CHAPTER 3

Methodology
3.1. Study design

Parallel group Randomized Controlled Trial

3.2. Study duration

February 2010 to May 2014

3.3. Ethical Committee Approval

The proposal was submitted to the Manipal University Ethics committee (MUEC) and was approved before the commencement of the study (UEC/55/2009). The subsequent renewal was obtained for five consecutive years.

3.4. Trial Registration

Clinical Trial Registry of India (CTRI No: CTRI/2011/07/001893).

3.5. Study settings

- Cardiopulmonary sciences laboratory, Department of Physiotherapy, SOAHS, Manipal University
- Fitness Laboratory, Department of Physiotherapy, Dr TMA Pai Hospital, Udupi
- Kasturba Hospital, Manipal & Department of Physiotherapy, SOAHS, Manipal

3.6. Sample size

Echocardiographic Epicardial Adipose Tissue Thickness (EEATT) was used to calculate the sample size. It was calculated based on the following formula.

\[ n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2}{d^2} \]

- \( Z_{1-\alpha} = 1.96 \) at 5 % level of significance
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- \( z_{1-\beta}=1.28 \) at 90% power
- \( d \) (clinical significance) = 0.8 mm
- \( \sigma(SD) = 1.4 \) mm

Keeping clinical significance difference of 0.8 and standard deviation (SD) to be 1.4, the minimal sample required was 64 in each group.

After incorporating an attrition rate of 25%, the sample size required was 84 in each group.

3.7. Participants

The participants were primary prevention population with no known diseases and expressed willingness to participate in the study. All the participants were recruited by advertisement, mainly by conducting awareness programs in the institutions and during disease prevention camps conducted by the Department of Physiotherapy in association with Kasturba Medical Hospital, Manipal. The awareness programs were also conducted for the students and faculty of the constituent institutions of Manipal University with prior permission from respective head of the institutions.

3.7.1. Inclusion criteria

- Overweight to mild obesity (BMI 25-35 kg/m²) according to WHO
- Asian-Indian ethnicity
- Age between 20-45 years
- Both males and females
- Sedentary individuals (not doing exercise regularly or exercising less than 3 days in a week or and with a peak VO2 < 40 ml/kg/min)
3.7.2. **Exclusion criteria**

- Smokers
- Chronic alcoholism
- Pregnancy
- Medicines influencing lipid metabolism
- Participants who are on a prescribed / specific diet for weight reduction
- Diabetes mellitus (fasting blood glucose > 120 mg/dl)
- Hypertension (Resting systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mmHg)
- Known cardiovascular diseases
- Musculoskeletal conditions contraindicated for exercise testing
- Psychiatric disorders who are on medications
- Not willing to participate in the study or not able to give commitment for 12 weeks intervention

3.7.3. **Subject acceptance**

All the interested participants were explained about the purpose of the study and benefits of participating in the study. They were asked to read an information sheet and sign on a written informed consent form.

3.7.4. **Screening**

264 interested participants were screened and a total of 170 eligible participants were recruited based on inclusion and exclusion criteria. Physical activity readiness questionnaire (International Physical Activity Questionnaire - Short version) was used for initial screening.
The interested participants not known to have any medical conditions or on any drugs for the same were included and explained about the purpose of the study. The screening form had a brief questionnaire included to know about the participant’s weekly physical activities. The screening also included the detailed family history of all participants to know if they are predisposed to cardiovascular diseases. Those who had family members with known CVD history were mentioned to have positive family history.

3.8. Randomization

After evaluation by the physiotherapist, participants were randomly allocated to one of the two groups (Group A and B). Group A served as the study group (n=85) in which participants followed an individually tailored supervised exercise program, whereas group B served as Control group (n=85). The allocation of the participants in the study group or control group was done using block randomization

3.8.1. Sequence Generation

The sequence was determined by randomly permuted blocks of equal size. Each block had 10 allotments

3.8.2. Allocation Concealment

After the completion of sequence generation, the envelopes were arranged in seventeen blocks (10 in each block). Randomization was implemented with the numbers assigned in Sequentially Numbered Opaque Sealed Envelopes (SNOSE)
3.8.3. Implementation

The Principal Investigator generated the sequence, enrolled the participants and allocated them to groups based on the number retrieved from the envelope. The assessment and exercise prescription were catered free of cost for all the participants in the study. A total of 17 blocks were completed with 10 participants in each block.

3.8.4. Duration of data collection

4 years (March 2010 to April 2014)

3.9. Outcome Measures

- Echocardiographic Epicardial Adipose Tissue Thickness in millimeters (EEATT)
- Weight in kilograms
- Body Mass Index in kg/m²
- Waist Circumference in centimeters
- Body fat assessment (percentage of total body fat, visceral fat levels)
- Lipid profile (High Density Lipoproteins (HDL-C), Low Density Lipoproteins (LDL-C), Total Cholesterol (TC) and Triglycerides (TG) in mg/dl; ratio of TC/ HDL-C)
- Fasting blood glucose in mg/dl

3.10. Procedure

170 obese Asian-Indian individuals (BMI ranging from 25.00 to 34.9) aged between 20-45 years were randomized to study group or control group. The estimated sample size was 64 in each group (MCID-0.8 and SD-1.4), but considering the high attrition rate, 85 participants were included in each group. The eligible participants were invited for an orientation session where the explanation and clarification about the study was given. The
participants who agreed to participate and sign the informed consent were assigned to one of the two groups and were taken through anthropometric assessment, body composition assessment, exercise testing, echocardiography and blood tests.

A thorough evaluation of all the participants was done as described below.

3.10.1. Anthropometrics

Each participant underwent the basic screening at our exercise laboratory. The height was measured using a wall mounted tape. The tape was calibrated to 0.1 cm and the participant stood with shoes removed. Body weight was measured using electronic weighing scale (Model DS-215 series, Essae- Teraoka limited -2003) wearing light clothing to the nearest 0.1 kg and no footwear. BMI was calculated using a standard formula and was expressed in kg/m$^2$. WC was measured in centimetres as per the guidelines given by National Institute of Health (NIH). The measurement was recorded after normal expiration using a snugly fitting inch tape placed between the lower rib margin and the iliac crest with the subjects standing with their heels together. NIH risk categorization recommended for South-Asians was used for interpretation. WC values > 80cms were considered high for females and > 90cms were considered high males.
3.10.2. Blood Tests

The participants were asked to undergo a blood test after an overnight fasting for 10–12 hours. They were asked to refrain from alcohol and intense physical activity for 24 hours prior to the blood test. Total cholesterol (TC), Low density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C) and Triglycerides (TG), fasting blood sugar levels and measurement of HS-CRP (Immuno-turbidimetric method) were estimated at the biochemistry laboratory, Kasturba Hospital. The blood lipid levels were interpreted according to the 2010 updated guidelines by National Cholesterol Education Program - ATP III\textsuperscript{221,222}.

3.10.3. Body fat measurement

Four electrodes Bio Impedance Analyzer (HBF- 362 Omron Karada Scan) with digital display system was used to record the total body fat percentage and visceral fat levels. It was
ensured that the participants have not consumed food or water for at least one hour prior to the measurement.

![Participant using Bio Impedance Analyzer for body fat measurement](image)

*Figure 3: Participant using Bio Impedance Analyzer for body fat measurement*

### 3.10.4. Echocardiographic epicardial adipose tissue thickness measurement

All the participants underwent trans-thoracic two-dimensional M-mode echocardiogram (Philips iE 33) in the department of Cardiology, Kasturba Hospital. The measurements were taken in standard parasternal and apical views in the left lateral decubitus position by an experienced sonographer. The measurement was taken perpendicularly on the free wall of the right ventricle at end systole in three to ten cardiac cycles. The images with poor window and undetectable epicardial adipose tissue were not recorded for further measurement. Two measurements with poor window were not considered and were not part of the images those were stored in the device for accurate measurement. The standardization of the method (proposed by Iacobellis et al) was done prior to the commencement of the study by sonographers. Inter-rater and intra-rater reliability of the measurements was found to be good.
(less than 4% difference between the readings) in the study conducted at our laboratory. All the measurements were taken by single tester who was blinded for the allocation of intervention.

3.10.5. Assessment of physical activity and cardio respiratory fitness

All the participants underwent complete fitness appraisal including flexibility assessment and cardio respiratory endurance test along with the body composition assessment using the Bio impedance analyzer. Flexibility assessment was done to prescribe the stretching exercises as part of the warm-up and cool down sessions of the supervised exercise program each day.

The exercise capacity test was conducted using Bruce protocol which is an indirect test to measure endurance, an incremental treadmill protocol bearing three minutes stage-wise progression. The increments of exercise test were in the form of inclination and speed in miles per hour as per the standard protocol. Gender specific equations were used to calculate the exercise capacity (Vo₂ peak) which is expressed in ml/kg/m² at baseline and after twelve
weeks. Familiarization to the treadmill walking was given for those who never used treadmill prior to the testing. A polar heart monitor (Polar FT 1-Polar electro OY, Finland) was used to monitor the heart rate response to the incremental exercise. The testing was stopped as soon as the client reached the age predicted MHR or as soon as clients expressed their inability to continue the test to the tester. Participants reported their physical activity levels in a log book during the twelve weeks of intervention.

Figure 6: polar heart rate monitor used in the study  

Figure 7: Participant with polar heart rate monitor during the exercise test  

Figure 8: Determination of intensity of exercise using Rate of perceived exertion scale
The Bruce Protocol Formulae for Estimating VO2 Max

- For Men VO2 max = 14.8 - (1.379 x T) + (0.451 x T²) - (0.012 x T³)
- For Women VO2 max = 4.38 x T – 3.9

(T = Total time on the treadmill measured as a fraction of a minute, T²=double the duration of exercise testing and T³= thrice the duration of exercise testing)

3.11. Treatment for the study group

American College for Sports Medicine (ACSM) guidelines were followed for the exercise prescription in the exercise or study group. Moderate intensity (60-85% of heart rate maximum or HRmax) aerobic exercise program on treadmill was given to the study group participants. The continuous aerobic exercise was preceded by 5-15 minutes of warm up session and was followed up by 5 to 10 minutes of cool down session post exercise. Stretching exercises and low-intensity treadmill walking exercises were mainly the part of warm up and cool down sessions. The appropriate heart rate range was calculated following the exercise test. The first two weeks of exercise was of lighter intensity (40-60% HR max) as most of the participants were sedentary prior to the inclusion into our study. The intensity was gradually progressed to 60-85% of HR max by third week and it was continued for the next nine weeks. The frequency of exercise was set as minimum of three sessions in a week to seven sessions and each session lasted for 40-60 minutes including the warm up and cool-down sessions. The prescribed heart rate range was maintained by adjusting the speed and inclination in the treadmill. A log book was maintained to track the duration, intensity and frequency of the exercise sessions done by each participant. The adherence to the exercise program was calculated for each participant and average for all participants was calculated. The total exercise minutes and energy expenditure in terms of METs - hours was calculated.
Figures 9a & b: Stretching exercises of lower limb during exercise session

Figures 9c & d: Stretching exercises of upper limb during exercise session
3.12. Control Group

The Participants in control group were asked to continue with their current level of physical activity during the twelve week study period. The activity logs were given to all the participants and were monitored every week either by a telephonic call or by personal contact. The participants were asked to report any unusual changes in their lifestyle which might have an impact on their body weight. The participants were also offered free exercise program in the laboratory after the completion of the study period.

*Figure 10: Participant doing aerobic exercise on motorized treadmill in the laboratory*
3.13. Follow-up of the participants

All the participants of our study were offered the extended supervised exercise program at our fitness laboratory. Only 13% (23/170) of all the participants recruited in the study agreed to attend the program after the study period at their own cost. However, we failed to monitor the EEATT changes at regular intervals following the study period due to lack of feasible time and interest among the participants. The required sample was achieved at the end of the study.
3.14. CONSORT flow Diagram

Figure 11: Flow diagram-procedure according to CONSORT guidelines
3.14. Statistical analysis

The continuous variables were summarized using mean and standard deviation or median and inter quartile range. The categorical variables were summarized by frequencies and percentages.

Independent t-test was used to compare the baseline anthropometric parameters, EEATT, lipids, FBS and VO2 max between the study and control group.

Repeated measures ANOVA was used to compare the changes in epicardial adipose tissue thickness, anthropometrics, BIA values, fasting blood sugar, lipid levels and HS-CRP values at the baseline and after 12 weeks between the two groups. The mean percentage change from baseline to 12 weeks and Cohen’s ‘d’ effect size was used to summarize the strength of association in study and control groups. The effect size was considered as small if d is >0.2 and <0.5; medium d > 0.5 and <0.8; large d > 0.8.

Mann Whitney U test was used compare the baseline values of HS-CRP between study and control groups, also median percentage change in HSCRP values from baseline to 12 weeks between study and control groups.

Pearson’s correlation coefficient test was used to study the correlation between EEATT values and anthropometrics, BIA values and VO2 max.

SPSS version 15.0 was used for data analysis, p value of less than 0.05 was considered to be statistically significant.