CHAPTER III
METHODOLOGY

Introduction

The details regarding the selection of subjects, selection of experimental variables, selection of exercise, administration of exercise and collection of data and training programme and statistical analysis have been presented in this chapter.

SELECTION OF SUBJECTS

The aim of the study is to evaluate the effect of sub-maximal exercise on Blood lactate and Recovery heart rate in three specified age groups. Twenty men were selected into each group. The sixty subjects of this study were categorised into three age groups viz., 18 - 22, 28 - 32, 38 - 42.

After finalising the list of subjects all the selected subjects were briefed about the nature and purpose of the study, the exercises and the tests they would be undergoing during the course of study. All of them whole heartedly co-operated with the investigator.

The age group from 18-22 were the students of Maharajas College, Ernakulam and were staying in the hostel. The other groups were the working men living in and around Ernakulam. All subjects were selected after a
detailed examination by a qualified doctor and were actively participating in the sports and games. No attempt was made to equate their daytoday life and the exercise pattern with regard to the concerned study.

**SELECTION OF VARIABLES**

Differing exercise modalities may have unique effects on exercise response variables eliciting different physiologic and metabolic responses. The common psychological responses that are used to evaluate effort during maximal and sub maximal exercise are maximum oxygen consumption, heart rate, respiratory exchange ratio and blood lactate. Oxygen update, heart rate and blood lactate are the indication of exercise intensity as the present study investigates the effects of sub-maximal exercise at various age groups. The parameters that indicates the level of exercise intensity was studied.

According to Stephen and Arthur and Arther Baltimore (1979) in the longitudinal study the difference of age category was ten. In the present study also evaluation is done in different age responses to sub-maximal exercise.

The purpose of the study has to investigate how the same exercise pattern affects the different age groups. The parameter to measure fatigue level is to measure the lactic acid produced. That is why lactic acid is selected as a variable for this study. Similarly the heart rate is also an indicator of exercise intensity. Blood lactate accumulation during exercise and the heart
rate response and the recovery pattern following exercise are vital indicators of exercise intensity and fitness adaptation of individuals.

**SELECTION OF EXERCISE**

There are three main parameters for measuring exercise intensity. They are Oxygen uptake, Heart rate and Blood lactate. According to Brog, rating of perceived exertion (RPE) can also indicate exercise intensity. Using this approach the exerciser rates on a numerical scale how it feels in relation to the level of exertion.

<table>
<thead>
<tr>
<th>R.P.E. Scale</th>
<th>Description</th>
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<tbody>
<tr>
<td>6</td>
<td>Very very light</td>
</tr>
<tr>
<td>7</td>
<td>Very light</td>
</tr>
<tr>
<td>8</td>
<td>Fairly light</td>
</tr>
<tr>
<td>9</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>10</td>
<td>Hard</td>
</tr>
<tr>
<td>11</td>
<td>Very Hard</td>
</tr>
<tr>
<td>12</td>
<td>Very very hard</td>
</tr>
</tbody>
</table>


According to Berg's (1982) studies, the exercise levels that correspond to higher levels of energy expenditure and physiological strain result in higher RPE rating. Exercise that feels 'Somewhat Hard' consider with the exercise heart rate of about 70% HR Max (Maximal Heart Rate).

Noonan and Dean have described the reliability of maximal versus sub-maximal exercise tests. They have several reasons for opting sub-maximal exercise tests rather than Maximal Exercise tests for assessing individual fitness level.

Venessa Noonan and Elizebath Dean have described several sub-maximal exercise test Bout.

1. Modified Bruce treadmill test.
2. Single stage sub-maximal Treadmill-walking test.
3. Astrand hytheming cyclke Ergometer test.
4. Canadian Aerobic fitness test
5. 12 minute run test
6. 20 metre shuttle test
7. One mile track walk test/Rock port fitness test.
8. Modified shuttle walking test.
Among these tests, the 12-minute run test and the single stage treadmill walking test were selected for this study. Twelve minute run test developed by Hopper (1968) based on the work of Balke (1987) indicates that various run/walk tests could relate VO\textsubscript{2} to either the distance covered in a given period of time or the time taken to cover a given distance. Reliability and validity of the test was obtained by Venessa Noonan and Elizabeth Dean (2000).

Four minutes treadmill run at the grade of 5% was selected for this study. Single stage sub-maximal walking test is another sub-maximal exercise for the present study which was developed by Edbling et al. (1997). Validity and reliability of the test was obtained by Venessa Noonan and Elizebath Dean (2000).

- Twelve minute run test developed by Hopper in 1968. This test is based on the work of Balke which indicate that various run/walk tests could relate VO\textsubscript{2} to either the distance covered in a given period of time or the time taken to cover a given distance. Reliability and validity of both the test was obtained by Aneesa Noonan Elizebath Dean.
ADMINISTRATION OF THE TESTS AND
COLLECTION OF DATA

Oxygen update, Heart rate, Blood lactate are the indication of exercise intensity. As the present study investigates the effects of sub-maximal exercise at various age groups. The parameters that indicates the level of exercise intensity was studied.

Parameters like accumulation of lactic acid and recovery heart rate after 15 seconds, 30 seconds and 45 seconds among the three age groups of 18 to 22, 28 to 32, and 38-42 years were studied. To serve this purpose sixty subjects were selected in random among the three age groups. All the subjects were selected after a detailed examination by a qualified doctor and were actively participating in sports and games.

Prior to the testing procedure the normal heart rate of the subjects of 18 to 22 years were taken in three times and the average heart rate obtained were recorded. After getting the normal heart rate the subjects were asked to go for twelve minutes continuous run and after the run again the pulse rate had taken in 15 seconds, 30 seconds and 45 seconds and were recorded on the next day the same subjects were underwent a detailed physical check up and 5 ml of blood samples were collected in a heparanised test tube for the measurement of resting blood lactate and after collecting the blood samples the subjects were asked to go for twelve minutes continuous run and soon after the
completion of the run 5 ml of blood samples were collected by a qualified technician within 3 to 5 minutes and kept it in the laboratory.

In the same way recovery heart rate measurements and the blood lactate measurements were taken for the age group of 28 to 32 years and 38 to 42 years of age after the twelve minutes run test.

After three days the subjects of 18 to 22 years of age were asked to report for the four minutes treadmill run. Before the commencement of the test the subjects were examined by a qualified doctor and the normal pulse rate were taken and after collecting the normal heart rate the subjects were asked to go for four minutes treadmill run and soon after the completion of the exercise the recovery heart rate were taken after 15 seconds, 30 seconds and 45 seconds for the purpose of this study.

In the same day prior to the exercise 5 ml of blood samples were taken for the measurements of resting blood lactate and after collecting the blood samples, the subjects were asked to perform the four minutes treadmill run and after the completion of the exercise 5 ml of blood samples were collected for the measurements of the recovery heart rate.

After three days in the same way the other groups like the age group of 28 to 32 and 38 to 42 were underwent the four minutes treadmill run and collected the data. The blood samples collected were given to the laboratory for the lactic acid estimation.
Estimation of Lactic acid

The estimation of lactic acid was done through the Randox kit method and the procedures done are given below.

Testing Procedures of Blood Lactate

Wage length: 550 nm
Curette: 1 cm light path
Reaction temperature: 20 – 20°C, 37°C
Measurement: against reagent blank pipette into test tubes

<table>
<thead>
<tr>
<th></th>
<th>Reagent Blank/μ</th>
<th>Standard (μ)</th>
<th>Sample (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Reagent</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Maximum incubate for 10 minutes at 20-25°C or 5 minutes at 37°C. Measure the absorbance of the sample (A simple) and standard (A standard) against the reagent blank with in 30 minutes

Calculation:

\[ L - \text{Lactate Concentration} = \frac{A \text{ Sample}}{A \text{ Standard}} \times \text{Stanford Concentration (mg/dl)} \]
\[
\frac{\text{A Sample}}{\text{A Standard}} \times \text{Stanford Concentration (mmol/l)}
\]

**Quality control**

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily control. Two levels of controls should be assayed at least once a day. If these values falls outside the range and repetition excludes error the following steps. Before the estimation of lactic acid the investigator checked the instrument settings and light sources and the cleanliness of all the equipments in use. Then checked the water contaminants bacterial growth which might contribute to inaccurate results and the reaction temperature.

**Linearity of the Test**

The test is Linear up to a L-lactate concentration of 12.21 mmol/l (110 mg/dl), Dilute samples above this concentration 1+1 with 0.9% NaCl and multiply the result by 2.

The apparent used for the test is Semi auto analyses 5010 flame-photo metre.

(1 ml reagent + 10 micro plasma)

**Colorimetric Method to determine the Reaction**

The concentration of L-lactate in the sample is determined according to the following reaction.
\[
\text{L–Lactate} + \text{O}_2 \xrightarrow{\text{Lactate Oxidase}} \text{Pyruvate} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{– aminoantipyrite} + \text{Toos} \xrightarrow{\text{Peroxidase}} \text{Purple product} + 4\text{ H}_2\text{O}
\]


L–Lactate is one of the ran materials of the glucogenesis produced by the active skeletal muscle and erythrocytes and it is metabolised by the liver. When tissue can not be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAS\(^+\) is regenerated from NADH by reduction of pyruvate to lactate.

**Experimental design**

The experimental design used for this study was similar to a random group design involving three different age groups who were randomly drawn into three age groups of twenty each. This study consisted of three different age groups and all the three age groups were underwent the 12 minutes continuous run and four minutes treadmill run for the collection of data. All the subjects were tested prior to and after the experimentation on recovery heart rate and blood lactate.
**Statistical Technique Used**

The data collected from the three groups before and after the exercise were statistically examined for significant difference in means by using analysis of co-variance. Wherever the F-ratio was found to be significant, the Least Significant Difference (LSD) post-hoc test was used to determine which of the paired mean differed significantly. In all cases, the level of significance was set at 0.05 level.