CHAPTER III

METHODOLOGY

In this chapter, selection of subjects, selection of variables, experimental design, pilot study, criterion measures, orientation of the subjects, reliability of data, instrument's reliability, tester's reliability, administration of tests, administration of training schedule, and statistical techniques employed for analyzing the data have been described in this chapter.

3.1 SELECTION OF SUBJECTS

The purpose of the present study was to find out the effect of spinning cycle exercise and protein supplementation on lipid profile and testosterone level on obese men software professionals.

For the purpose of the study, sixty (N=60) obese software professionals from Chennai city were selected as subjects at random and their age ranged between 27 and 40 years. They were divided into four groups consisting of fifteen (n=15) subjects each. The selection of control and experimental groups were done at random. Experimental group I underwent spinning cycle exercise for 45 minutes in a day for three days per week for 12 weeks. Experimental group II underwent protein supplementation for 12 weeks. Experimental group III underwent the combination of both spinning cycle exercise and protein

supplementation for 12 weeks. And IV group acted as control group. Subjects who were in the control group were not exposed to experimental treatment. Throughout the experimental period the subjects were allowed to take their normal food.

Obesity of the subjects were determined through Body fat percentage can be estimated from a person's BMI by the following formula:

$$Bodyfat\% = (1.2 * BMI) + (0.23 * age) - 5.4 - (10.8 * gender)$$

where gender is 0 if female and 1 if male and BMI was calculated using the following formulae

$$BMI = \text{weight in kilograms / height in meters}^2$$

For the purpose of this study, men with 30% and above of Body Fat percentage is considered as obese men.

The investigator explained the proposed research work, nature of the study and subjects involvement, testing as well as exercise schedules so as to avoid any ambiguity. Prior to the administration of the study, the investigator got the individual consent from each subject.

3.2 SELECTION OF VARIABLES

The investigator reviewed the available scientific literatures pertaining to the spinning cycle exercise study from books, journals, periodicals, magazines

and research papers. Based on the consideration of feasibility criteria, availability of instruments and the relevance of the variables to the present study, following variables were selected.

Dependent Variables

Lipid Profiles

1 Triglycerides
2 Low Density Lipoprotein
3 Very Low Density Lipoprotein
4 High Density Lipoprotein
5 Total Cholesterol
6 Testosterone

Independent Variable

1. Spinning cycling exercise for 12 weeks
2. Protein supplementation for 12 weeks
3. Combination of both Spinning cycling and protein supplementation for 12 weeks

3.3 PILOT STUDY

The pilot study was conducted before analysis of training programme to ensure the suitability, frequencies and duration of exercise. Further it helps to

know the subjects' capacity to know the satisfactory effects and know the difficulty of conducting training programme and to set a clear understanding about the duration of time which is required for conducting the test.. It also helped the investigator to know the administration of different spinning cycle exercise training and protein supplementations.

3.4 CRITERION MEASURES

The following criterion measures were adopted to measure the test.

1. Triglycerides were estimated by enzymatic calorimetric method. Bio-chemistry analyses (Model RA –50) was used for this purpose

2. LDL –C was calculated from TC, TG and HDL-C levels using the following Friedewald's equation.

$$LDL\text{-}C = TC - TG/5 \text{ - } HDL - C$$

3. VLDL was calculated from TG following Friedewald's equation.

4. HDL was estimated by applying phosphtungstate method, as recommended by Castelli, et al., Bio-chemistry analyzer (Model RA-50) Bayer Diagnostics was used for this purpose.

5. Enzymatic calorimetric method recommended by Siedal et al., and Kuattermann et al., was applied for estimation of Total cholesterol. Bio-chemistryanalyser (Model RA-50) was used for this purpose.

6. Testosterone was calculated using Direct immunoenzymatic determination of Free Testosterone in serum or plasma.

3.5 EXPERIMENTAL DESIGN

Randomly selected 60 (N=60) men software professionals from Chennai were randomly divided into four groups. Experimental group I, which underwent 12 weeks spinning cycle exercise training, experimental group II, which underwent 12 weeks protein supplementation, group III underwent combination of spinning cycle exercise and protein supplementation and group IV acted as control group, which did not participate in any of the special training. Prior to the experiment, blood samples of all the subjects were collected to determine the selected variables, triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and testosterone, which forms the initial scores of the subjects. After the completion of experimental period of twelve weeks, blood samples were collected from the subjects and determined the scores of the final scores. The difference between the initial and final scores was considered as the effect of respective experimental treatments. To test the

significance of the difference were subjected to statistical treatment using ANCOVA. In all cases 0.05 level was fixed to test the significance.

3.6 RELIABILITY OF DATA

The reliability of data was ensured by establishing the instrument reliability, tester's competency and subject reliability.

3.6.1 RELIABILITY OF INSTRUMENTS

The research scholar collected the blood samples from the subjects through qualified lab technicians and the tests were conducted in Chennai Clinical Laboratory. The lab is a reputed one and the instruments used by them are standard ones. All the instruments were in good working condition. Their calibration were tested and found to be accurate enough to serve the purpose of the study. Hence, the reliability of the instruments were accepted for the purpose of this study.

3.6.2 TESTER'S RELIABILITY

To determine the reliability of measurements involved in this study, the data were collected from the college men of three groups. To ensure that the investigator was well versed in the technique of conducting the test, the investigator had a number of practice sessions in the testing procedures. The investigator took all the measurements with the assistance of persons well

acquainted with the tests and their procedures. Tester's competency and reliability of tests were established by Test, retest, process. As very high correlation was obtained, the tester competency in taking measurement and test reliability were accepted.

The co-efficient of reliabilities were significant at the 0.01 level for all the test under investigation.

3.6.3 SUBJECT RELIABILITY

To determine the reliability of the subjects of software professionals, selected subjects were divided into four groups as experimental group I, Experimental group II, experimental group III, and control group. The test conducted for tester's reliability ensured the subject reliability.

Before the commencements of experiment, the reliability of the data were established through test and retest method. The correlation of coefficient correlation obtained for the testes variables were given in Table I.

Table I

Intra Class Correlation Coefficient of Test – Retest Scores

S.No	Variables	Coefficient of Correlation
1	Triglycerides	0.93*
2	Low density lipoprotein	0.92*
3	Very Low Density Lipoprotein	0.89*
4	High density lipoprotein	0.91*
5	Total Cholesterol	0.92*
6	Testosterone	0.91*

***** Significant at 0.01 level.

3.7 SPINNING CYCLE EXERCISE TRAINING

The following five core movements in the Spinning programme were practiced by experimental group I for twelve weeks

Seated Flat:

The subjects cycled with hands at the center part of the handlebars. This was Hand Position one. This position should be used only when seated, for flat road simulations and during the warm-up and cool down. Cadence between 80 and 110 rotations per minute.

Standing Flat (also known as **running**):

The subjects with hands wide on the back 12-14" part of the handlebars that crosses the rider's body made this exercise. This was hand position two. Proper form for standing while running required the body to be more upright and the back of the legs touching or enveloping the point of the saddle, with the center of gravity directly over the crank. The pressure of body weight should never rest excessively on the handlebars. Cadence is between 80 and 110 rotations per minute.

Jumps, (also known as **Lifts**):

The performed this exercise as a combination of seated and standing with riders hands at position two for durations of between two and eight seconds. Cadence between 80 and 110 rotations per minute.

Seated Climb

The subjects performed this exercise with hands at position two, increased resistance and lower cadence of 60-80 rotations per minute.

Standing Climb

The subjects performed this exercise with hands wide and forward so the thumb tips were touching the far end of the handlebars (hand position three)and

the rider was canted slightly forward so that maximum force can be exerted onto the pedals with heavy resistance and a cadence of 60-80 rotations per minute.

These five movements each work a different part of the body and focus on different leg muscle groups. The rider should always maintain control of the flywheel by having resistance applied and remaining below a cadence of 110 rotations per minute.

Table II shows the schedule of spinning cycle exercise training protocol for experimental group I.

Table II (A)

Training Schedule for I to IV Weeks

S.No	Name of Exercise	Cadence	Duration	Rest	Repetitions
1	Seated Flat	70 – 90 RPM	2 mts	1 mt	2
2	Standing Flat	70 – 90 RPM	2 mts	1 mt	2
3	Jumps	70 – 90 RPM	2 mts	1 mt	2
4	Seated Climb	50 – 70 RPM	2 mts	1 mt	2
5	Standing Climb	50 – 70 RPM	2 mts	1 mt	2

Training Schedule for V to VIII Weeks

S.No	Name of Exercise	Cadence	Duration	Rest	Repetitions
1	Seated Flat	80 – 100 RPM	2 mts	1 mt	2
2	Standing Flat	80 – 100 RPM	2 mts	1 mt	2
3	Jumps	80- 100 RPM	2 mts	1 mt	2
4	Seated Climb	60 – 80 RPM	2 mts	1 mt	2
5	Standing Climb	60 – 80 RPM	2 mts	1 mt	2

Training Schedule for IX to XII Weeks

S.No	Name of Exercise	Cadence	Duration	Rest	Repetitions
1	Seated Flat	90 – 110 RPM	2 mts	1 mt	2
2	Standing Flat	90 – 110 RPM	2 mts	1 mt	2
3	Jumps	90 – 110 RPM	2 mts	1 mt	2
4	Seated Climb	70-90 RPM	2 mts	1 mt	2
5	Standing Climb	70-90 RPM	2 mts	1 mt	2

3.8. PROTEIN SUPPLEMENTATIONS

Experimental Group II was given protein Whey supplementation for twelve weeks. The drink was made up of protein powder mixed with water. The subjects were provided with two scoops, morning one scoop and before bed time one scoop. Each scoop contained 28.4 grams of protein. The contents of the Whey supplementation were as follows.

Table III
Contents of Protein Supplementation

NUTRITION FACTS : Strawberry		
Serving Size 1 oz = 1 scopp (28.4 g) Servings per container: 80		
	Amount Per Serving	% Daily Value
Calories	104	
Calories from Fat	1	
Total Fat	0 g	0%
Saturated Fat	0 g	0%
Trans Fat	0 g	0%
Cholesterol	4 mg	1%
Total Carbohydrats	1 g	0%
Dietary Fiber	0 g	0%
Soluble Fiber	0 g	
Sugars	1 g	
Calcium	120 mg	12%
Phosphorus	59 mg	6%
Sodium	42 mg	2%
Potassium	83 mg	2%
Protein	25 g	50%
* Percent Daily Values are based on 2000 calories. Your daily values may be higher or lower depending on your calorie needs		
	Calories	
	2000	2500%
Total Fat Less than	65 g	80 g
Saturated Fat Less than	20 g	25 g
Cholesterol Less than	300 g	300 g
Sodium Less than	2400 mg	2400 mg
Potassium	3500 mg	3500 mg
Total Carbohydrats	300 g	375 g
Dietary Fiber	25 g	30 g
Protein	50 g	65 g
Calories per gram Fat = 9, Protein = 4; Carbohydrate = 4 **INGREDIENTS:** Pure Whey Protein isolate. Natural and Artificial Flaver. Xanithan Gum, Sucralose (Splenda), Peanut Ingrediants. Allergen Information Contains Milk, and Soy (Lacithin) Ingredients.		

3.9 COMBINED EXPERIMENTAL TREATMENT

Experimental Group III was given spinning cycle programme as scheduled in Table II along with protein supplementation as stated above for twelve weeks. Thus, group III underwent combination spinning cycle exercise and protein supplementation. Experimental group III was provided with one scoop (28.4grams) of protein immediately after spinning cycle exercise and one scoop before bed time.

3.10 TEST ADMINISTRATION

3.10.I. Estimation of Lipid Profile

Lipid profiles were measured using blood samples. The blood sample was collected before training and immediately after the 12 weeks training for all the four groups.

Blood Collection

The subject was asked to sit on an arm chair comfortably. An examination of the superficial vein of the left fore arm was made to select the site for venous puncture. The skin was cleared 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 cleared area and gentle traction was given to fix the vein. A

3 ml sterilized syringe with needle was used to puncture the vein and blood flowed in the syringe. Five millimeter of blood was collected from each subject and stored in a stoppered container with antioogulant. The collected blood samples were subjected to the following estimations.

a. Triglycerides

Triglycerides were estimated by enzymatic calorimetric method. Bio-chemistry analyses (Model RA –50) was used for this purpose

Principle

Triglycerides + H_2O $\xrightarrow{\text{Lipoprotein Lipare}}$ Glycerol + Fatty Acid

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol –3-Phosphate + ADP

$2H_2O_2$ + 4 Aninoantipyrine + ADPS $\xrightarrow{\text{Peroxidase}}$ Red quinone +4 H_2O

GPO - Glycerol – 3 – Phosphate Oxidine

ADPS –N-Ethyl – N- Self propyl-n-onisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and is me asured at 546 nm.

Procedure

To ten µl of the sample, one ml of the reagent was added and mixed and incubated for 5 minutes at 37^{o}C. The readings were taken and the final colour is stable for at least 30 minutes.

Triglycerides is expressed as mg/dl.

b. Low Density Lipoprotein Cholesterol

LDL –C was calculated from TC, TG and HDL-C levels using the following Friedewald's equation.

LDL-C = TC – TG/5 - HDL – C

LDL-C was expressed as mg/dl.

c. Very Low Density Lipoprotein Cholesterol

VLDL-C was calculated from TG using the formula

VLDL – C = TG/5

VLDL-C was expressed as mg/dl.

d) High Density Lipoprotein

HDL was estimated by applying phosphtungstate method, as recommended by Castelli, et al., Bio-chemistry analyzer (Model RA-50) Bayer Diagnostics was used for this purpose.

Principle

Chylomicrons, VLDL and LDL fractions in serum are separated from HDL by precipitating with phosphtumgstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is arrayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen Aninoantspyrine.

Precipitating Reagent

Phosphotungstic acide - 2.4 mmol/l

Magnesium chloride - 39 mmol/l

Procedure

To 0.02 ml of sample, 0.20 ml of precipitating reagent was added and mixed well. The tubels were centrifuged at 4000 rpm for 10 minutes, 100 mg/dl clear supernatant was separated immediately to determine HDL cholesterol content by enzymatic cholesterol method and the readings were taken.

Serum HDL cholesterol was expressed as mg/dl.

e. Total Cholesterol

Enzymatic calorimetric method recommended by Siedal et al., and Kuattermann et al., was applied for estimation of cholesterol. Bio-chemistryanalyser (Model RA-50) was used for this purpose.

Principle

Cholesterolesters + H_2O $\xrightarrow{\text{Cholesterolestrase}}$ Cholesterol + RCOOH

Cholesterold + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Δ^4 – cholesterone + H_2O_2

$_{2H}2_O2_{=4}$ Aninophenazone + Phenol $\xrightarrow{\text{POD}}$ 4 p Benzoquinone – Monoiminol – phenazone + 4 H_2O_2

Procedure

Ten µl of serum, standard and distilled water was incubated with 1000 µl of reagent at 37^o C for 5 minutes and the absorbance of the sample and standard were read at 546 nm within one hour against reagent blank.

Serum cholesterol is expressed a mg/dl.

f. Measurement of Testosterone

Purpose

To measure testosterone of the subjects.

Method

Direct immunoenzymatic determination of Free Testosterone in serum or plasma method was used.

Principle

Free Testosterone (antigen) in the sample compets with horseradish peroxidase testosterone(enzyme-labeled antigen) for binding onto the limited number of anti- testosterone (antibody) sites on the microplates (solid phase). After incubation the bound/free separation is performed by a simple solid-phase washing. The enzyme substrate (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance is determinated. Free Testosterone concentration in the sample is calculated based on a series of standard. The color intensity is inversely proportional to the Free Testosterone concentration of in the sample. Testosterone in the blood is bound to SHBG (60 %) and in lower quantity to other protein. Only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active.

Reagent

1. Free Testosterone Standards 6x (1 vial = 1 mL)

STD0 **REF DA002/1506-0**

STD1 **REF DA002/1507-0**

STD2 **REF DA002/1508-0**

STD3 **REF DA002/1509-0**

STD4 **REF DA002/1510-0**

STD5 **REF DA002/1511-0**

2. Conjugate (1 bottle) 22 mL **REF DA002/1502-0**

Testosterone-HRP conjugate

3. Coated Microplate (1 microplate breakable) **REF DA002/1503-0**

Anti-Testosterone IgG adsorbed on microplate

4. TMB-substrate (1 bottle) 12 mL **REF DA004-0**

H2O2-TMB 0.25gr/L (avoid any skin contact)

5. Stop solution (1 bottle) 12 mL **REF DA005-0**

Sulphuric acid 0.15 mol/L (avoid any skin contact)

Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_5), two for each sample, one for Blank.

Reagent	**Standard**	**Sample**	**Blank**
Standard So-S_5	20µL		
Sample		20µL	
Conjugate	20µL	20µL	
Incubate at 37°C for *1 hour.* Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely			
TMB substrate	100µL	100 µL	
Incubate at room temperature 22÷28°C for *15 minutes* in the dark			
Stop Solution	100µL	100 µL	100 µL
Read the absorbance (E) at 450 nm against Blank.			

The units are measured in ng/dl.

3.11 STATISTICAL TECHNIQUES

Design is the key for controlling the outcomes from experimental research. The independent variables are manipulated in an attempt to judge their effects on the dependent variable. The experimental design used in this study was pre test, post test random group design. Here, the groups were randomly formed but the groups were taken a pre test as well as post test. The major purpose of this type of design was to determine the amount of change produced by the treatment, that is, does the experimental group change more than the control group.

The collected data on selected lipid profiles and testosterone variables prior to and after 12 weeks of spinning cycle exercise training and protein supplementation were statistically analysed using Analysis of Covariance (ANCOVA) 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.

Scheffe's confidence interval was calculated to find out the significance of mean differences where F value of the obtained ANCOVA is greater than the required value to be significant.