Chapter - III

METHODOLOGY

In this chapter the selection of subjects, selection of variables, reliability of instruments competency of tester, reliability of data, orientation of subjects, collection of the data, administration of the tests, the experimental design and the statistical procedures used have been presented.

Selection of Subjects

Prior to the test, the investigator visited various places around Kumbakonam, Thanjavur District, Tamilnadu State and met the middle aged men explained to them about the purpose and nature of the study and requested them to volunteer for the study. Only middle aged men those who were aged between 35 and 40 years were contacted and around two hundred and forty four subjects gave their voluntary consent to work as subjects for the study. A qualified medical doctor of the Government Hospital, Kumbakonam, examined two hundred forty four middle aged men and declared one hundred ninety eight of them were fit for this study, and from the one hundred ninety eighty subjects only forty five were selected. They
were selected by lot method and they were divided randomly into three groups as two experimental groups and one control group. Their written voluntary consent was obtained after clearly explaining the nature of the study, the training programme and the variables under which they would be tested and they were assured that the data would not be used for any purpose other than the present study. They were also assured that the results would be kept strictly confidential. They were also informed that they were free to opt out of the study at any time if they feel any discomfort or difficulty in continuing with the training programme.

The selected forty five subjects were randomly divided into three groups of fifteen each, out of which group - I (n = 15) underwent Swami Satyananda Saraswathi yoga practice, group - II (n = 15) underwent B.K.S. Iyengar yoga practice for six days per week (Monday to Saturday) for twelve weeks and group - III(n = 15) remained as control. All the subjects have revealed that they have no ailments of any sort and were taking medicines for treatment after a general medical check up done on them. The physician confirmed
this and the subjects were given clearance to take part in the various packages of yogic practices.

**Selection of Variables**

It has been universally accepted that several factors either directly or indirectly enhance the risk of developing health related problems. As mentioned in the previous sessions, these risk factors, which are believed to increase the chance of developing health related problems, are either curable or incurable. There are certain other risk factors, which are self induced by the individual and can be avoided or altered voluntarily at any given moment.

Heredity, age, gender, race are some of the factors which cannot be altered and over eating, physical inactivity and psychological stress are some of the factors which a person consciously adapts to in spite of knowing the health hazards associated with them. Here the individual needs sheer determination to either avoid these habits or alter accordingly. No external help can do any better to them unless they themselves eat as much as required and involve themselves in physical activity.
After eliminating the risk factors, which are mentioned above, the researcher’s task was to identify, which may be altered as a consequence of yogic practices.

Taking into consideration all these factors, a set of variables was selected to test on the selected subjects, for observing the variations in their levels due to the training effect. The variables selected and tested were:

**I. Body Composition Measures:**

1. Percentage of Body Fat  
2. Body Mass Index (BMI)

**II. Physiological Variables:**

1. Blood Pressure (Systolic and Diastolic)  
2. Resting Pulse Rate  
3. Breath Holding Time

**III. Biochemical Variables**

1. Total Cholesterol  
2. Triglycerides  
3. High Density Lipoprotein  
4. Blood Glucose
Test Items

The present study was undertaken primarily to assess the effectiveness of varied yogic packages on selected body composition measures, physiological and biochemical variables. The investigator analyzed literatures and also consulted with physical education professional to use most suitable tests for the purpose of the study and it was presented in Table - I.

Table – I

TEST ITEMS FOR THE SELECTED VARIABLES

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Dependent Variables</th>
<th>Test Items</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Percentage of Body Fat</td>
<td>Deurenberg et al formula</td>
<td>Percentage</td>
</tr>
<tr>
<td>2.</td>
<td>Body Mass Index</td>
<td>Quetelet index</td>
<td>Kg/m²</td>
</tr>
<tr>
<td>3.</td>
<td>Blood Pressure</td>
<td>Sphygmomanometer</td>
<td>mmHg</td>
</tr>
<tr>
<td>4.</td>
<td>Resting Pulse Rate</td>
<td>Counting the pulse rate / min</td>
<td>Numbers</td>
</tr>
<tr>
<td>5.</td>
<td>Breath Holding Time</td>
<td>Holding the breath for maximum duration</td>
<td>Seconds</td>
</tr>
<tr>
<td>6.</td>
<td>Total cholesterol</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>7.</td>
<td>Triglycerides</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>8.</td>
<td>High Density Lipoprotein</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>9.</td>
<td>Blood glucose</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
</tbody>
</table>
**Orientation to the Subjects**

The subjects were allowed to familiarize with the techniques involved to execute the Swami Satyananda Saraswathi yoga practice and BKS Iyengar yoga practice. It helped them to perform well in both Swami Satyananda Saraswathi yoga practice and BKS Iyengar yoga practice and they were informed of the benefits both the training schedules. The control group had no specific training and was advised not to involve themselves in any sort of exercise related to yoga practices. Further, they were informed of the seriousness of this project, which needed good orientation and co-operation on the part of the subjects.

**Collection of Data**

Data on the selected body composition measures, physiological and biochemical variables were collected as per the method prescribed in test administration one day prior to the commencement of the practice session and one day after the completion of the practice session. 10 ml of blood was collected from each subject by venous puncture method and the blood thus collected was stored in small bottles containing heparin.
Tester Reliability

Prior to the commencement of the study, the investigator had undergone training in various techniques and testing procedures under expert’s guidance. Estimation of total cholesterol, triglycerides, high density lipoprotein and fasting blood glucose was done under the constant supervision of a biochemist and two laboratory technicians.

Reliability of Instrument

All the instruments and equipment used for the study were standard ones and of high quality. None had any functional defect and were being used for the same purposes. Each instrument was tested several times and was used on subjects only after being satisfied with the performance of the instrument. Skinfoldcalipers, measuring tapes, etc., were acquired from a physician who has been using it for diagnostic purposes on his patients for quite some time. Estimation of total cholesterol, triglycerides, high density lipoprotein and fasting blood glucose was done with the help of experts in a professional laboratory and all the instruments such as centrifuge and
auto analyzer were of high quality manufactured by companies of repute and showed excellent accuracy in giving results.

**Reliability of the Data**

The reliability of the data was established by test and re-test method. The Univariate Co-efficient was used to find out the reliability of the data on each criterion variables separately and they are presented in Table - II.

**Table – II**

Univariate Co-efficient on Selected Criterion Variables

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Variables</th>
<th>r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Percentage of Body Fat</td>
<td>0.94*</td>
</tr>
<tr>
<td>2.</td>
<td>Body Mass Index</td>
<td>0.99*</td>
</tr>
<tr>
<td>3.</td>
<td>Blood Pressure</td>
<td>0.99*</td>
</tr>
<tr>
<td>4.</td>
<td>Resting Pulse Rate</td>
<td>0.87*</td>
</tr>
<tr>
<td>5.</td>
<td>Breath Holding time</td>
<td>0.89*</td>
</tr>
<tr>
<td>6.</td>
<td>Total Cholesterol</td>
<td>0.99*</td>
</tr>
<tr>
<td>7.</td>
<td>Triglycerides</td>
<td>0.99*</td>
</tr>
<tr>
<td>8.</td>
<td>High Density Lipoprotein</td>
<td>0.99*</td>
</tr>
<tr>
<td>9.</td>
<td>Blood Glucose</td>
<td>0.99*</td>
</tr>
</tbody>
</table>

* Significant at .05 level of confidence. (The table value required for significance at .05 level of confidence with df 9 is 0.767).
Training Programme

The subjects were divided into three groups, namely Swami Satyananda Saraswathi yoga practice schedule and BKS Iyengar yoga practice schedule and control group. The control group was not given any training. The experimental groups underwent the respective training programmes weekly six days, i.e. Monday to Saturday, between 6.00 a.m. to 8.00 a.m., for a period of twelve weeks.

Training Schedule

Two experimental groups (Swami Satyananda Saraswathi yoga practice and BKS Iyengar yoga practice groups) underwent their respective training programmes six days per week (Monday to Saturday) for twelve weeks. Group I underwent Swami Satyananda Saraswathi yoga practice and group II underwent BKS Iyengar yoga practice. Group III was instructed not to participate in any strenuous physical exercise and requested to do regular work throughout the study.

The two experimental groups (Swami Satyananda Saraswathi yoga practice group and BKS Iyengar yoga practice group) who
participated were informed to report at the early hours of the day around 5.30 A.M. at their respective practice places. The selection of yoga and pranayama practice was given in appendix – IV & V.

**TEST ADMINISTRATION**

**Body Mass Index (BMI)**

The weight in kilograms divided by the square of the height in meters, used in the assessment of underweight and obesity.\(^1\)

**Percentage of Body Fat**

Adult Body Fat \(\% = (1.20 \times \text{BMI}) + (0.23 \times \text{Age}) - (10.8 \times \text{gender}) - 5.4\)

Where as the gender: Male = 1, Female = 0.\(^2\)

**Estimation of Total Cholesterol**

For the purpose of the study, all the thirty subjects were tested for amount of total cholesterol in 150 ml. of blood.

**Procedure for cholesterol estimation:**

CHOD-POP method, recommended by Katterman\(^3\) was used for this purpose. The kit used for this purpose was Boehringer

---


Mannheim, West Germany with Photometer – 4010 (auto analyzer).

Boehringer Manheim kit consisted of one bottle.

Bottle 1: Cholesterol reagent (MPR1).

Preparation: MPR1 dissolve contents of one bottle 1 by adding 32 ml redistilled water. The reagent solution is ready to use after 10 minutes.

Procedure:

Wave length: Hg 546 nm (470 – 560 nm)
Spectrophotometer: 500nm
Cuvette: 1 cm light path
Incubation temperature: 20 – 25°C or 37°C
Measure against reagent blank (RB)
One reagent blank is sufficient for each assay series.
Sample material pipette into test tubes will 0.02 ml and reagent solution pipette into test tubes 2.00 ml of reagent blank (RB) and 2.00 ml of sample. Mix and incubate RB and sample for 10 minutes at 20 – 25°C or 5 minutes at 37°C. Read absorbance of sample against RB within 1 hour = A sample.

Dilution threshold: 1000 mg/dl.
At higher cholesterol concentrations, dilute 0.1 ml of sample material with 0.2 ml of 0.9% of NaCl solution and repeat assay (result x 3).

**Calculation:** Cholesterol concentration (C) in the samples was calculated by the following formula:

\[
\text{Hg} \ 546 \ nm - c = 853 \times A \ \text{sample} \\
500 \ nm - c = 575 \times A \ \text{sample}
\]

**Estimation of HDL-Cholesterol**

HDL – Cholesterol was estimated by using CHOD – PAP method recommended by Lopes – Virella *et al* (1977).

Serum must be separated from the blood clot as rapidly as possible.

**Bottle 1: Cholesterol (reagent)**

Dissolve Contents of one bottle 1 by adding 32 ml redistilled water.

Sample preparation: Precipitation – sample pipette into centrifuge test tubes 200 ml and precipitant 500 ml.
Mix and let stand for 10 minutes at room temperature, than centrifuge for 10 minutes at 4000 rpm or more or for 2 minutes at 12000 rpm.

After centrifugation, separate the clear supernatant within two hours and determine the cholesterol contend by the CHOD-PAP method.

Assay procedure:

Wave length : Hg 546 nm

Spectrophotometer : 500nm

Cuvette : 1 cm light path

Incubation temperature : 20 – 25 °C or 37 °C

One reagent blank is sufficient for each assay series. Redistilled water, pipette into test tube 100 ml of reagent blank supernatant pipette into test tubes with 100 ml of sample. Reagent solution pipette into test tubes with 1600 ml reagent blank and 1000 ml sample. Mix and incubates at 37 °C then measure absorbance of sample \( A_{\text{sample}} \) against blank with in 1 hour.

Calculation:

\[ \text{Wavelength} - \text{Hg 546 nm} = 325.1 \times A_{\text{sample}} \]
500 nm = 219.2 x A_{Sample}

**Estimation of Triglycerides**

Triglycerides were estimated by using GOP – PAP method recommended by Searcy\(^4\) contains two sample bottles

- Bottle 1: Buffer
- Bottle 1a: 6 Reagent strips

**Preparation and stability of solution:**

Do not touch the reagent patches or the surrounding area. Immerse one reagent strip in one bottle of buffer solution and use to stir the bottle contents for Ca – 10 seconds. Leave to stand in buffer solution for 5 minutes stir once again for Ca – 10 seconds and then discard reagent strip with 2\(^0\) c.

**Procedure:**

- Wave length : Hg546 mm
- Spectrophotometer: 500 nm
- Cuvette : 1cm light path

---

Incubation temperature: 20 – 25 °C or 37 °C

Measure against reagent solution: One time is sufficient for each series (increase in absorbance).

Serum or plasma pipette into test tubes with 0.02 ml and reagent solution with 2.00 ml.

Mix and incubate for 10 minutes at 20 – 25 °C. Read absorbance of sample against reagent solution within 60 min = $A_{\text{Sample}}$

Dilution threshold: 1000 mg/dl

Calculation via factor: calculated the concentration (c) of triglycerides as follows: 20 – 25 °C

Wavelength: Hg 546 nm - $c = 1040 \times A_{\text{sample}}$

500 nm – $c = 760 \times A_{\text{sample}}$

calculation via standard: calculated the concentration (c) of triglycerides as follows

Incubation temperature: 20 – 25 °C or 37 °C

$$C = 200 \times \frac{A_{\text{Sample}}}{A_{\text{Sample}}}$$
Estimation of Glucose

GOD-PAP method, recommended by Trinder\textsuperscript{5} was used for this purpose. The kit was used for this purpose was Boehringer Mannheim, West Germany with Photometer – 4010 auto analyzer.

The kit consists of 2 bottles:

Bottle 1: Buffer/Enzymes/Chromogen (5 bottles for 5 x 20 ml)
Bottle 2: Phenol (5 bottles for 5 x 200 ml).

Preparation and stability of Reagent solution:

Dissolve the contents of one bottle by adding 200 ml distilled water. Then add the contents of one bottle la (Phenol) store in amber glass bottles.

Sample Preparation:

Note: Deproteinise immediately

Plasma should be separated from cellular constituents immediately if possible, not later than one hour after collecting the blood specimen.

URAC (deproteinizing solution) pipette into a centrifuge tube with 1.00 ml sample pipette into a centrifuge tube with 0.10 ml.

Flush the pipette with the mixture several times. Centrifuge the suspension and use 0.20 ml of the supernatant for the assay.

**Procedure:**

- **Wave length:** Hg 546 nm (470-560 nm)
- **Spectrophotometer:** 510 nm
- **Cuvette:** 1 cm light path
- **Incubation temperature:** 20 – 25°C.

Measure against blank. One standard and one blank are sufficient for each assay series. Use the standard in the assay in the same way as the supernatant.

Distilled water pipette into test tubes .2 ml with blank, standard pipette into test tubes 0.2 ml with standard, supernatant pipette into test tubes 0.2 ml sample, reagent solution pipette into test tubes 2.0 ml with standard and 2.0 ml with sample.

Mix and incubate at 20 – 25°C. Avoid exposure to direct sunlight, after 35 – 60 minutes read the absorbance’s of the sample (\(A_{\text{sample}}\)) and standard (\(A_{\text{standard}}\)) against the blank.

**Concentration of Reagent Solution:**
GOD ≥ 18 U/ml; POD 1.1 U/ml; phenol 11 mol/l; 4 –aminophenazone: 0.77 mmol/l

Calculation the concentration (C) glucose in blood plasma.

\[
C = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 100 \times \text{(mg/dl)}
\]

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS**

The experimental design used for the study was similar to random group design involving forty five subjects, who were divided into three groups such as Swami Satyananda Saraswathi yoga practice group, BKS Iyengar yoga practice group and control group of fifteen subjects each.

This study consisted of two independent variables such as Swami Satyananda Saraswathi yoga practice and BKS Iyengar yoga practice. Among the three selected groups, group - I was treated with Swami Satyananda Saraswathi yoga practice; group - II was treated with BKS Iyengar yoga practice and group - III acted as control group. The subjects in all the three groups were tested prior (pre-test), and after twelve weeks (post test) on selected dependent variables, such as, percentage of body fat, body mass index (BMI),
blood pressure (both systolic and diastolic), resting pulse rate, breath holding time, total cholesterol, triglycerides, high density lipoprotein, and fasting blood glucose.

The data collected from the three groups prior to experimental treatment as pre-test data and after twelve weeks of yoga practice on selected criterion variables were statistically examined for significant difference, by applying the analysis of covariance (ANCOVA). No attempt was made to equate the groups in any manner. In all the cases, 0.05 level of confidence was fixed to test the significance, which was considered as an appropriate.

Whenever, ‘F’ ratio for adjusted test was found to be significant for adjusted post-test means. Scheffé S test was followed, as a post-hoc test to determine which of the paired mean differ significantly.