CHAPTER - III

METHODOLOGY

Research methodology elaborate the systematic process by which researcher starts from the initial documentation of the problem to its final conclusions. The regulation of the methodology is to carry on the research work in a scientific and valid way. The purpose of the experiment was to discover whether there would be any significant outcome of floor aerobic exercises with and without low glycemic diet on physical physiological biochemical and hematological variables among male type II diabetic mellitus.

This chapter delivers an overview of the technique used for the research study selection of subject, selection of variables, experimental design, pilot study, selection of test, reliability of data, administration of test, collection of data, and statistical techniques employed.

3.1 SELECTION OF THE SUBJECT

To achieve the purpose of the study among 100 subjects 45 male type II diabetic mellitus were selected from Chennai district. They were randomly divided into three equal groups and each group consists of 15 subjects. Experimental group I underwent Aerobics with low glycemic diet, Experimental

group II underwent Aerobics without low glycemic diet control group was not undergone any training.

3.2 SELECTION OF VARIABLES

This study was to find out the effect of floor aerobic exercises with and without low glycemic diet on physical physiological biochemical and hematological variables among male type II diabetic mellitus.

The following variables were selected for this study,

3.2.1 DEPENDENT VARIABLE

PHYSICAL VARIABLES

- Body Mass Index

- Percentage of body fat

PHYSIOLOGICAL VARIABLES

- VO_2 Max

- Blood Pressure

BIOCHEMICAL VARIABLES

- Low Density Lipoprotein

- High Density Lipoprotein

HEMATOLOGICAL VARIABLES

- Hemoglobin

- Fasting Blood Sugar

- Postprandial blood sugar

3.2.2 INDEPENDENT VARIABLE

4. Experimental group I – Aerobics with low glycemic diet
5. Experimental group II – Aerobics without low glycemic diet

3.3 EXPERIMENTAL DESIGN

The research design of the study was random group design. The selected 45 subjects were assigned into three groups namely, experimental group I, experimental group II and control group, and each group consisting of 15 subjects. The pretest was administered to assess the selected variables among three groups. The experimental treatment was conducted for a period of 12 weeks on alternative days, thrice a week. Experimental group I underwent Aerobics with low glycemic diet, Experimental group II underwent Aerobics without low glycemic diet control group was not given any training. The training was given

for a period of 60 minutes per day. The post test was conducted to assess the selected variablesamong three groups.

3.4 PILOT STUDY

A pilot study was conducted before analyzing training programme to ensure the suitability and duration of exercise. Five subjects each from Experimental groups were utilized for the pilot study. Further it helps to training the exercises and knows the difficulty of conducting training programme and the duration which is requires for conducting the test.

3.5 CRITERION MEASUREMENTS

The following criterion measures were chosen for the testing the hypotheses.

TABLE – I

TABLE SHOWING SELECTION TESTS AND MEASURES

S.NO	VARIABLES	TEST	MEASURES
1.	BMI	Height Weight ratio	Kilo grams/ Meters^2
2.	Percentage of body fat	Skin fold measurements	%BF
3.	VO$_2$ Max	Harvard step test	Pulse rate per minute
4.	Blood Pressure	Portable Automatic Arm Blood Pressure Monitor	mmHg
5.	LDL	Blood test	mg/dL
6.	HDL	Blood test	mg/dL
7.	Hemoglobin	Blood test	grm/dL
8.	Fasting blood sugar	Blood test	mg/dL
9.	Postprandial blood sugar	Blood test	mg/dL

3.6 RELIABILITY OF DATA

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

3.7 RELIABILITY OF INSTRUMENTS

The research scholar used the following for measuring instruments of various tests. The Measuring tape, stop watch, Spigmomonometer, sthescope, 5ml dispoven, centrifuge test tube track, timer, heating block, spectrophotometer Test tubes, Pipettes, Boiling water bath, iced cold water bath and Cuvet.

3.8 SUBJECT RELIABILITY

To determine the reliability of the subjects of diabetic men selected as subjects were divided into three groups as control group, experimental group I and experimental group II. The initial test and final test were conducted. 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.

3.9 ORIENTATION OF THE SUBJECTS

Prior to the test the investigator explained to the subject about the purpose of the low glycemic diet and floor aerobics. Investigator explained brief introduction regarding the purpose, effects, and benefits of the training to the subjects participating on the study. The study with a view to get full. Co-operation and also to motivate them to exhibit their level of performance in the training to obtain maximum subjects reliability.

3.10 TRAINING PROGRAM

Throughout the training era, the experimental group undertook their particular training program three days per week for twelve weeks. Aerobics training with low glycemic diet were given to Experimental Group I and aerobics

training without low glycemic diet were given to experimental Group II. For the
Control Group III no training was given.

TRAINING PROGRAMME FOR AEROBIC TRAINING

Duration was 12 weeks on 3 alternative days

Training programme	: 12 weeks
Warm up & warm down	: 10 minutes & 5 minutes
Interval	: 5 minutes
Duration of training	: 30 minutes
Total time duration	: 45 minutes

S.No	Number of aerobic exercises
1	Marching on the spot
2	Bounding
3	Kick sideward
4	Kick front
5	Sideward bend
6	'V' step
7	Alternative elbow & knee touch

Table-II (A)

TRAINING PROGRAMME FOR I to IV WEEKS

Days	Number of exercises	Time for total exercise (Minutes)	In between break (Minutes)	No of set	Intensity	Total time duration (Minutes)
Mon	7	30	5	4	60%	45
Wed	7	30	5	4	60%	45
Fri	7	30	5	4	60%	45

*The intensity was increased based on the tempo of the music.

Table II (B)

RAINING PROGRAMME FOR V TO VIII WEEKS

Days	Number of exercises	Time total exercise (Minutes)	In between break (Minutes)	No of set	Intensity	Total time duration (Minutes)
Mon	7	30	5	5	70%	45
Wed	7	30	5	5	70%	45
Fri	7	30	5	5	70%	45

*The intensity was increased based on the tempo of the music.

TABLE II (C)

TRAINING PROGRAMME FOR IX to XII WEEKS

Days	Number of exercises	Time for total exercise (Minutes)	In between break (Minutes)	No of set	Intensity	Total time duration (Minutes)
Mon	7	30	5	6	80%	45
Wed	7	30	5	6	80%	45
Fri	7	30	5	6	80%	45

*The intensity was increased based on the tempo of the music.

3.11   AEROBICS TRAINING PROCEDURE

Warm-up exercises

The first of preparing the body for strenuous training activity is to warm-up. Stretching exercise increases readiness to perform and help prevent injuries. Care was to be taken to prevent injury by moving slowly, focusing on the muscle when changing the stretching position from one position to another.   The flexibility exercise were done in sequence from upper body to lower body.

Aerobics

After the warming up, `aerobics exercises were given. Aerobics exercise consisted of the following

1. Marching on the spot

The subject was asked to lift alternate leg up to a certain level and place it back on the same place. The arms with bent elbows were swing slightly with alternate leg movement. This was performed for 32 counts

2. Bounding

1. The subject was asked to stand with one leg comfortably with another leg bounding upward

2. For the 1" and 2" count the subject was asked to jump slightly upward with arms upward raise (arms close to the ear, palms closed upward, another hand was down stretched close to body. palm faced downward)

3. The subject was asked to bring the stretched position at shoulder level at the shoulder line

4. The subject was back to position and it was be repeated for 32 counts.

3. Kick sideward

1. The subject was asked to step up the left leg on stepper and kicked right leg sideward.

2. The subject was asked to bring back the right leg to the position.

3. Exercise was repeated with left leg and the subject was asked to bring back left leg to the position.

4. This was performed for 32 counts on both sides left and right alternatively.

4. Kick front

1. The subject was asked to kick the right leg forward.

2. Back to the position.

3. Exercise was repeated with left leg and the subject was asked to bring back left leg to the position.

4. This was performed for 32 counts on both sides left and right alternatively.

5. Sideward bend

1. The subject was asked to stand shoulder level feet apart.

2. The left leg spread out and slightly stretched. The body also slightly bends sideward. The arms close to the ear, palms faced downward.

3. The right leg spread out and slightly stretched. The body also slightly bends sideward. The arms close to the ear, palms faced downward.

4. The subject back to position and it was repeated for 32 counts.

6. 'V' step

1. The subject was asked to perform the 'v' step by placing.

2. The subject step or placed the left foot front and diagonally out left side on and place the hands forward bend in front of chest, fingers closed.

3. Right foot front and diagonally out right on the stepper.

4. Left foot back to the position.

5. Right foot back to the position.

6. It was repeated for 32 counts.

7. Alternative elbow & knee touch:

1. The subject was asked to stand with both the legs comfortably.

2. The subject step the right leg knee on the forward bend to touch the left hand elbow alternatively, in front of the body.

3. Another hand was in swinging action.

4. Right leg knee & left hand elbow back to the position.

5. Both the leg & hand alternatively doing the action continuously (elbow & knee touch)

COOL - DOWN EXERCISES

After every training session the subjects of experimental groups were given ten minutes cool down exercises by performing simple stretching without burning or a slow jogging similar to a warm-up. Warming down exercise are necessary to remove waste products that accumulate in the muscles are result of exertion. Stopping exercise abruptly can cause a sudden drop in blood pressure because when the heart stops pumping hard, the blood can pool in the veins.

TABLE III

DIET PRESCRIPTION FOR EXPERIMENTAL GROUP I

Time	Diet menu choice 1	Diet menu choice 2	Diet menu choice 3
Early morning	Tea / Skimmed milk without sugar 100ml	Tea / Skimmed milk without sugar 100ml	Tea / Skimmed milk without sugar 100ml
Break fast	Ragiidli 3 No's + Sambar 75ml	Broken wheat upma with Vegetables 150grm+ Mint Chutney 30ml	Multi grainsdosa 3 No's + Sambar 75ml
Mid-morning	Butter Milk 100ml + Boiled vegetables 50grm	Mixed vegetables soup 100ml + Fruit salad 50grm	Butter Milk 100ml + Vegetable Salad 50grm
Lunch	Rice 200grm + Sambar 50ml + Greens kootu 50grm + Mixed Veg Porial 50grm + Skimmed curd 25ml	Rice 100grm +Chapathi 3 No's + Sambar 25ml + Dhal subji 50ml +Gourd veritiesPorial 50grm + Boiled Egg white 1 No	Mixed Veg. Biryani 200grm +Raitha 30ml + Cucumber Salad 25grm
Evening snacks	Sundal 50grm + Tea without sugar 100ml	Sprouts 50grm + Tea without sugar 100ml	RagiAdai 1 No + Tea without sugar 100ml
Dinner	Chapathi 3 No's + Veg. Subji 75ml	Multi grains rotti 3 No's + Dhal Subji 75ml	Mixed grain dosa 3 No's + Sambar 75ml

FOODS SHOULD BE AVOIDED

Banana, Jack fruit, custard apple, mango, potato, yam and carbonated beverages.

3.12   TEST ADMINISTRATION

3.12.1 MEASURING BODY MASS INDEX

Height

Objective:

To measure height

Apparatus used:

Stadiometer and Anthropometric rod

Test Description:

Height was measured by anthropometric rod. The subject stood erect bare footed on a plane horizontal surface against a wall with heels, back of the shoulder and head touching the wall and stretch the body. Stretched upwards as much as possible without the heel leaving the ground. Than anthropometric rod was kept in front of the subject and the crossbar of the anthropometry was

adjusted so that the lower edge touches the highest point of the subject's head. Height was recorded in meters.

Weight:

Objective:

To measure weight

Apparatus used:

Weighing Machine

Test Description:

The weights of the subjects were taken on a weighing machine with the subjects wearing short and vest only. They stood on the weighing machine and weight was recorded nearest to half a kilogram. (Yobu, 1983).

Calculation of Body Mass Index

From the height and weight obtained following above procedures, body mass index of each subject was calculated.   Body mass index was calculated by dividing the subject's body weight in kilogram by their height in metres squared. (Palanivel, 2004)

3.12.2 BODY FAT PERCENTAGE

Fats are the mechanisms of carbon, hydrogen and oxygen, mainly three fatty acid molecules involved to one glycerol molecule. Neutral fats are better sources of energy giving about nine calories for each gram of fat taken.(Sharma 1987)

Testing

The skin-fold size was used to assess valued body fat percentage or more precisely the level of fatness in an individual. The skin-folds in the present study were measured with "Slim Guide skin-folds Caliper" and the instruction manual for this resolve by Wallace C. Donoghue (1987) was followed.

Biceps

The subject was requested to let the arm droop freely and the skin-folds were elevated over the biceps muscles.

Triceps

The subject was requested to flex the arm at the elbow to 90°. The skin-folds dimension was taken over the triceps muscles.

Subscapular

Dimension was made at the fold crosswise from the vertebral column upward the inferior angle of the right scapula, about 2 cm below the inferior angle and somewhat towards the midline of the body.

Suprailiac

The site was placed direct above the crest of the left ileum. The thumb was positioned over the iliac and the fold lifted medial to the midline at a slight angle to the vertical along the normal fold line.

Body Fat Percentage

After density has been assessed, the percentage of fat is intended, based on the known alterations in density of fat and lean tissue. Siri's (1956) equation is used for altering body density to percentage of fat:

Estimated Body Density=: 1.1620 - 0.630 Kg x Log of the sum of the four

skin-folds (Biceps+ Triceps+ Suprailiac + Subscapular)

Estimated body fat percentage = {(4.950/Density) - 4.500} x 100

(Johnson and Nelson, 1982)

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3.12.3   VO2 MAX

COOPER'S 12 MINUTES RUN OR WALK TEST

Purpose

To measure the VO_2 max (cardio respiratory endurance)

Equipment

Whistle, stopwatch, 400 meters track.

Description

Subjects assemble behind the starting line .at the starting signal, they, run or walk as far as possible with in the 12 minutes time limit. An experienced pacer should accompany performers around the running area during the actual test. At the signal 'to stop 'performers should remain where they finished long enough for test administrators to record the distance covered. Ample time should be given for stretching and warm-up as well as cool down.

Scoring

The distance in meters covered in 12 minutes

The VO_2 max in ml/min/kg was calculated based on the formulae suggested by Cooper (1960) was:

$$\text{VO}_2 \text{ max} = \frac{d_{12} - 505}{45}$$

Where, d_{12} is the distance (in meters) covered in 12 minutes. And the scores recorded in ml/kg.min.

3.12.4 BLOOD PRESSURE

Purpose: -

The determination of the test was to found the the systolic blood pressure at rest.

Equipment:

Diamond makes Sphygmomanometer.

Procedure:

The blood pressure was recorded at the beginning and end of the training periods using the equipment in a sitting relaxation position. The left upper arm was concentrated by an inflatable rubber bag which was connected to pressure pump and manometer. By driving air, the pressure in the bag was rapidly raised about to 180mmHg which was adequate to completely obliterate the brachial artery so that no blood comes over and the radial pulse disappeared. The stethoscope is placed over the brachial artery in the cubital fossa. The pressure is released at a rate of approximately 2 mm per second. The pressure at which the

first sounds are heard (systolic pressure) and the pressure once all sounds fade (diastolic pressure) are recorded. At this precise point pressure shown on the dial was measured to be the systolic pressure. The pressure on the brachial artery was then gradually reduced until the arterial pulse rate beats could be distinctly heard and particular point at which the sound extinct was taken as the diastolic pressure. (AmbikaShanmugam 2001).

Scoring:

The blood pressure was indicated on the Sphygmomanometer scores recorded in mmHg..

3.12.5 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
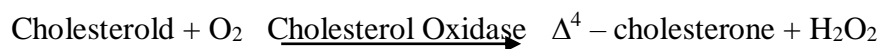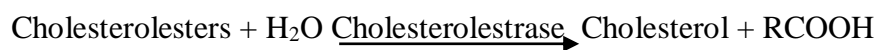
Procedure

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.
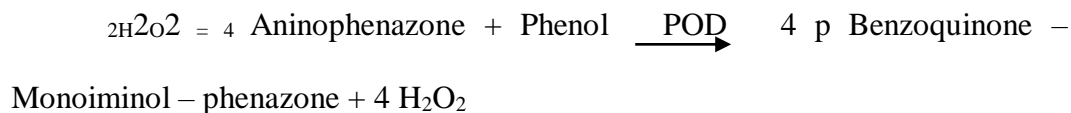
Serum HDL cholesterol was expressed as mg/dl.

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.
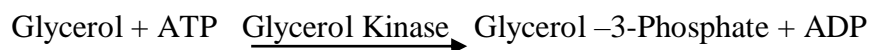
Principle

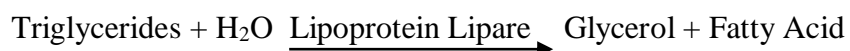Cholesterolesters + H_2O $\xrightarrow{\text{Cholesterolestrase}}$ Cholesterol + RCOOH

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

$2H_2O_2 = 4$ 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° 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.

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 measured 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 37ºC. The readings were taken and the final colour is stable for atleast 30 minutes.

Triglycerides is expressed as mg/dl.

3.12.6 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.

3.12.7 HEMOGLOBIN

Hemoglobin was estimated by the acid hematin method using Sahle's haemocytometer.

Principle

The hemoglobin was converted into acid haematin 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 hematin 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.

3.12.8 BLOOD SUGAR

The O-Toluidine method was used to find the glucose level in the blood. The random blood sugar level was determined initially for all the subjects and it was again taken at the end of eight weeks training schedule.

Principle:

The quantitative determination of glucose in body fluids by use of O-Toluidine as a colour reagent was first reported by Hultman in 1959. The method has been proved to be simple and reliable. O-Toluidine reacts with aldehyde group of glucose in hot acetic acid solution to form an equilibrium mixture of glucosylamine and corresponding schiffs base. The blue-green coloured end product has maximum absorption at 630 mm. Intensity of colour developed is proportional to the original concentration of glucose present in the specimen.

Procedure without deprotenization

Pipette into three test tubes labelled as B-Blank, S-Standard, T-Test (unknown).

Reagent	For/m/ procedure		
	B	S	T
1. Glucose Reagent	1.0ml	1.0ml	1.0 ml
2. Distilled water	20 ml	20 ml	20 ml
3. Glucose standard	20 ml	20 ml	20 ml
4. Specimen(Serum or plasma)	20 ml	20 ml	20 ml

Mix by lateral shaking. Put all tubes into vigorously boiling water both (100°c) for exactly nine minutes. Quickly remove the tubes and cool to room temperature by placing in cold water for three minutes.

Transfer the contents of tubes to avette and measure Absorbance (O.D.) of all tubes against blank adjusted to zero at 630+20mm. In case of LAB SYSTEM ANALYSER use 620 mm ie. 62 on the filter wheel.

Calculation

The concentration of glucose in the unknown sample is calculated using,

RT/RS x 100 mg/dcd

RT –Optical density of test

RS- Optical density of standard

Blood sugar levels determined from the blood samples taken when the subject was fasting was considered as fasting blood sugar.

Blood sugar levels determined from the blood samples taken 2 hours after meal of the subject was postparandial blood sugar.

Blood sugar fasting and post parandial were determined initially for all the subjects and it war again taken at the end of twelve weeks training schedule.

3.13 STATISTICAL TECHNIQUES

The analysis of covariance (ANCOVA) statistical method was used to find out the outcome of aerobic training with low glycemic diet on physical physiological biochemical and hematological variables amongst male type II diabetic mellitus over SPSS software. Scheffe's post-hoc test was engaged for inter group assessment, where 'F' ratio initiate significant (Thirumalaiswamy, 1995).