CHAPTER III

METHODOLOGY
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The methods adopted for the selection of subjects, variables used, experimental design, training programme, procedure for test administration and methods employed for statistical treatment of data have been presented in this chapter.

SELECTION OF SUBJECTS

A total of 60 male students of Madras Veterinary College, Chennai who were apparently normal were chosen for the present study. They belonged to the age group of 19 to 22 years and were not exposed to any regular resistance training programme. They were subjected to medical examination conducted by an expert physician and were certified to be fit for the present training programme. The subjects were randomly selected and assigned into four groups (Group I, II, III and IV) with each group comprising of fifteen subjects. Group I, II and III served as experimental groups, while group IV served as control group. All the subjects completed an Informed Consent Form (Appendix - I) and a Health History Questionnaire (Appendix - II).
EXPERIMENTAL DESIGN

The experimental research attempts to establish cause and effect relationship between an independent variable which is manipulated to determine its effect upon the dependant variable (Thomas and Nelson, 1990). The research design used in this study was a pre test – post test randomised groups design.

SELECTION OF VARIABLES

The investigator referred various relevant literature, consulted with experts from the field of sports and biochemistry to identify the variables that could produce cause and effect change due to resistance training.

DEPENDENT VARIABLES

The following dependant variables were chosen in the present study to assess the changes due to 12 weeks relative resistance training programme.

Physical:  
  a. Muscular strength (kgs)
  b. Muscular endurance (Nos)

Anthropometrical  
  a. Percent body fat
  b. Girth measurements
     i. Chest circumference (cms)
ii. Thigh girth (cms)
iii. Forearm girth (cms)
iv. Upper arm girth (relaxed) (cms)
v. Upper arm girth (flexed) (cms)

Haematological
a. Haemoglobin (g/dl)
b. Red blood cells (millions/mm³)

Biochemical
a. Blood glucose (mg/dl)
b. Total protein (g/dl)
c. Albumin (g/dl)
d. Globulin (g/dl)
e. Blood cholesterol (mg/dl)
f. Blood lactate (mg/dl)

The blood samples were analysed in the Clinical Laboratory of Madras Veterinary College, Chennai - 7, with standard equipments. All the equipments confirmed to the scientific standards and have been in use continuously for medical research. The equipments were calibrated prior to testing the subjects.

EXPERIMENTAL GROUPS

The isotonic resistance training was used by the three experimental groups with the following intensity of loads.

a. Group I: 40% of 1 RM
b. Group II: 60% of 1 RM

c. Group III: 80% of 1 RM

**DETERMINATION OF ONE REPETITION MAXIMUM (1 RM)**

The subjects were made to sit comfortably in the chest press station of the multigym apparatus. Each subject was asked to perform chest press with a predetermined standing weight of 60 Kgs. Then five kilogram weight were added or reduced by the investigator until the subject was just able to lift the absolute maximum weight. This maximum weight was recorded as one repetition maximum (1 RM), for chest press.

Similarly, the 1 RM of each subject was determined for leg press and shoulder press in the multi gym apparatus. The 1 RM for arm curl was determined using free weights.

**ISOTONIC RESISTANCE TRAINING PROGRAMME**

The resistance training programme used in the present investigation for the three experimental groups are described below.

- Isotonic resistance exercises
  - a. chest press
  - b. leg press
  - c. shoulder press
  - d. arm curl
b. Volume of load: Group I: 40% of 1 RM
     Group II: 60% of 1 RM
     Group III: 80% of 1 RM

c. Training frequency: Three days per week

d. Number of sets in each unit of exercise: Two sets of exercises were conducted, for chest press, leg press, shoulder press and arm curl individually. Rest period of one minute were given in between the two sets, while two minutes periods were given in between each station.

e. Number of repetitions: Depending upon the subjects individual capacity, varied from three to twelve repetitions.


PILOT STUDY

A pilot study was conducted to assess whether the intensity and duration of the relative isotonic resistance training were within the limits of the subject's capacity.

A total of 12 subjects who did not participate in the research study performed chest press, shoulder press and arm curl resistance training exercise.
For this purpose, four subjects were chosen for the three different intensity load of resistance training, namely, 40% of 1 RM, 60% of 1 RM and 80% of 1 RM and they performed three to 12 repetitions per set depending upon their individual capacity. The investigator and the tester assistant obtained field work experience in a setting similar to that of the research study which adequately prepared them to give proper training and to collect data.

TESTER RELIABILITY

Before the commencement of experiment the reliability of the tester was established. For this purpose 12 male students were selected randomly from the nonathletes of Madras Veterinary College, Chennai - 7. The subjects were from the age group of 19 to 22 years.

To test the reliability, test and retest method was followed. The variables selected in the present investigation were measured twice on the same subject by the same testing personnel by using the same equipments under similar conditions and the reliability coefficients are presented in Table I. Care was taken to administer the test and retest in identical conditions. As suggested by Thomas and Nelson (1990), univariate correlation (inter class correlation) was computed separately for each criterion variable for the test and retest scores to establish reliability.
TABLE I

RELIABILITY COEFFICIENT FOR THE DEPENDENT PHYSICAL, ANTHROPOMETRICAL, HAEMATOLOGICAL AND BIOCHEMICAL VARIABLES

<table>
<thead>
<tr>
<th>S.No</th>
<th>Dependent Variables</th>
<th>Coefficient of Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Muscular strength</td>
<td>0.912</td>
</tr>
<tr>
<td>3</td>
<td>Muscular Endurance</td>
<td>0.882</td>
</tr>
<tr>
<td></td>
<td>Anthropometrical</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Percent body fat</td>
<td>0.892</td>
</tr>
<tr>
<td>5</td>
<td>Chest Circumferences</td>
<td>0.914</td>
</tr>
<tr>
<td>6</td>
<td>Thigh Girth</td>
<td>0.902</td>
</tr>
<tr>
<td>7</td>
<td>Forearm Girth</td>
<td>0.899</td>
</tr>
<tr>
<td>8</td>
<td>Upper Arm Girth (Relaxed)</td>
<td>0.872</td>
</tr>
<tr>
<td></td>
<td>Upper Arm Girth (Flexed)</td>
<td>0.916</td>
</tr>
<tr>
<td>9</td>
<td>Haematological</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Haemoglobin</td>
<td>0.921</td>
</tr>
<tr>
<td></td>
<td>Red blood cells</td>
<td>0.933</td>
</tr>
<tr>
<td>11</td>
<td>Blood glucose</td>
<td>0.924</td>
</tr>
<tr>
<td>12</td>
<td>Total protein</td>
<td>0.934</td>
</tr>
<tr>
<td>13</td>
<td>Albumin</td>
<td>0.902</td>
</tr>
<tr>
<td>14</td>
<td>Globulin</td>
<td>0.894</td>
</tr>
<tr>
<td>15</td>
<td>Blood cholesterol</td>
<td>0.912</td>
</tr>
<tr>
<td>16</td>
<td>Blood lactate</td>
<td>0.867</td>
</tr>
</tbody>
</table>

INSTRUMENT RELIABILITY

In the present study, standard equipments such as multi gym (Thunderfit, India), skinfold calliper (Lange, USA) and non extensible measuring tape (Freeman, India) available at Madras Veterinary College and at
the YMCA College of Physical Education, Chennai were used. The haematological and biochemical variables were measured with standard equipments purchased from reputed companies. The equipments like, Photoelectric calorimeter (Erma – AIII Japan), Centrifuge (Remi, England) and chemical reagents of fine quality were used. The equipments have been in use continuously for research purposes in the clinical laboratory of the Madras Veterinary College, Chennai.

**ORIENTATION OF SUBJECTS**

Before the commencement of the resistance training programme, several sessions were spent to familiarise the subjects with the correct techniques involved in the lifting of weights and executing the resistance training programme. It helped the subjects to perform the resistance exercises in a proper technique. The subjects were motivated to attend the practice sessions regularly. Further, the control group was specially oriented and advised to avoid practice any kind of resistance training programme during the experimental period. The subjects of all the groups were sufficiently motivated to perform to their maximum level during the testing periods and the training sessions.
EXPERIMENTAL PROTOCOL

The isotonic resistance training programme was scheduled for three sessions per week in the morning 6.30 a.m. to 8.00 a.m. for all the three experimental groups. In every session the workout lasted approximately 60 minutes. The training was given under the direct supervision of the investigator. Proper warming up exercises were performed by the subjects individually prior to the training session.

During the experimental period, the control group did not participate in any type of resistance training programme. This was carefully monitored by the testing personnel who were exclusively allotted for this purpose, since all the subjects were residents of the college hostel.

DESCRIPTION OF ISOTONIC RESISTANCE TRAINING

The following resistance training exercises were given to the subjects during the experimental period in a multigym (Figure 1).
Figure - 1

Multigym Apparatus
Chest Press

The subject laid down on the bench in supine position with the feet straddling the bench and placed on the floor. The head, shoulders and buttocks were positioned on the bench. Five to ten centimeters space was allowed between the head and the weight stack. The subject grasped the handle over the shoulders with a closed overhand grip (Figure II). Then the subject was asked to push the handle away from the chest to a fully extended elbow position while maintaining the same body position on the bench (Figure III).

After the upward movement, the subject was asked to lower the weight slowly to the starting position.

Leg press

The subject assumed a seated position on the leg press machine with the feet flat on the pedals. The thighs, lower legs and feet were positioned parallel to each other, with knees flexed at an angle of less than 90°. The subject was asked to grasp the handles and to keep the torso erect (Figure IV). The foot pedals were pushed forward until the legs were fully extended while maintaining the erect position on the seat. The subject was advised to avoid forceful locking of knees, while doing the exercise (Figure V).
Figure - II
Chest Press - Initial Position

Figure - III
Chest Press - Final Position
Figure - IV
Leg Press - Initial Position

Figure - V
Leg Press - Final Position
The subject allowed the foot pedals to return slowly to the starting position while maintaining the same body position on the seat.

Shoulder Press

The subject was asked to sit on the seat and assumed a flat back position and grasped the handle with a closed, pronated grip. The feet were positioned on the floor with the eyes focussed straight ahead (Figure VI). Then the subject was asked to push the handles up with the elbows pointed out to sides until arms were fully extended, without forcefully locking the elbows. (Figure VII).

The handles were slowly lowered to the shoulder level to the starting position.

Arm Curl

The arm curl with free weights was performed by the subject using a closed, supinated grip with the arms extended at shoulder width. The subject was asked to stand erect with feet apart and knees slightly flexed. The subject rested the bar on the anterior thigh with the little finger touching the outer thigh. The upper arms were positioned against the ribs and perpendicular to the floor (Figure VIII). The bar was raised in an arc by flexing the arms at the
Figure VI
Shoulder Press - Initial Position

Figure VII
Shoulder Press - Final Position
Figure VIII
Arm Curl - Initial Position

Figure IX
Arm Curl - Final Position
elbows, while the upper arms and elbows were stationary. The bar was raised to within 10 to 15 cm of the anterior deltoid (Figure IX).

The subject then lowered the bar slowly until the elbows were fully extended.

COLLECTION OF DATA

The data on physical, anthropometrical, haematological and biochemical parameters were collected prior to the commencement of experiment (pre test), and after 12 weeks post-test of training. Care was taken to administer the pre and post-tests under identical conditions and the same apparatus, testing personnel and testing procedures were used.

The procedure adopted for the collection data are described below.

**Muscular Strength**

The muscular strength was assessed by one repetition maximum performed by each subject in chest press. The performance was recorded in kilograms.
Muscular Endurance

Muscular endurance was assessed by maximum number of repetitions performed at a baseline intensity corresponding to 60% of 1 RM in chest press. The performance was recorded as the number of maximum possible repetitions in one set.

Percent Body Fat

The following equipments and procedures were used in measuring the skinfold thickness of chest, abdomen and thigh.

Equipment

Skinfold caliper (Lange, USA).

Procedure

The skinfold caliper was used to measure skinfold thickness of each site listed below and the measurements were recorded to the nearest millimeters.
Chest

A diagonal fold was taken one half of the distance between the anterior axillary line and the nipple of the subject.

Abdomen

A vertical fold was taken at a lateral site, approximately two centimeters from the umbilicus.

Thigh

A vertical fold on the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella was taken.

The sum of the skinfolds of the chest, abdomen and thigh was compared with the age of the subject and the percent body fat was calculated from the index table (Pollock, et al., 1980).

Girth Measurements

The procedures as described by Jackson, et al., (1978) were used for girth measurement. Adequate care was taken to measure the circumference at the bulkiest part of the muscle without applying too much pressure.
Equipment

Gulich tape

Procedure

The Gulich tape was used to measure each site listed below, and the measurements were recorded in cms.

Chest Circumference

The subjects were made to stand with the arms slightly abducted so as to permit to pass the gulich tape around the chest. The measurement was taken at the nipple level at the end of a normal expiration.

Thigh Girth

The thigh girth was measured at the maximum circumference of the thigh in a plane at right angles to its body axis, midway between the hip and knee.

Forearm Girth

The forearm girth was measured at the point of greatest circumference between the elbow and wrist in an extended, relaxed position.
Upper Arm Girth (Relaxed)

The upper arm girth was measured at the maximum circumference in a relaxed position at right angles to the body axis.

Upper Arm Girth (Flexed)

The upper arm girth was measured at the maximum circumference when in a flexed position at right angles to the body axis.

HAEMATOLOGICAL AND BIOCHEMICAL ESTIMATIONS

The following haematological variables, namely, haemoglobin, red blood cell count and the selected biochemical variables, namely, blood glucose, total protein, albumin, globulin, cholesterol and lactate were measured.

Pre and Post Test Blood Sampling

The blood sample was collected at rest and immediately after the maximal resistance exercise test with the resistance fixed at 60% of 1 RM for all the subjects.
Blood Collection

The blood sample was collected by venous puncture. A 20 ml sterilized syringe with needle, a stoppered container with anticoagulant, tourniquet cotton soaked in methylated spirit were used for collection of blood sample from each subject.

Procedure

The subject was asked to sit on an arm chair comfortably. An examination of the superficial vein of the left forearm was made to select the site for venous puncture. The skin was cleaned with spirit and allowed to dry. A tourniquet was tied around the upper arm. The subject was asked to flex and extend the wrist joint to make the veins more prominent. Thumb of the left hand was placed on the lower part of the cleaned area and gentle traction was given to fix the vein. As the vein was punctured, blood flowed in the syringe. A sterile cotton wool was placed on the puncture site and pressed gently. Five milliliter of blood was collected from each subject just prior to experimental trials and also at the end of the experimental trials from cubital vein using disposable syringe and needle (Sisco, India). Due considerations were advocated to maintain sterility and preferred anticoagulants were weighed exactly before collection.
Plasma for blood glucose estimation was obtained from whole blood collected in sodium fluoride in order to preserve the glucose in the plasma from being used up by the red blood cells, blood cholesterol and lactate estimations were carried out in plasma from the blood collected in heparin. Total protein, albumin and globulin assay were carried out in serum samples. Blood was collected for these parameters without any anticoagulants and it was made to clot upon standing. Oozed out serum on clot retraction was used to assay total proteins, albumin and globulin. Other blood parameters like haemoglobin and red blood cells were carried out in the blood collected using Heller and Paul mixture as anticoagulant. Plasma for estimation was obtained after centrifugation of collected blood and was stored in an airtight containers at – 20° C until analysis.

Blood samples were subjected to the following estimations

(a) **Hematological** : a) Haemoglobin and b) Red blood cells.

(b) **Bio-chemical** : a) Blood glucose b) Total protein, albumin and globulin c) Blood cholesterol and d) Blood lactate.
HAEMATOLOGICAL ESTIMATION

1. Haemoglobin

Haemoglobin was estimated by the acid hematín method using Sahle's haemocytometer.

Principle

The haemoglobin was converted into acid hematín by reacting with dilute hydrochloric acid. The resulting brownish mixture was matched with a standard in a colorimeter.

Procedure

Upto two marks of the square tube, the 0.1N hydrochloric acid was taken. To this 20 micro liter of blood was added and then mixture was allowed to stand until acid hematín was developed. Distilled water was added drop by drop till the colour matched with standard colour of the haemometer. Once the colour matched, the readings were recorded directly.

Result:

The results were expressed in gram percentage.
2. Enumeration of Red Blood Cells

The number of red blood cells (RBC) were determined by suitable dilution and enumerated over a definite area.

Procedure

Blood was drawn upto 0.5 mark of red blood cells pipette. The blood on the sides of the pipette was wiped off. Hayem's fluid was drawn into red blood cells pipette upto 101 mark carefully avoiding air bubbles. The contents were mixed gently taking care to avoid haemolysis. First few drops were discarded and then a small drop of mixture was placed at the edge of the cover slip, placed on the haemocytometer, which was focussed under the microscope. It was allowed undisturbed for five minutes so that the cells could settle on the haemocytometer. The number of cells in five small squares were counted.

Calculation

Number of cells in 5 small squares  = X

Number of cells in a square millimeter area (25 small squares) = X x 5

Depth (height between the coverslip and counting chamber, = 0.1mm

Dilution factor  = 200
Number of cells in 1 cu.mm = \( X \times 5 \times 10 \times 200 \)
\[ = X \times 10000 \]

The results were expressed in millions / cu.mm of blood.

**BIOCHEMICAL ANALYSIS**

The procedure used for determining the blood parameters are described below. Estimations were carried out by colorimeter (Erma photoelectric colorimeter, AE-III, Japan).

1. **Blood Glucose**

Blood glucose was estimated colorimetrically by O-Toluidine method as described by Winckers and Jacobs, 1971.

**Principle**

Glucose reacted with ortho-toluidine in the presence of hot acetic acid forming a green colored complex which was measured colorimetrically at 620 nm (wave length).
Procedure

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O - Toluidine reagent</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Glucose standard (100 mg)</td>
<td>--</td>
<td>0.1 ml</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Plasma</td>
<td>--</td>
<td>--</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water</td>
<td>0.1 ml</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

The tubes were kept in boiling water bath for 10 minutes. After cooling, the optical density was recorded at 620 nm.

\[
\frac{\text{O.D. of Test} - \text{O.D. of Blank}}{\text{O.D. of Std} - \text{OD of Blank}} \times 100 \text{ mg}
\]

The obtained value was expressed as mg/dl.

2. **Total Protein and Albumin**

Total protein and albumin were estimated by modified Biuret and Dumas method (1972).
a. Total Protein

**Principle**

Protein in the serum reacted with copper of Biuret reagent in alkaline medium to form a blue purple complex with an absorption maximum at 530 nm.

**Procedure**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
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<tbody>
<tr>
<td>1</td>
<td>Biuret reagent</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Protein standard</td>
<td>--</td>
<td>0.1 ml</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>--</td>
<td>--</td>
<td>0.1 ml</td>
</tr>
</tbody>
</table>

The tubes were kept at room temperature for 5 minutes and the optical density was recorded at 530 nm.

**Calculation**

\[
\text{O.D. of Test} - \text{O.D. of Blank} \times \text{Concentration of standard}
\]

\[
\text{O.D. of Std} - \text{O.D. of Blank}
\]

The obtained value was expressed as mg/dl.
b. Albumin

Albumin in the serum bound with the dye Bromocresol green at pH 3.8 to form a green coloured complex with absorbance of which was measured at 620 nm.

Procedure

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffered dye reagent</td>
<td>4.5 ml</td>
<td>4.5 ml</td>
<td>4.5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>--</td>
<td>0.03 ml</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Serum</td>
<td>--</td>
<td>--</td>
<td>0.03 ml</td>
</tr>
</tbody>
</table>

The tubes were kept for one minute and read on a colorimeter using red filter.

Calculation

\[
\text{O.D. of Test} - \text{O.D. of Blank} \times \text{Concentration of standard} / \text{O.D. of Std} - \text{OD of Blank}
\]

The obtained value was expressed as g/dl.

a. Globulin

\[
\text{Globulin} = \text{Total Protein} - \text{Albumin}
\]

The obtained value was expressed as g/dl.
3. Blood Cholesterol

Blood cholesterol was estimated as per the method described by Wybeng and Pileggi (1970).

Principle

Cholesterol reacted with hot solution of ferric perchlorate, ethylacetrate and sulphuric acid and gave a lavender colored complex which was measured at 530 nm.

Procedure

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Blank (B)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol reagent</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Working cholesterol standard (200 mg%)</td>
<td>--</td>
<td>0.025 ml</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Plasma</td>
<td>--</td>
<td>--</td>
<td>0.025 ml</td>
</tr>
</tbody>
</table>

The tubes were kept in boiling water bath for 90 seconds and cooled at room temperature. The optical density of test, blank and standard was recorded at 530 nm.
Calculation

\[
\text{O.D. of Test} - \text{O.D. of Blank} \times \frac{\text{X 100 mg}}{\text{O.D. of Std} - \text{OD of Blank}}
\]

The obtained value was expressed as mg/dl.

**Estimation of Blood Lactate**

Blood lactate was estimated as per the procedure, described by Barker and Summerson (1941).

**Procedure**

A protein free filtrate of 0.5 ml plasma was taken. To this, 1.0 ml of 20 per cent copper sulphate solution was added. The mixture was made up to 10 ml using distilled water. One gram of powdered calcium hydroxide was then added. The mixture was kept at room temperature for half an hour with occasional shaking and then it was centrifuged. One ml of the supernatant was transferred into a clean dry test tube. To this 0.05 ml of 4 percent copper sulphate solution and 6.0 ml of concentrated sulphuric acid were added. The tube was placed in a boiling water bath for five minutes. It was then removed, cooled to below 20°C using ice water bath and 0.1 ml of p-hydroxy diphenyl was added. Precipitated mixture was disbursed at least once during the time.
Then it was placed once again in the boiling water bath for 90 seconds. After cooling, it was read at 539 nm.

The results were expressed in mg/dl.

STATISTICAL ANALYSIS

The collected data on the selected physical, anthropometrical, haematological and biochemical parameters prior to and after 12 weeks of relative isotonic resistance training were statistically analysed using the Analysis of Covariance (ANACOVA) as recommended by Clarke and Clarke, (1972) and Best and Khan, (1986). In all the cases 0.05 level was fixed as level of significance, which was considered as appropriate.