Chapter – III

PROCEDURE

In this chapter, the procedure for selection of subjects, selection of variables, criterion measures, experimental design, reliability of data, collection of data, procedure for administration of tests, administration of training programme and the statistical technique employed for analysis of data have been described.

Selection of Subjects

The subjects for the study were sixty middle aged men, age ranging between forty to fifty years selected from among the residents of Pattom residential area of Trivandrum; and another group from among the male teaching staff of M.G. College, Trivandrum. The sixty subjects consisted of thirty sedentary and thirty occasional participants in physical activities, randomly categorized into two experimental and two control groups, each comprising of fifteen subjects. Thirty subjects who were labelled occasional participants were mainly those who were involved in walking and few others who were involved in playing badminton and tennis occasionally. All the subjects selected for the experimental groups were subjected to medical evaluation and certification from a doctor ensuring their health capacities to undergo the training programme.

The requirement of the project were explained to all the subjects and all of them agreed voluntarily to undergo the testing and training programme. A special talk for
orienting the subjects on the research project was conducted, while a thorough picture was given regarding the experimental procedure, testing, as well as the exercise schedules to be carried out so that there was no ambiguity of what effort was required on their part and what hardship they might have to endure.

Selection of Variables

Having gone through both critical and allied literature related to the study, and keeping in mind the feasibility criteria for conducting such a study, the following variables were selected for the study.

Serum Lipoprotein Profiles

1- Total Cholesterol
2- High Density Lipoprotein Cholesterol (HDL-C)
3- Low Density Lipoprotein Cholesterol (LDL-C)
4- Triglycerides
5- Very Low Density Lipoprotein Cholesterol (VLDL-C)

Body Composition Variables

1- Total Body weight
2- Fat Weight
3- Lean Body Weight.
**Criterion Measures**

1- Total Cholesterol was recorded in mg %

2- High Density Lipoprotein Cholesterol was recorded in mg %

3- Low Density Lipoprotein Cholesterol was recorded in mg %

4- Triglycerides was recorded in mg %

5- Very Low Density Lipoprotein Cholesterol was recorded in mg %

6- Body Weight was recorded in kilograms.

7- Body Fat was recorded in kilograms.

8- Lean Body Weight was recorded in kilograms.

**Experimental Design**

Random group Design was used for the study as it was found most appropriate. The thirty sedentary and thirty occasional participants were randomly assigned to the experimental and control groups, in such a way so that one experimental group comprised of fifteen sedentary subject and other group comprised of fifteen occasional participants. The control groups were also in the same pattern. Both the experimental groups were given aerobic training programme (brisk walking/jogging) for a period of sixteen weeks, excluding the period utilised for pre-test and post test. The control groups did not participate in any training programme during the experimental period.
Reliability of Data

The reliability of data was established following the instrument reliability and tester competency.

Instrument Reliability

Estimation of lipoprotein variables were done with the help of a bio-chemist and laboratory technicians. All the instruments such as centrifuge and auto-analyzer were of high quality, manufactured by reputed companies and the results showed excellent accuracy. The testing procedures were started only after establishing the instrument reliability.

The skinfold caliper used for taking the skinfold measurements were also of standard type, which was obtained from the Physiology Laboratory of Lakshmibai National College of Physical Education, Trivandrum.

Tester Competency

Extraction of blood and measurement of lipoprotein was done by two laboratory technicians under the supervision of a biochemist at SRVS Diagnostic and Research Institute, Medical College P.O., Trivandrum.

In case of body composition assessment, the investigator had many practice sessions under the expertise of Dr. (Mrs.) Usha Nair, Reader, LNCPE, Trivandrum. The competency of the investigator in the skinfold measurement assessment was established
following high reliability co-efficients for test retest correlations obtained for the skinfold measurements on a sample of fifteen subjects obtained by the tester and the expert.

The reliability coefficients obtained for the test-retest data on the body composition variables are shown in table-1.

Table –1

RELIABILITY COEFFICIENTS FOR TEST RE-TEST SCORES OF SELECTED VARIABLES

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body Weight</td>
<td>0.99</td>
</tr>
<tr>
<td>Body Fat</td>
<td>0.82</td>
</tr>
<tr>
<td>Lean Body Weight</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Collection of Data

For the purpose of collection of data the subjects were asked to report during early morning, one day prior to the commencement of training and one day after the training with an overnight fast for 12 hours. The subjects had also abstained from exercise for 48 hours prior to the collection of blood samples for lipoprotein determination. 10 ml. of blood samples were drawn from a vein near the antecubital fossa twice, once before the training programme (pre-test) and later after the training programme (post test). Post test
blood samples were drawn 48 hours after the last exercise bout in an attempt to minimise
the potential of acute exercise effect on the lipoprotein variables.

The skinfold measurements were also taken one day prior and one day after the
experimental period.

**Procedure for Administration of Tests**

**Estimation of total cholesterol**

Blood cholesterol was determined by end point estimation using cholesterol
esterase and peroxidase using reagents supplied.

**Procedure:**

In 1 ml of the working reagent 10 μl of serum, standard and distilled water were
added, in separate tubes. This was incubated for 5 minutes at 37°C. The absorbance of
the test and standard are noted against blank at 500 nm.

**Calculation:**

**Cholesterol concentration in serum**

\[
\text{Cholesterol concentration in serum} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200 \text{ mg/dl.}
\]

**Estimation of HDL Cholesterol**

This was also done by end point method using phosphotungstic acid as
precipitating reagent.
Procedure: The VLDL cholesterol and LDL cholesterol fractions were precipitated using phosphotungstic acid and then HDL cholesterol is separated by centrifugation and measured for its cholesterol content.

Concentration of HDL cholesterol

\[
\text{Absorbance of test} \times 100 \text{ mg/dl.} = \frac{\text{Absorbance of standard}}{\text{Absorbance of standard}}
\]

Estimation of Serum Triglyceride

This was done by end point method using reagents supplied by Ranbaxy.

Procedure:

In 1 ml of the working reagent 10 µl each of test, standard and distilled water were added, and this was incubated at room temperature for 20 minutes. The absorbance of the test and standard were noted against blank at 540 nm.

Concentration of triglyceride

\[
\text{Absorbance of test} \times 100 \text{ mg/dl.} = \frac{\text{Absorbance of standard}}{\text{Absorbance of standard}}
\]

Estimation of Serum VLDL Cholesterol and LDL Cholesterol

From the values of total cholesterol, HDL and triglycerides, the values of LDL cholesterol was determined using Friedwald’s formula.

\[
\text{LDL Cholesterol} = \text{Total Cholesterol} - (\text{HDL} + \text{TG/5})
\]

1/5 of Triglyceride value gives the amount of VLDL cholesterol in plasma.
Body Composition

Body Fat

The Lange's Skinfold Caliper was used to assess the body fat. The instrument consists of accurately calibrated dial which indicates in millimetres the thickness of the skinfold when the jaws are open, holding the skinfold.

To eliminate error, the reading was made between three to four seconds, when essentially all compression have taken place. If this precaution was not taken, the skinfold would gradually decrease the tissue squeezed out form the jaws of the calipers.

The right side of the body was used to determine the percentage of fat. The thickness of the skin and subcutaneous fat was grasped between the thumb and index finger and measurements were taken to the nearest millimeter from four different specific sites using the caliper.

The following were the sites used for taking skinfold measurements:

1. Biceps
2. Triceps
3. Subscapular region
4. Supra-iliac region
Biceps Skinfold:

With the subject standing erect with arm hanging loosely, a fold of skin was picked up on the anterior of the mid part of biceps and the skinfold thickness was measured. The position of the fold was vertical and reading to the nearest half millimeter was recorded.

Triceps Skinfold:

The skinfold thickness was taken over triceps muscles at a point half way between the tip of the shoulder (acromial process) and the tip of the elbow (Olecranon Process). The point was located with fore-arm flexed to 90 degrees and while taking the measurement the arm was kept hanging free. The fold of skin was lifted parallel to the long axis of the arm and the reading to the nearest half millimeter was recorded.

Subscapular Region Skinfold:

The skinfold thickness was taken at the tips of the scapula (interior angle) with the subjects in a relaxed standing position. The fold was lifted in the diagonal plane at about 45 degree from vertical and horizontal planes and the reading to the nearest half millimeter was recorded.

Supra-iliac Skinfold:

The skinfold thickness was taken three to five centimetre above the anterior – superior iliac spine on diagonal line going downward and inward and the reading to the nearest half millimetre was recorded.
The sum of the skinfold of four sites of the body was converted into percentage body fat with the help of standard conversion table suggested by Durnin and Rehman. From each subject’s body weight, the weight of the fat possessed was calculated by using the following formula:

\[
\text{Fat Weight} = \frac{\text{Body Weight \times Percentage Value of Fat}}{100}
\]

\text{Lean Body Weight}

The total body weight minus the weight of the body fat is called Lean Body Weight (LBW).

\[
\text{LBW} = \text{Total Body Weight} - \text{Weight of Fat.}
\]

\text{Total Body Weight}

The weight of the subjects were taken on a weighing machine. The subjects wearing minimal clothing stood on the weighing machine and weight was recorded nearest to half a kilogram.

\textbf{Administration of the Training Programme}

The two experimental groups underwent the training programme with the training schedule prepared by the investigator. The training was personally supervised by the investigator with the help of properly trained physical education teachers who strictly followed the instructions of the investigator. The training was carried out four days a
Prior to the actual training programme a pilot study was conducted selecting ten subjects randomly from the experimental groups. Initially the subjects were asked to run at a uniform pace and later the running pace was varied so that the pulse rate was maintained between 110 and 130 beats per minute, (60 to 70% of the maximum heart rate of the subjects). The duration up to which the subjects could exercise maintaining the above pulse rate was recorded. On the basis of the pilot study result, the duration of the training programme was initiated from 15 minutes with progressive increase of 3 minutes after every two weeks considering that the duration of two weeks was sufficient for adaptation of the body to the given work-load.

The bi weekly schedule of the aerobic training load is given in Table-2.
### Table - 2

**BI-WEEKLY SCHEDULE OF AEROBIC TRAINING PROGRAMME FOR SIXTEEN WEEKS**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Training Duration</th>
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<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>15 minutes</td>
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<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; and 4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>18 minutes</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt; and 6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>21 minutes</td>
</tr>
<tr>
<td>7&lt;sup&gt;th&lt;/sup&gt; and 8&lt;sup&gt;th&lt;/sup&gt;</td>
<td>24 minutes</td>
</tr>
<tr>
<td>9&lt;sup&gt;th&lt;/sup&gt; and 10&lt;sup&gt;th&lt;/sup&gt;</td>
<td>27 minutes</td>
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<td>11&lt;sup&gt;th&lt;/sup&gt; and 12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>30 minutes</td>
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<td>13&lt;sup&gt;th&lt;/sup&gt; and 14&lt;sup&gt;th&lt;/sup&gt;</td>
<td>33 minutes</td>
</tr>
<tr>
<td>15&lt;sup&gt;th&lt;/sup&gt; and 16&lt;sup&gt;th&lt;/sup&gt;</td>
<td>36 minutes</td>
</tr>
</tbody>
</table>

**Statistical Technique**

The following statistical techniques were employed for the analysis of data:

To find out the significance of difference between the pre-test and post-test means of the experimental and the control groups, the “t” test was employed.

To compare the significance of differences in the means among the experimental and control groups on the selected variables, the analysis of covariance was applied. The level of significance chosen to test the hypothesis was 0.05.