mondays, Tuesdays, Thursdays and Fridays. It was therefore decided to conduct the survey on these four days every week, from 6 a.m., and finishing completely by 9 a.m., so that the personnel were easily available to their respective army units after 9 a.m. Thus the survey work had minimum interference with the functioning of the army units.

4.6.3.3. Most of the actual data collection was to be done by the worker himself, with small amount of support from his team members in the form of documentation, drawing of blood samples and recording of ECG. Considering this aspect, it was felt that a maximum of 5 subjects could be assessed in detail on a given day of survey. Thus, 5 subjects were studied on every day of survey.

4.6.4. **Briefing of selected subjects and informed consent**

4.6.4.1. From every randomly selected unit / subunit (selected by multistage random sampling as described under sampling procedure in para 4.3.5. earlier), the commanding officer of the unit was contacted one month in advance of the date from which the survey was to start in his unit. The details, scope and utility of the research work was once again explained to him. Since the various commanders had already been briefed in detail (as explained in para 4.6.2. above), no difficulty was encountered during this meeting and all help and co-operation was provided by the commanding officers.

4.6.4.2. A list of all persons physically available in the unit was obtained. The list was serially numbered and a 1 in 4 sample was selected by random sampling from this
list using random number table. The administrative officer of the army unit (adjutant) was given the detailed list (termed as a “nominal roll”) of subjects who were finally selected for the survey. A time-schedule was made so as to cover 5 subjects in a day for the survey. This time table, showing the personal details of the selected subjects, and the date time and place on which they were to report to the survey site was given to the administrative officer for issuing the necessary instruction. A copy of this time table was kept with the worker.

4.6.4.3. All subjects, selected by random sampling, were contacted 3 days prior to their appointment for survey. They were briefed personally, by the worker regarding the scope of the study. They were also briefed in detail regarding the fact that they should have normal meals for the next 3 days, and on the evening before the survey, they should finish eating their dinner by 7 pm, and subsequently, they should not consume anything (except plain water) as also that they should not consume any early morning bed tea on the day of survey. Thus, the subjects were explained in detail about coming to the survey site in an overnight fasting state. The subjects were also asked not to smoke or consume tobacco in any other form, on the day of coming for the survey, as per requirements of the oral glucose tolerance test of WHO (37).

4.6.4.4. The subjects were also provided with one clean bottle each of 1 liter capacity and were asked to pass urine into this bottle from 7 pm onwards, on the evening prior to the survey, and all urine overnight, till next day morning. They were asked to bring the bottle containing their overnight urine collection on the day of the survey, where it was collected and labeled at the registration counter.
4.6.4.5. Once again, one day prior to the day when the subjects were to report for the survey, they were again contacted and reminded about the various procedures explained above. Fine-tuning of their requirements of transport for the next day was also done in liaison with the respective administrative officers of the unit.

4.6.4.6. In case a person could not report physically, or could not report in proper fasting state, or else was having mild sickness on the day of survey, efforts were made to get the same subjects at a later date by maintaining liaison with the administrative officer.

4.6.4.7. In case a subject who was selected by random sampling could not be made available by the unit for administrative reasons (e.g., having left on transfer or long leave etc), then the next serial number on the list of subjects was taken up. However, this accounted for only a small proportion, since out of the 614 subjects finally studied, 608 were those who were selected by original simple random selection. As regards the remaining six, 4 were the ones who were selected as the next serial number on the list, since the originally selected subjects was not available (as described in para 4.3.6.(e) earlier). Another two were those subjects who already had diabetes or hypertension (vide para 4.3.6.(c) earlier) and hence were replaced by the next subject on the list.

4.6.4.8. In case any subjects had symptoms or signs of acute infection (fever, headache, bodyache, nausea, vomiting, loose motions etc.), his interview was undertaken, but his clinical examination and drawing of body samples was undertaken at a later date, when he was asymptomatic. Only 2 out of the 614 subjects had to be postponed for this reason.

4.6.5. Selection of the survey sites

4.6.5.1. The survey sites were selected with two broad considerations in mind:
FIG-5
LAY-OUT OF SURVEY SITE

ENTRY

ASSEMBLY & WAITING

REGISTRATION COUNTER AND DEPOSITION OF OVERNIGHT URINE SAMPLE

AREA FOR CENTRAL COLLECTION OF FORMS & SPECIMEN

URINE

DRAWING OF FASTING BLOOD

AREA FOR HEALTH EDUCATION, PERSONAL ADVISE & DISPERSAL

FASTING BLOOD

FILLING OF QUESTIONNAIRE, PHYSICAL EXAMINATION

E.C.G. RECORDING

ROOM NO. 1

ROOM NO. 2

ROOM NO. 3

ROOM NO. 4

EXIT

E.C.G. TRACING

MOVEMENT OF SUBJECTS

FLOW OF SAMPLES & DOCUMENTS

LEGEND
(a) They should be close to the various army units whose subjects were being covered in the survey.

(b) They should provide adequate facilities for undertaking the various procedures.

4.6.5.2. With the above consideration, a total of \( x \) different sites were selected. These sites had an already established large Medical Inspection Room (MI Room) with facilities like adequate space, electricity, refrigerator, water, furniture, facility for ice packs, telephone etc. After the selected subjects from a group of army units were studied at the particular site, the survey site was shifted to the next location and due intimation about the new site was given to administrative officers of all army units which were to be covered from the particular location.

4.6.6. **Layout of the survey site**

4.6.6.1. With a view to ensure an orderly flow of subjects as well as to collect all the required data and samples for investigations, the layout of the survey site was organized according to standard guidelines of the WHO (328). The sequence of movements of subjects is described in succeeding paragraphs. The same is also presented in Figure-5 which is placed opposite.

4.6.6.2. **Room No 1**

4.6.6.2.1. The subjects were given the time of assembly as 6 a.m. The first counter in Room No 1, which was a large room, was the assembly and waiting area, where chairs were positioned for the subjects to sit comfortably. The next counter in this room was the registration counter which was manned by a trained Health Assistant (a paramedical worker with specialized training in public health, including Sanitary Assistant's Diploma). The health assistant was already given a copy of the nominal roll of the
subjects who were to report for the survey on that particular day. After confirming that all
the subjects who were to come on that day had arrived, registration of the subjects was
done at this counter. This included filling up their personal particulars as well as the
survey serial number on the proforma which was given to the subjects. These entries
were also made on another register called as the “Survey Register”. The overnight urine
samples brought by the subjects were also collected and labeled here. From the
registration counter, the subjects moved to the next counter in the same room where
fasting blood sample was drawn and collected into different vials, for fasting blood sugar,
insulin, lipids, uric acid and TLC. The details of method of drawing these samples have
already been described in para 4.5.15. The sample vials were labeled and a tick mark was
placed on the subject’s proforma, in front of the heading for these investigations,
indicating that these samples have been drawn. This counter was manned by a well
trained Laboratory Assistant.

4.6.6.2. From the counter for drawing the fasting blood sample, the subject, along
with his proforma, moved to the next counter within this room, which as managed by
another trained Health Assistant. Here, 75 gram of glucose dissolved in 250 ml of water
was administered orally. Glucose packets of requisite quantity were made before hand by
weighing the glucose in chemical balance of public health chemistry laboratory of
AFMC. Similarly, measuring tumbler was used to measure 250 ml of water which was
poured into separate glass tumblers and one packet of 75 grams glucose was dissolved
using a clean spoon. Adequate number of tumblers, spoons and plates were carried along
with the survey equipment. The subject was asked to drink the solution over a period of 5
minutes. The subjects were also instructed not to eat or drink anything, or smoke, for the
next 2 hours. Till such time their second blood sample was drawn. A nominal roll, in duplicate, was made at this counter and the time at which the subjects started drinking the solution was noted in both the copies. All subjects were asked to wait in the waiting area of Room No 4 for giving the 2 hours blood sample. After all the subjects had taken oral glucose solution, the duplicate copy of this nominal roll was sent to the counter in room no 4 for drawing 2-hours blood sample, so that the presence of the subjects may be checked by the in charge of this counter and the 2 hours sample could be drawn, right in time.

4.6.6.2.3. Room no 4 also had a counter for central collection of specimen and forms. All the specimen (overnight urine, fasting blood samples, 2 hours blood samples) as well as the questionnaire, duly filled, and the ECG strips were sent to this counter. This counter was managed by a senior paramedical official (a junior commissioned officer) who had adequate experience in both administrative work as well as health survey work. All the questionnaire were checked at this counter for any missing data. The specimen were checked at this counter for proper labeling and then kept in the fridge which was available in the vicinity. The ECG strip was also checked for proper labeling and then attached with the questionnaire. Laboratory investigation requisition slips were also made for each subject at this counter and attached to the questionnaire. Finally, an entry was made by the in charge of this counter on the nominal roll obtained from the registration counter that all samples had been received and all entries were checked and found complete. In case of any missing data entry or any difficulty with the samples, the in-charge of this counter immediately informed the principal worker who was available in the next room.
4.6.6.3. **Room No. 2**: This room was meant for filling up the questionnaire, including details of physical exercise, tobacco use, alcohol use and other risk factors. Measurement of anthropometric parameters, as well as physical examination was also conducted here. This room was manned by the principal worker himself and the entire history taking, and physical examination was conducted personally by the principal worker, in full privacy. After completion of the history taking and physical examination, the questionnaire was checked again for any missing information. Thereafter the questionnaire was sent to “central specimen and forms counter” in room no. 1 and the subjects moved to room no. 3.

4.6.6.4. **Room no. 3**: From room no. 2, the subjects moved to Room no 3. The resting ECG was conducted in this room. This was conducted personally by a senior paramedical worker of the rank of a Non Commissioned Officer (NCO), who had been trained, tested and certified by a faculty member from Department of Medicine in the method of recording ECG. The method of recording the ECG has been described earlier in para 4.5.14. After the ECG was recorded, a check was made by the in-charge about the correctness of the tracings, and the personal particulars of the subjects as well as his survey serial no. was written on the ECG strip. The tracing was sent to the central counter for collection of specimen and forms, while the subjects was asked to proceed to Room no 4.

4.6.6.5. **Room no. 4**: As the subjects entered room no. 4, they were asked to sit down comfortably in the waiting area. The time of their having taken oral glucose solution (as mentioned in the copy of nominal roll which was sent by the oral glucose load counter) was checked with the current time and the subjects was advised to wait here till the exact
time when 2 hours following oral glucose would be over. Exactly when two hours were over, the 2 hours blood sample was drawn, labeled, and sent to central specimen collection counter while the subjects was asked to wait in the health education and dispersal area in the same room. When all the subjects had been examined and specimen collected, the principal worker personally came and talked to the subjects who were sitting in the dispersal area. The method of communication was a “small group discussion”, in which the subjects were educated regarding various risk factors for cardiovascular diseases and NIDDM, and their prevention. They were also asked to spread this message to their colleagues and family members. In addition, they were asked to contact their unit administrative officer after 1 week to take the results of their investigations and referral forms, in case any subjects needed further referral to the hospital. Finally, all subjects were thanked for their cooperation.

4.7. **REFERAL SUPPORT**: Within a week of the examination, all investigation results were obtained and entered in the questionnaire. A summary of the findings was made for each subject and handed over personally to the administrative officer of the unit for further handing over the to the subject. In addition, for any subject who needed referral to the specialist for further evaluation, a referral case sheet was made and handed over, along with the investigation results / ECG strip, personally to the subject who was also advised on the details of further action to be taken by him. The specialists in the referral hospital were also informed regarding these subjects who were referred to them.

4.8. **ETHICAL ISSUES**: The study was purely an observational study without any active intervention being undertaken. Thus the problem of interference with ethical issues was, in any case, minimal. In the present study. In fact, on the contrary, the study was
ethically strong since subjects who were detected to be having any abnormality were given the benefit of further evaluation/treatment by specialists, and, in addition, all subjects were provided with health education. To further safeguard the ethical aspects, the protocol was submitted to the higher army authorities whose prior approval was taken to conduct this study. Moreover, each and every subject was informed of the scope of the study and his verbal, informed consent was taken, as has been mentioned earlier. Finally, full confidentiality of data provided by subjects was maintained.

4.9. **DIAGNOSTIC CRITERIA**

4.9.1. **Criteria for insulin resistance and hyperinsulinaemia**

4.9.1.1. Various workers have used raised fasting insulin levels (fasting hyperinsulinaemia) as the measure of insulin resistance in large scale epidemiological studies. It is accepted in the scientific circles that fasting plasma insulin level is a highly correlated and effective surrogate measure of insulin resistance, with higher plasma insulin levels indicating a lowered insulin sensitivity, i.e. insulin resistance (30,40,94,99,100,101,102,103). This criteria has been used by various workers in several large scale epidemiologic studies (39, 43, 47, 48). Based on this strong scientific evidence already available, raised levels of fasting insulin (fasting hyperinsulinaemia) were taken as the measure of insulin resistance in the present study.

4.9.1.2. **Criteria for raised levels of fasting insulin (fasting hyperinsulinaemia)**: As on today, there seems to be no standard, accepted definition of "hyperinsulinaemia" (104). Majority of the studies undertaken on syndrome X till now have defined "fasting hyperinsulinaemia" as subjects who were have fasting insulin levels in the highest quintile (i.e. upper 20%) of insulin values in the data set. These studies include various
large scale and methodologically strong scientific studies (44, 48, 105, 291). With the above background, the criteria of fasting hyperinsulinaemia was kept as subjects having fasting insulin values in the upper quintile (upper 20%) of the fasting insulin values.

4.9.2. **Criteria for definition of “syndrome X”**

4.9.2.1. The factors to be included in syndrome X were identified on the basis of the original definition of syndrome X as proposed by Reaven (21). As per this criteria, insulin resistance (as measured by hyperinsulinaemia) is the key issue which initiates a chain of pathophysiological events which result in clustering of certain specific cardiovascular risk factors. The specific risk factors, besides hyperinsulinaemia (as defined above, as subjects in the upper most quintile of fasting insulin), include raised blood pressure, impaired glucose tolerance, elevated plasma triglycerides and lowered HDL-cholesterol (21). Against this background, as well as based on the results of other studies (39, 68, 333), it was felt that at least three factors should be present together in constellation i.e., hyperinsulinaemia plus two more of the following three- hypertension, IGT, dyslipidaemia (either raised triglycerides or low HDL or both ), so as to define a person as having syndrome X. In addition, it was also decided to study the specific combinations of various risk factors and to assess the statistical probability of such combinations occurring by random variation (chance).

4.9.3. **Definition of the levels of individual risk factors**

4.9.3.1. Having decided that at least three risk factors should be present in a subject for him to be brought under the criteria of syndrome X (hyperinsulinaemia and two more factors), and having defined hyperinsulinaemia as fasting insulin levels in the
uppermost quintile, the cut off criteria for other risk variables were defined as per standard criteria, as follows:

(a) **hypertension**: hypertension was defined as systolic BP \( \geq 140 \) mm Hg, and/or diastolic BP \( \geq 90 \) mm Hg as per guidelines of the recent WHO expert committee on hypertension (7) and other standard texts (334).

(b) **Impaired glucose tolerance (IGT)**: IGT was defined as per the recent WHO guidelines (37), as fasting sugar (venous plasma) < 140 mg/dl and 2 hours PP levels between 140 to 200 mg/dl.

(c) **Dyslipidaemia**: A large number of studies in this field have used the definition of dyslipidaemia as either hypertriglyceridaemia or low HDL-cholesterol or both (39,44). The cut off points for triglycerides and HDL were decided as follows:

(i) **Triglycerides** - Keeping in view the overall evidence and criteria used by different workers (39, 43, 44, 56, 274, 285), a cut off criteria of > 200 mg/dl was used to define hypertriglyceridaemia, since this criteria has been used by most of the workers.

(ii) **HDL-cholesterol** - Keeping in view the overall scientific evidence, it was decided to use a cut off criteria of < 35 mg/dl for defining low HDL-cholesterol levels, since this cut off point has been used by most of the workers (39, 43, 47, 56, 74, 76, 274, 285).

4.9.3.2. Thus, to summarize the criteria for syndrome X in this study, the same was defined as hyperinsulinaemia (fasting insulin level in the upper most quintile) with any two of the following three risk factors:

(a) **hypertension**: SBP \( \geq 140 \) mm Hg and/or DBP \( \geq 90 \) mm Hg.
(b) **IGT**: Fasting blood sugar (venous plasma) < 140 mg/dl and 2 hours PP levels between 140 to 200 mg/dl.

(c) **dyslipidaemia**: TG > 200 mg/dl or HDL < 35 mg/dl or both.

4.9.4. **NIDDM**: The two major disease outcomes for which this risk factor clustering in syndrome X acts as strong risk factor are CHD and NIDDM. The methodology for their diagnosis, as used in this study is described in the succeeding paragraphs.

4.9.4.1 **Diagnosis of NIDDM**: As regards NIDDM, the diagnosis was based on fasting and 2-hours post oral glucose blood sugar (venous plasma levels), using the methodology, as well as the criteria, defined by WHO (8, 37). These criteria were:-

“Fasting blood sugar ≥ 140 mg/dl or 2 hours PP blood sugar ≥ 200 mg/dl.”

4.9.4.2. Since the study was in an adult population aged ≥ 35 years, it was reasonable to assume that all cases detected by the above criteria would be those of NIDDM and no case of IDDM would be there. This assumption was based on the justification provided by Warram et al in Joslin’s textbook of diabetes mellitus wherein it is mentioned that it is extremely unlikely for IDDM cases to be living in adult population aged more than 30 years (168).

4.9.5. **Diagnostic Criteria for CHD**: For the diagnosis of CHD, the available scientific evidence based on large scale epidemiological studies conducted by earlier workers and recommendations of standard agencies like WHO were carefully considered. As per WHO guidelines, recording of resting ECG and classification according to Minnesota code is regarded as an objective method of assessing the frequency of IHD in populations, especially in large epidemiological surveys on persons aged 35 to 64 years (328). Though the resting ECG does has its problems of specificity as well as of
sensitivity, still this is the only method of assessing the prevalence of IHD in large population based epidemiological studies. Thus, resting ECG has been used as criteria of prevalence of IHD in the large scale population based studies, including those undertaken in our country (76, 160, 335, 336, 338, 339). It was, therefore, decided to use resting ECG as a criteria for IHD in the present study. The details of criteria for interpretation of ECG are presented in the subsequent paragraphs.

4.9.5.2. The recording and interpretation of ECG was as per the standard guidelines of WHO, provided in their monograph titled “Cardiovascular Survey Methods” (318). Twelve lead resting ECG as recommended in population studies was used (leads I, II, III, aVR, aVL, aVF, precordial leads VI to V6). The machine used was standard portable ECG machine manufactured by BPL - India. The machine was standardized initially against another machine used in the office of a faculty member in the Dept of Medicine and subsequently, once a week, on every first working day of the week. Repairs, if any required, were undertaken by the army engineers specialized in repair and maintenance of electro-medical equipment. Standard paper speed of 25 mm per second was used. The coding of ECG was as per the standard “Minnesota code” described in detail in the WHO monograph (318). The standard criteria of labeling a subject as IHD on the basis of resting ECG were as per the criteria used in several large scale population based studies (76, 160, 201, 335, 336, 337, 338, 339), as well as the guidelines for using the Minnesota code as published in WHO monograph on Cardiovascular survey methods (318). The following Minnesota codes were used as diagnostic:

<table>
<thead>
<tr>
<th>Minnesota code</th>
<th>Details</th>
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<tbody>
<tr>
<td>1.1.1. Q/R amplitude ratio &gt;= 1/3, plus Q duration &gt;= 0.3 sec, in any of the sites.</td>
<td></td>
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</tbody>
</table>
1.1.2. Q duration $\geq 0.4$ sec in leads I or v6 (anterolateral) or leads II (posterior) or any of the anterior site leads (v1 to v5).

1.1.3. Q duration $\geq 0.4$ sec plus R amplitude $\geq 3$ mm in lead avL.

1.1.4. Q duration $\geq 0.5$ sec in lead III plus Q wave amplitude of $\geq 1.0$ mm in majority of the beats in lead avF.

1.1.5. Q duration $\geq 0.05$ sec in lead avF.

1.1.6. QS pattern when initial R wave is present in adjacent lead, to the right on the chest, in any of the leads v1 to v5.

1.1.7. QS pattern in all of the leads v1 to v4 or v1 to v5.

1.2.1. Q/R amplitude ratio $\geq 1/3$, plus Q duration $\geq 0.02$ sec and $< 0.03$ sec in leads I or v6 (anterolateral) or lead II (posterior) or any of v2, v3, v4 and v5 (anterior site).

1.2.2. Q duration $\geq 0.03$ sec and $< 0.04$ sec in lead I or v6 (anterolateral) or lead II (posterior) or any of leads v2, v3, v4 and v5 (anterior site).

1.2.3. QS pattern in lead I (anterolateral) or lead II (posterior). (Do not code in presence of 7.1.1, i.e., complete LBBB).

1.2.4. Q duration $> 0.04$ sec and $< 0.05$ sec in lead III, plus a Q wave $>1.0$ mm amplitude in the majority of beats in lead avF.

1.2.5. Q duration $\geq 0.04$ sec and $< 0.05$ sec in avF.

1.2.6. Q amplitude $\geq 5.0$ mm in leads III or avF.

1.2.7. QS patterns in all the leads of v1, v2 and v3 (do not code in presence of 7.1.1, i.e. complete LBBB)
4.1.1. ST junction depression $\geq 2.0$ mm and ST segment horizontal or downward sloping in any of the leads I, aVL, or v6 (anterolateral sites); or in lead II or avF (posterior); or in any of the leads v1 to v5 (anterior site).

4.1.2. ST segment junction depression $>1.0$ mm but $<2.0$ mm, and ST segment horizontal or downward sloping in any of the leads I, aVL, or v6 (anterolateral site) or lead II or avF (posterior); or in any of the leads v1, v2, v3, v4, or v5 (anterior site).

5.1. T amplitude negative $5.0$ mm or more in either of leads I or v6, or II or any of v2, v3, v4, or v5; or else T amplitude negative $5.0$ mm or more in either of avL or avF provided R amplitude is $\geq 5.0$ mm.

5.2. T amplitude negative or diphasic (positive - negative or negative - positive type) with negative phase at least $1.0$ mm but not as deep as $5.0$ mm in lead I or v6 (anterolateral), or lead II (posterior) or any of leads v2, v3, v4, or v5 (anterior site), or else T amplitude negative or diphasic with negative phase at least $1.0$ mm but not as deep as $5.0$ mm in lead avL, provided R amplitude is $\geq 5.0$ mm, or in avF provided QRS is mainly upright.

4.9.5.3. The subjects labeled as having IHD based on the above defined criteria were referred to the concerned specialist for further necessary diagnostic evaluation. As a method of standardization and quality control, all ECGs which were labeled as IHD, and a randomly selected sample of equal number of ECGs which were diagnosed as not having IHD, were read independently by a physician from faculty of medicine and the results were cross checked. Out of 36 ECGs read as having IHD by the principal worker of this study, 2 were read as "normal" by the physician and thus they were taken as "normal", thereby leaving 34 subjects with ECG diagnosis of IHD. Out of the random
sample of 75 ECGs which were read as normal by the worker of this study, all were read
as normal by the physician also. Thus, there was a very high correlation of both positive
and negative results.

4.10. DATA MANAGEMENT AND STATISTICAL PROCEDURES

4.10.1. Data entry procedures:

4.10.1.1. All proforma were personally checked by the worker for completeness.

All the results of investigations and ECG were personally entered by the worker himself.

Data was entered personally by the worker into the computer of the standard
configuration of 2.1 GB hard disk drive, 32 MB RAM, and 133 MHz speed. The entries
were made into a data base file on the “dbase III plus” program which is one of the most
standard database programs, developed by Ashton-Tate. Screen entry program, including
checks for validation were written personally by the principal worker who was himself
trained on computers. Standard dbase III plus reference books were also used for this
purpose (340,341). In addition, computer print outs of the entire data set were taken out
after entry and checked manually against the data in the original proforma and any
mistake revealed during this extensive re-check was rectified.

4.10.1.2. All calculations described in methodology (e.g., calculation of average daily and
lifetime alcohol / tobacco consumption, calculation of energy expenditure in physical
exercise, and so on ) were done on the above mentioned computer package (dbase III
plus) by developing minor programs written by the worker himself.

4.10.2. Statistical methods: The various statistical procedures used for analysis of data
were as follows :-

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4.10.2.1. **Descriptive statistics**: Descriptive statistical procedures included calculations of means / proportions for the various study variables, and their 95% confidence intervals (95% CI) as described in standard text books on biostatistics (313,342,343).

4.10.2.2. **Association between variables recorded on qualitative scales**: The following procedures were used (342,343):

(a) Chi-square test for nominal data.

(b) Linear-trend chi-square for polychotomous, linearly arranged data for linear trend in proportions.

4.10.2.3. **Tests for differences between means / medians**: For variables recorded on numerical scale, tests for difference between means/medians were used as follows (342,343):

(a) 't' test / ANOVA for difference between means.

(b) Mann-Whitney 'U' test / Kruskal Wallis test for difference between medians.

(c) Bartlett's test was undertaken to assess the homogeneity of variances. While comparing the means, if the variances were not homogenous (as indicated by significant results on Bartlett's test), then non-parametric tests (e.g., Kruskal Wallis test) were used, rather than using the parametric tests like ANOVA.

4.10.2.4. **Association between continuously distributed variables**: This was worked out by calculating Pearson's product moment correlation coefficient, its 95% CI, and the coefficient of determination.

4.10.2.5. **Issues of confounding and their statistical control**: The potential confounding factors (PCFs) which could lead to indirect associations were identified during extensive review of literature. In addition, the various variables that can be
confounders in the studies on syndrome-X, and hence need to be controlled as covariates have been summarized by Ruige et al in their meta-analysis of the relationship between insulin and cardiovascular disease (109). The main confounders identified by them included age, sex, BMI, WHR, physical activity, smoking, alcohol consumption, social class, BP levels, glucose levels, TG, HDL, TC, LDL, heart rate, uric acid levels, and family history of cardiovascular disease. With this background, data on all these potential confounding factors was obtained, using scientific methods (as explained at various places, while describing the methodology of making measurements). The process of restriction automatically controlled sex since the study was undertaken on males only. All the other potential confounders were adjusted statistically, using standard procedures (343), as described in subsequent paragraphs.

(a) **Univariate analysis**: The association of the confounder with the outcome was assessed initially in a univariate analysis, by assessing the level of statistical significance.

(b) **Bivariate analysis, controlling for the effect of potential confounder**: A bivariate analysis between possible exposure and the outcome variable was undertaken, controlling for the effect of the potential confounder, using Mantel–Haenszel stratified analysis procedure to calculate the adjusted Odds Ratio (OR), its 95% CI, and the Mantel-Haenszel adjusted chi-square value. As and when the outcome was treated on a continuous scale, Two-way Analysis of variance was (ANOVA) undertaken.

(c) **Test for Effect Modification**: Effect modification (statistical interaction) was assessed using Woolf’s test for qualitative variables, as described by
Schlesselman (311), and by two way ANOVA for quantitative outcomes. Subsequently, effect modification was also assessed by mathematical models, after introducing interactive (multiplicative) terms, and assessing their beta coefficients, as described in standard texts (308,311,344). The procedure of multivariate analysis through regression models is being described in detail, subsequently.

4.10.2.6. **Statistical methods in developing the mathematical models**: The two main forms of multivariate analysis undertaken in this study were:

(a) **Multiple linear regression**

(b) **Multiple logistic regression**.

4.10.2.7. The details of these analyses were as per guidelines given by Klienbaum et al on multivariate methods (344). The worker had already developed mathematical models earlier (320,345,346,347), thereby gaining experience in undertaking these multivariate procedures. In general terms, the multiple linear regression for predicting the level of a continuously distributed outcome variable $Y$, given the several independent (predictor) variables $X_1, X_2 \ldots X_n$ was based on the model

$$Y = a + b_1 X_1 + b_2 X_2 + \ldots + b_n X_n$$

Where, “$a$” is the intercept (constant) and $b_1, b_2 \ldots b_n$ were the respective beta coefficients of $X_1, X_2 \ldots X_n$.

4.10.2.8. The significance level of beta coefficients was ascertained by dividing the beta coefficient by its standard error (SE) and evaluating the resultant ‘Z’ score. Using
the standard error and standard normal deviate (1.96 at 95% level of confidence), the 95% confidence intervals for the various beta coefficients were calculated.

4.10.2.9. The multiple logistic regression model for predicting the binary outcome variable, Y, given the general independent (predictor) variables XI, X2 ----Xn, took the following general format :-

\[
\text{Prob (D = 1 / X)} = \frac{\exp (a + b_1 X_1 + b_2 X_2 + \cdots + b_n X_n)}{1 + \exp (a + b_1 X_1 + b_2 X_2 + \cdots + b_n X_n)}
\]

Where, D = the dichotomous outcome, a = value of constant, X1, X2, ---- Xn are the various predictor variables and b1, b2, ---- bn are the values of beta coefficients for these respective predictor variables. The beta coefficients in a logistic regression represented the natural logarithm of the risk; their exponentiation value was equal to the risk (odds ratio) due to that independent variable for causing the outcome, after having adjusted for the linear relationship of the other predictor variable which were included in the model.

4.10.2.10. The broad methodology used in developing the regression models was based on already published works in this area, notably the study by Stern et al (348), Zopdey et al (349) and other studies done earlier by the principal worker of this study, on the building of mathematical models (320,345,346,347). All mathematical models were preceded by a univariate analysis between the outcome and individual predictor variables. The optimal predictive models were generated by stepwise multiple logistic regression (MLR) analysis. The variables that were allowed to compete in the stepwise regression analysis were those which, in the preliminary univariate analysis, had shown significant association at the probability level of less than 10% (p<0.1), as used by Stern et al in their
study (348). The stepwise MLR model was constructed by including the variables in descending order, starting with the variables which had shown the largest chi-square statistic / Z statistic value in the preliminary univariate analysis. The process continued, successively entering the variables with next lower values of Chi-square / Z statistic value, until all variables with $p < 0.1$ in preliminary analysis were entered. At each step, the fit of the model was evaluated. In addition, the variables which had entered the model on previous step were required to meet the specified level of significance for staying in the model ($p = <0.05$) as used by Stern et al (348), or else they were removed from the model. In addition to the "optimal models" described above, a series of "reduced models" were evaluated that were based on a restricted list of potential risk factors. Specifically, the reduced list was confined to risk factors that are commonly measured in ordinary clinical and health practice. If the results between optimal models and reduced models were different, both these types of models were presented in the findings and discussed.

4.10.2.11. ROC Curve Analysis: Receiver Operating Characteristics (ROC) curve analysis was undertaken to decide to optimum cut off for various anthropometric measures, and for developing risk scoring system and predictive rules. The procedure was based on standard guidelines available in textbooks on clinical epidemiology (309). Detailed procedures were also based on the method of ROC analyses described in standard, international published articles (350,351). In addition, the worker had himself worked on ROC analysis and published papers in this field, thereby gaining experience in undertaking this form of analysis (346,347). The values of sensitivity obtained from diagnostic test characteristics were plotted along the Y-axis while their corresponding False Positive Fractions (1-Specificity) were plotted along the X-axis. The
corresponding points were joined to give the ROC curve and the point on this curve nearest to the top left hand corner of the box was taken as the optimum operating point for cut off. The class interval corresponding to this optimum operating point was taken as the cut off point, and predictive rules were subsequently developed.

4.10.2.12. **Validation of predictive models**: Validation of predictive models was done as described by Stern et al in their study (348). The complete data set was divided into two random halves, consisting of a “developmental” set and a “validation” set. The development set was used to estimate the parameters (intercept and beta coefficients) by stepwise MLR procedure. These parameter estimates from the developmental set were than applied to subjects in the validation set to predict the risk of outcome for each individual. These individual risks so calculated were then aggregated into the deciles and the predicted, i.e. expected number of cases with the outcome in each decile, were computed by summing the individual risks in the respective deciles. The resultant expected number of cases were then compared with the actual (observed) number of cases with the outcome, in each decile, using the Hosmer-Lemeshow procedure. Such comparison provided an internal validation test of how well the parameters estimated from the developmental set (randomly selected from the total set) predicted the outcome in the validation set (348).

4.10.2.13. **Statistical packages used for analysis**: All univariate analysis as well as bivariate analysis including Mental – Haenszel procedure for control of confounding and tests for linear trends were undertaken manually, by the worker personally. For this purpose, the worker was earlier trained in Epidemiology and Biostatistics at the post doctoral level, on a full time fellowship, for 2 years. The procedures were in conformity
with the guidelines provided in the standard textbooks in biostatistics, as referred in the 
foregoing paragraphs. For making mathematical models including multivariate analyses, 
correlational analysis, multiple linear regression, multiple logistic regression and factor 
analysis, assistance of specialized computer based statistical programs for analyzing the 
data was taken. The following two standard statistical packages were used: - 

(a) SPSS: This is a very widely used statistical program for use in social and health 
sciences. Its window based version, a copyright of Microsoft Company, was used. 

(b) EPI-Info version 5: This is another widely used, standard statistical package 
developed jointly by the Centers for Disease Control, Atlanta and the WHO.

4.11. PILOT STUDY: A pilot study was undertaken with a view to pretest the entire 
methodology, including sampling procedure, questionnaire, clinical and laboratory 
procedures. This pilot study was conducted on 25 subjects. Changes, as required, were 
made to further refine the methodology, based on the experience of the pilot study. The 
25 subjects of pilot study have not been included in the analysis of the final study sample 
of the 614 subjects.