Chapter III

Materials and Methods

3.1 Study Design:

Randomized, active controlled, outcome assessor blind, parallel group, multiple arm trial

3.2 Trial Registration:

The trial was approved by Manipal University Ethics Committee (UEC 16/2008) and was registered in the Clinical Trial Registry of India (CTRI No: CTRI/2009/091/001005).

3.3 Participants:

We recruited 98 young adults between the age group of 18 – 40 years who were sedentary, otherwise healthy. All participants with hs CRP value more than 1 mg per liter and less than 15 mg per liter were included. Participants were excluded if they had any,

- Current illness associated with systemic inflammatory response,
- History of hypertension and diabetes,
- Cardiovascular medications (including aspirin) / NSAIDs / steroids/ statins/ niacin atleast 48 hours prior to testing,
- Other cardio-pulmonary and neuromusculoskeletal impairment that contraindicated exercise testing and training.

3.3.1 Recruitment: The first participant was recruited in May 2009 and recruitment was stopped in February 2012.
3.4 Sample size:

Assuming a reduction in hs CRP of 2 mg/l in eight weeks with a standard deviation of 2.9 mg/l, it was estimated that a minimum of 22 participants in each group would provide 90% power at an α-level of 0.05.

3.5 Randomization:

Participants were randomly allocated to one of the four groups (Group A, B, C, and D). Group A served as the control group with no exercise training and the remaining groups (B, C, and D) underwent exercise training at different exercise intensities.

3.5.1 Sequence Generation: Sequence generation was performed on the website: Random.org.

3.5.2 Allocation Concealment: Allocation concealment was executed by Sequentially Numbered Opaque and Sealed Envelopes (SNOSE) method. We used brown envelopes which are opaque. After the sequence generation was completed, the envelopes were arranged in four blocks (24 in the first three blocks and the last block had 28 envelopes). When the participant picked the envelope, before opening the envelope the name of the participant was written.

3.5.3 Implementation: The principal investigator generated the sequence, enrolled the participants and allocated them to groups based on the number found in the envelope.
3.5.4 Blinding: The clinical biochemist (assessor of the primary outcome measure) was blinded to group assignment.

3.6 Setting:

The trial was conducted at the Human Performance Laboratory (HPL), Department of Physiotherapy, MCOAHS. Manipal University. This center performs about 20 submaximal tests in a month with clients of various needs, to estimate their exercise capacity and has prescribed structured exercise program to more than 850 clients in the last four years.

3.7 Intervention:

The control group was instructed not to change their levels of physical activity throughout the study period. Participants in the exercise groups underwent eight weeks of supervised exercise training. The program consisted of five days per week and thus had 40 exercise sessions before the post intervention measurement. Each session consisted of a four minute warm up, aerobic exercise (treadmill walking) and four minutes of cool down. The intensity of exercise for Groups B, C and D were calculated based on the Heart Rate Reserve method recommended by American College of Sports Medicine\textsuperscript{121}. Target heart rate zone was calculated using Karvonen’s Equation.

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\text{Target Heart Rate} = (\text{Intensity}) \times (\text{Maximal Heart Rate} - \text{Resting Heart Rate}) + \text{Resting Heart Rate}
\]

Maximal Heart Rate was noted at the termination of exercise test performed to estimate exercise capacity.
Resting Heart Rate was measured by the participants early in the morning before they got off the bed.

Group B, C and D received an exercise program with an intensity of 50 – 60%, 60 – 70%, 70 – 80% respectively. Intensity range of 50 – 80% HRR was chosen for the exercise groups as there are no physiologic benefits below 50% HRR and training above 80% HRR crosses the anaerobic threshold and is thus not an intensity to improve aerobic capacity. Intensity of exercise was monitored continuously using POLAR radio telemetric heart rate monitor one day in a week. As a result of training, when the participants got adapted and when their heart rate during the session dropped below the intensity prescribed, either the speed or the grade of treadmill (based on participant’s preference) was increased to maintain the target heart rate range.

All exercise groups performed aerobic training for 30 minutes every day for the first two weeks. 30 minutes of aerobic exercise was progressed to 40 minutes after two weeks (10 sessions) and further progressed to 45 minutes after four weeks (20 sessions).

Flexibility training and basic core strengthening exercises (appendix 6) were also administered, with an objective of preventing overuse injuries associated with regular aerobic training. Flexibility training consisted of stretching exercises for gastro-soleus, quadriceps, hamstrings, ilio-tibial band and gluteus maximus. Stretches were held for 40 seconds at the point of discomfort and performed twice on each extremity.

Core strengthening exercises administered include, alternate arm and leg raise in quadruped, supine bridging with unilateral leg raise and crunches. Participants performed the first two exercises by holding the position to 15 seconds and thrice on each side.
Crunches were performed for two sets of 15 repetitions. Pictorial representation of these exercises can be found in the appendix-6.

3.8 Outcome Measures:

The primary outcome measure used in this study is the hsCRP and the secondary outcome measures consisted of blood glucose, plasma lipids, aerobic capacity, waist circumference and fat percentage.

3.8.1 Hs CRP Measurement:

A venipuncture was performed at the ante-cubital vein to draw the blood sample and the sample was sent to the clinical biochemistry laboratory of Kasturba Medical College for analysis. The hs CRP was measured using immunoturbidimetry (Auto-analyzer - HITACHI - 912). This measurement was done at baseline and after eight weeks. If the participant reported a positive history of recent infection and inflammation the test was deferred for a minimum of two weeks following subsidence of symptoms and signs of infection/inflammation. Post intervention measurement was done 48 hours after the last exercise session in the participants belonging to the exercise groups.

3.8.2 Plasma lipid measurements:

A blood sample was obtained from the participants after an overnight fast of a minimum of 12 hours. Blood was drawn at the clinical laboratory of Kasturba Hospital and then analyzed at the biochemistry lab. Lipid profile consisted of Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) are measured in mg/dl. The method used for TC was CE-CHOD-POD enzymatic method, for TG was GPO Trinder, for HDL-C was Direct
Homogenous methods while LDL was calculated. The post intervention measurement was done 48 hours after the last exercise session. Fasting Blood Glucose (FBG) also was measured (hexokinase method) simultaneously.

### 3.8.3 Anthropometric and body composition measurements:

Body composition measurements were done prior to measuring aerobic capacity of the participants. Height and body weight were measured in metric units. Waist circumference was measured at the level of iliac crest with the feet one foot apart and hands by the side. The inferior border of the measuring tape corresponded to the superior lip of the iliac crest. The participant was instructed to take a normal tidal breath and the measurement was taken.

Fat percentage was estimated using the bio-electric impedance analysis (OMRON Body Fat Monitor HBF-302) in the following manner. The age, height, weight and gender of the participant were fed into the instrument. Following this, the participant was made to stand erect holding the electrodes of this device for about five seconds. At the end of five seconds, the display shows the fat percentage of the individual. The device passes low electric current through the body, current passes easily through body fluids but encounters resistance with fat. This resistance is proportional to the amount of fat present in the body and is thus used to estimate fat percentage.

### 3.8.4 Measurement of Aerobic capacity:

Aerobic capacity of participants was measured using the Bruce protocol in the Human Performance Lab and at the treadmill test area of the Department of Cardiology, Kasturba Medical College, Manipal. All participants were familiarized about the test
procedure and were assisted to wear the radio telemetric heart rate monitor. Participants were instructed to walk as long as possible on the treadmill until exhaustion and they were encouraged to convey any other symptoms experienced during the test. The test was terminated at volitional fatigue. Time recorded at the termination of exercise test, was used in the regression equation to estimate the aerobic capacity. The measure of aerobic capacity is Peak oxygen uptake (Vo2 peak) and is expressed in ml/kg/min. Time of testing at baseline and post intervention was kept constant to avoid circadian influence.

3.9 Instruments:

- Auto-analyzer (HITACHI - 912)
- Assay kit
- Whispermill with concept Integration Software
- Training Treadmill
- Polar radio-telemetric tester
- OMRON Bio-Electric Impedance Analyzer (Body Fat Monitor HBF-302)
- Measuring tape
- Weighing Machine (Essae)
- Stadiometer
3.10 Procedure:

Ethical approval for the trial was sought from the Manipal University Ethics Committee (appendix 4). The trial was registered in clinical trial registry of India (appendix 5). Following this, verbal and printed advertisements were done at various institutes of Manipal University. Volunteers who responded to the advertisement were assessed for their candidacy in this trial using Physical Activity Readiness – Questionnaire (appendix 1). History related to recent infections, systemic inflammation, musculoskeletal, cardiopulmonary conditions, drugs and other comorbid illness were collected.

Volunteers who were found eligible were given a requisition form for measuring hsCRP after taking a verbal consent. If the participants fulfilled the criteria of having hsCRP more than 1 and less than 15mg/L, a written informed consent (appendix 2) was obtained and the requisition form for FBG and lipid profile was given. After the participants gave the blood sample, the secondary outcome measures were analyzed on the same day. On the same day, the participants were randomly allocated to one of the four groups, by the method explained in section 3.5.

Participants in the control group were instructed to stay sedentary for the next eight weeks and were asked to refrain from any new form of physical activity. All participants in the exercise groups attended supervised exercise sessions in HPL. After each exercise session, participants were encouraged to sign in the adherence record to monitor compliance. Participants were given sporting apparel as an acknowledgement of their
willingness to participate in this study. A comprehensive exercise program was given for all the participants including control group at the end of eight weeks.

3.11 Preliminary Work:

3.11.1 To explore the prevalence of high level of hsCRP, 21 sedentary, otherwise healthy volunteers between the age group of 21 to 40 years who responded to a verbal advertisement gave a blood sample for measurement. Volunteers were both sedentary individuals and recreational exercisers. The results of the preliminary study revealed that 15 out of 21 had CRP values higher than 1 mg/l (i.e. average or high risk). In spite of the small sample size for assessing prevalence, this finding indicated that sub clinical inflammation is common in sedentary Indians.

3.11.2 To establish the stability of hsCRP and to determine inter-test variability in immunoturbidimetry, two blood samples were taken at the same time from 10 volunteers and were assessed separately. Maximum variability obtained from this sample was 0.1mg/l. This finding helped us ascertain the precision and reproducibility of the results from immuno-turbidimetry.
3.12 Statistical Methods:

Descriptive statistics of each variable were calculated and reported as mean and standard deviation. We performed rank transformation of the data as the hsCRP data was skewed even after logarithmic transformation. Repeated measures ANOVA was used to determine the effects of eight weeks of exercise training across groups. One way ANOVA was used to determine if the changed scores in hsCRP between groups was different after removing the outliers. Intention to treat principle was adopted in the primary analysis; baseline value of the participant was carried forward when the outcome was missing.

Spearman’s rank correlation was performed to investigate the relationship between hsCRP and the other secondary outcome measures. Kruskal – Wallis one way ANOVA was used to analyze the difference in hsCRP levels across different BMI categories. Multivariate Linear modeling with stepwise variable selection was performed to determine the best/ best set of predictors of hsCRP. Level of significance was set at p ≤ 0.05. Analyses were performed using the statistical software SPSS version16.