CHAPTER 3- METHODS AND MATERIALS

This chapter deals with the methods and tools employed to carry out the present study. The broad objective of the study was to generate data on nutritional status, body composition, fitness level and nutrition awareness among the Elite Cricketers (14 years or above) of Urban Vadodara. Impact evaluation of a cocoa flavanol rich drink on the muscle recovery of the participants was also studied. The methods used in the study are as follows:

PHASE-1 (SAMPLING, NUTRITIONAL STATUS ASSESSMENT, TRACKING ENERGY EXPENDITURE, MORBIDITY INJURY PROFILE, FITNESS ASSESSMENT AND HEMOGLOBIN ESTIMATION)

Sampling technique

All the sports associations located in Vadodara involved with cricket were visited. Elite cricketers from these associations were identified. The operational definition of elite male cricketers is those who train for a minimum of 5 hours for at least five days a week. The operational definition of elite female cricketers is those who train for a minimum of 4 hours for at least five days a week. The following inclusion criteria were used to enrol the participants in the study.

Inclusion Criteria

The participants fulfilling the following criteria were included in the study.

- Elite Male Cricketers 19 to 30 years of age.
- Elite Female Cricketers 14 to 30 years of age.
- Willing to participate.
- Cricketers who are not differently abled or who do not suffer from any disease.
- Non-pregnant and non-lactating females.
- Those who are not under any drug treatment.
- Those who do not have abnormally high Creatine Kinase levels at the baseline.

Exclusion criteria

The following participants were excluded from the study

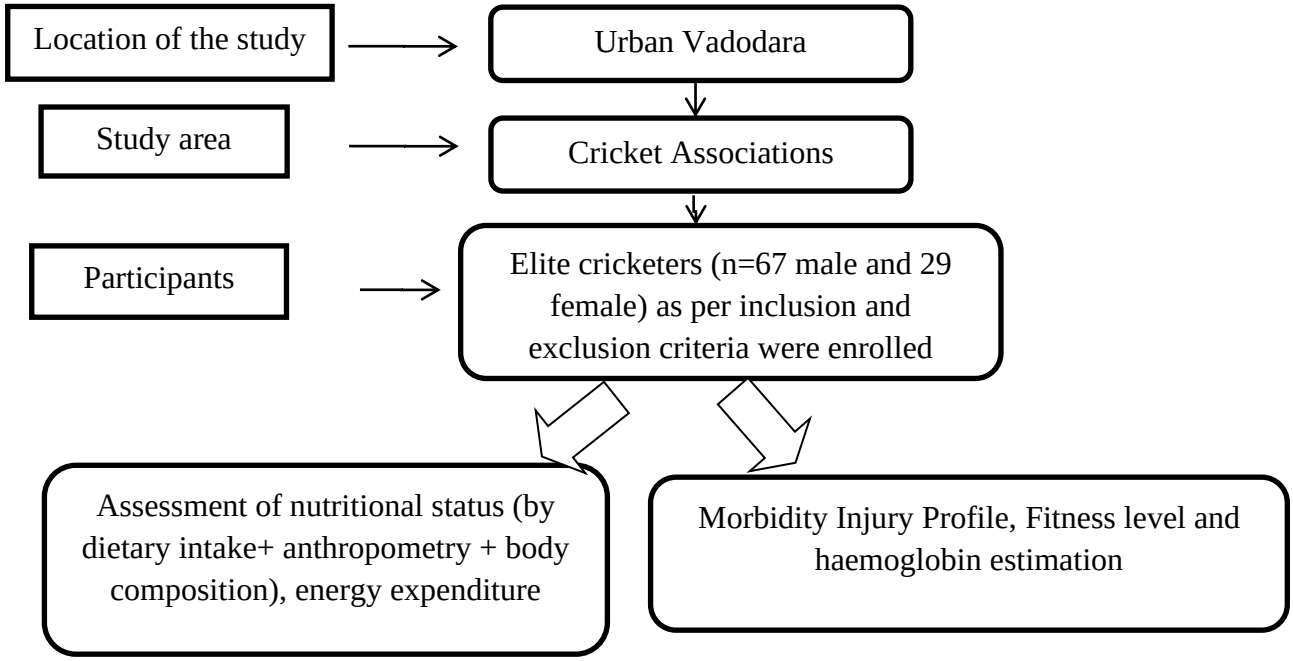
- Elite cricketers who were not residing in Vadodara.
- Elite cricketers who were not a part of any of the Baroda Cricket Association squads during enrolment.

- Purposive sampling of the participants was carried out based on the above-mentioned inclusion criteria.
- In all, 96 participants (males-67, females-29) fulfilled these criteria. All of them play through the Baroda Cricket Association (Appendix 1).
- After receiving well-informed, written consent (Appendix 2-A, B, C, D), the participants were enrolled in the study.

STUDY PLAN – PHASE -1

Figure 3.1 illustrates the study design of this phase.

Figure 3.1: Study design of phase 1



The tools and techniques used in phase 1 are mentioned in table 3.1

Table 3.1: Tools and Techniques (PHASE 1)

PARAMETERS	INDICATORS	METHODS
Nutritional status	Anthropometric measures	Standard methods for Height, weight, waist circumference and hip circumference
	Body composition	Bioelectrical impedance (Bodystat Quadscan 4000 unit)
	Dietary intake	24-hr dietary recall (3 day- during 2 training days and one rest day) and semi-structured food frequency questionnaire

Iron Status	Haemoglobin	Sodium lauryl sulphate method (Sysmex XN- 1000)
Energy expenditure	Accelerometer sensing intensity of movements	Fitness tracker (MI band 2)
Morbidity injury profile	-	Pretested questionnaire
Fitness	Cardio Respiratory	Yo yo test, Cardio vascular endurance test -2 km and Repeated sprint ability
	Power	Vertical Jump test
	Speed	20 m Sprint, 40 m Sprint test
	Agility	Run a three test
	Muscular endurance	Prone hold

DESCRIPTION OF METHODS (PHASE 1)

Background information (Appendix 3)

It was collected using a semi-structured questionnaire.

Socio-Economic Information (Appendix 3)

It was assessed using the per capita income and evaluated based on the B G Prasad scale (Dalvi et al, 2020).

Anthropometric Measurements

Height

It is a linear measurement of the body. It is an indicator of long-term under-nutrition. A non-stretchable measuring tape perpendicular to the floor is fixed on a smooth wall. The participant is asked to stand erect and look straight ahead with his shoulder and back portion of his head touching the wall with arms hanging loosely by the sides. With help of a measuring scale, the height is noted to the nearest mm.

Weight

Weight is the key anthropometric measurement of body mass. It indicates the current nutritional status. A standardized weighing platform scale is used for taking measurements. Balance is placed on a hard flat surface and checked for zero balance before each measurement. Participants are asked to stand erect on the scale with minimum clothing, looking straight and without taking any support from

the wall and then measurements are noted down. The balance was calibrated with a set of standard weights regularly (after every 10 measurements) throughout the study period.

Body Mass Index (BMI)

Body mass index indicates the current nutritional status. The Asia Pacific cut-offs were used for classifying participants (≥18 years) as underweight, normal, overweight or obese (Table 3.2). BMI for age values by WHO were used for adolescents (14-17 years). BMI was calculated using the following formula:

BMI = Weight (kg): Height (m^2)

Table 3.2: Asia Pacific Classification for BMI for Adults

Classification	BMI (kg/m^2)
Underweight	<18.50
Normal	18.50-22.9
Overweight	23.0-24.9
Obese	35.0 and above

Source: Gallagher, 2004.

Waist Circumference (WC)

Waist circumference assesses abdominal obesity. Participants are asked to stand straight and then with help of non-stretchable tape, measurements are taken by placing tape horizontally mid-way between the lowest rib margin and the iliac crest. International Diabetes Federation (IDF) for South Asians cut off values as depicted in Table 3.3 were used for classification.

Table 3.3: International Diabetes Federation classification for Waist Circumference for South Asians

Gender	Cut offs
Men	≥90 cm
Women	≥80 cm

Hip Circumference

For measurement of the hip circumference (HC), the participant is asked to stand erect with arms at the side and feet together. Then measurements are taken with help of non-stretchable measuring tape held horizontally from the point yielding the maximum circumference over the buttocks.

Waist Hip Ratio (WHR)

This index measures the central distribution of fat. It is the ratio of waist circumference to hip circumference (Jelliffe, 1966). Table 3.4 indicates cut off points for assessing abdominal obesity by WHR. WHR was calculated by using the formula:

WHR= Waist circumference (cm): Hip circumference (cm)

Table 3.4: Cut off values for assessing abdominal obesity by WHR

Gender	Cut offs
Men	> 0.90
Women	> 0.80

Source: World Health Organization, 2005.

Waist to Height Ratio (WHtR)

This index measures central obesity. It is a simple, easy, accurate and age independent ratio of Waist Circumference to Height.

WHtR= WC (cm): Height (cm)

Cut off values

A waist-to-height ratio of ≥0.5 is considered a risk for both men and women and characterises the short fat phenotype (Hsieh SD et al, 2003).

Body Composition Analysis by Bioelectrical Impedance Analysis

With help of the Bodystat Quadscan 4000 instrument, which is based on multi-frequency Bioelectrical Impedance Analysis (BIA) technology, body composition analysis was done. The Quadscan 4000 unit is battery-operated and light in weight. The principle behind the BIA technique is that lean tissue, which consists of electrolyte-containing water, will conduct electric current and yield low impedance, whereas the body's fat compartment which is very low in body water content acts as an insulator with high Impedance to the high-frequency current. The impedance measurement, therefore, reflects the degree of resistance to the flow of current in the body. Within a few seconds, Quadscan 4000 unit can measure a fat weight, lean weight, dry lean weight, water volume, Extra Cellular Water volume, Intra Cellular Water volume, their percentages with normal and optimal ranges of each along with Basal Metabolic Rate (BMR), BMI and normal range of Body Fat Mass Index (BFMI), Fat Free Mass Index (FFMI) and WHR.

Procedure:

- Participants are asked to lie down in the supine position on a non-conductive surface for 3-4 minutes so that the fluid levels stabilize in the body before a measurement is taken.
- Care has to be taken that no parts of the body are touching each other.
- A new set of self-adhesive disposable electrodes are placed on the right hand and right foot in order to avoid the battery current passing through the left side of the body where the heart is situated.
- The unit has two main lead wires which are interchangeable. The crocodile clips are attached to the metal tab strip of the electrodes (black to wrist and ankle and red clip behind knuckles and toes).
- The QuadScan 4000 unit is then switched on.
- Then accurate participant data, i.e height, weight, etc. is entered into the unit.
- After that the Enter <> key is pressed to further carry out the process.
- The crocodile clips are disconnected from the electrodes and the electrodes are removed.
- Participants could then sit or stand up.
- The QuadScan 4000 unit is switched off.
- The Bodystat software is paired to a PC via Bluetooth.
- The data are downloaded into the software installed on the PC.
- The output in terms of, Body fat, Lean mass, TBW, BFMI, FFMI, etc. is obtained.

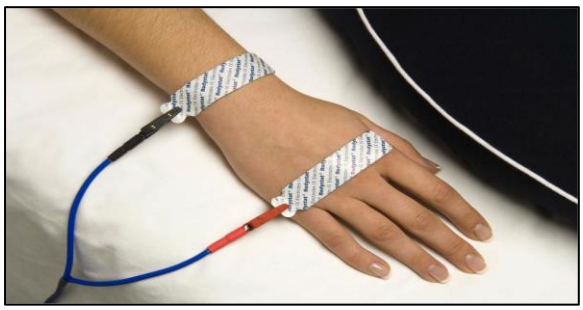
The following guidelines are followed in order to get accurate and reproducible results:

- No eating or drinking 4 to 5 hours prior to the test.
- No exercise 12 hours prior to the test.
- No alcohol and caffeine consumption 24 hours prior to the test.

The placement of electrodes is as follows:

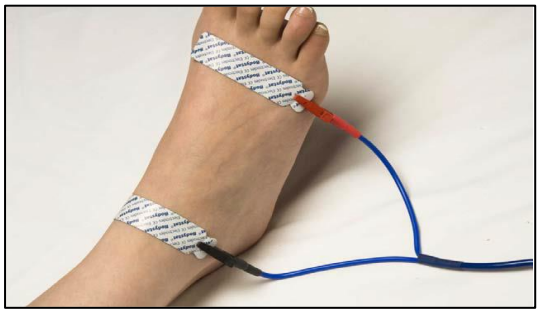
RED LEAD: Behind the knuckles

BLACK LEAD: On the wrist next to the ulna head



RED LEAD: Behind the toes.

BLACK LEAD: On the ankle at the level of and between the medial and lateral malleoli.



Fat free mass index (FFMI) and body fat mass index (BFMI)

The FFMI and BFMI indices are equivalent concepts to the BMI (Shutz et al, 2002). BFMI and FFMI can be calculated by the formula:

- FFMI = Fat free mass (kg):Height^2 (m^2)
- BFMI = Fat mass (kg):Height^2 (m^2)

BFMI and FFMI are better indicators compared to absolute fat and lean mass values as they take into consideration the height of the individual as well.

Dietary Intake Assessment

Twenty-four hour dietary recall (Appendix 4)

This is the most common method used for diet survey. It is based on the recall of food consumption over a period of 24 hours prior to the survey. It is used for assessing food and nutrient intake of populations.

Procedure:

Information related to various recipes, types of food preparations made during all meals in a day i.e. breakfast, lunch, snacks, dinner etc. and the food items consumed in each meal by the participant on previous day are obtained from the participants (for the accuracy in ingredients and quantities) in terms of measures of standard cups and spoons. Care is taken not to gather data on the day of fast, feasting and festivals. From this, participant's intake of raw ingredients was calculated followed by the calculation of various nutrients like energy, carbohydrate, protein, fat, Vitamin C and iron with help of "Diet Cal" software, a tool for dietary assessment and planning developed by Ms Gurdeep Kaur from the Department of Dietetics, AIIMS, New Delhi, India.

Food Frequency Questionnaire Method (Appendix 5)

It is a qualitative food frequency method, which gives an idea about the usual number of times each food in a checklist was consumed during a specific period. This method gives an idea about the frequency of consumption of foods rich in specific nutrients.

Procedure:

An exhaustive list of foods rich in iron and calcium was prepared. Then participants were probed as to how frequently they consumed each of the listed foods. The frequency was daily, thrice a week, twice a week, once a week, once in fifteen days, occasionally/once in a month or never. Data were entered into an excel sheet and then analysed.

Hemoglobin estimation

Instrument used-Sysmex XN- 1000

The method used - Sodium lauryl sulphate (SLS) method

In past, the mainstream methods for automatically measuring haemoglobin were the cyanmethemoglobin and oxyhemoglobin method. But these methods have both advantages and disadvantages when they are used with a large, fully automatic instrument. In the Cyanmethemoglobin method, since haemoglobin conversion speed is slow and multiple sample processing is an assumed requirement, this method is not really appropriate for automatic analysis. Moreover, since it uses cyanide compounds, which are poisonous as reagents, the liquid waste must be treated making the method undesirable from an environmental perspective.

In contrast, haemoglobin conversion speed of the oxyhemoglobin method is fast, as blood haemoglobin is instantly converted into oxyhemoglobin. And since it does not use poisonous substances such as cyanide, it is a suitable method for performing automatic analysis. It cannot however convert methemoglobin into oxyhemoglobin, which is not a problem for normal human blood, but will result in values lower than the true values for samples that contain large amounts of methemoglobin such as control blood samples. The SLS method is an analysis method that makes use of the advantages of the two aforesaid methods.

As with the oxyhemoglobin method, the haemoglobin conversion speed of the SLS haemoglobin method is fast and the method does not use poisonous substances making it suitable for automation. Further, since methemoglobin can be analysed, control samples such as blood containing methemoglobin can also be accurately analysed. (Manual of Sysmex Systems)

The normal range for Males is 13-18 and for females, it is 12.5-16 g/dl.

Fitness tracker

It was used to assess the energy expenditure of the participants. The device had to be worn on the wrist. The device used was MI band 2.

Principle- This tracker has in built sensor called an accelerometer. An Accelerometer uses capacity sensing to measure micro electro-mechanical movement and track the force of movements. A program converts this force into Metabolic Equivalent (METs). METs of basic activities like walking are known and so this code can be converted into the number of steps taken. It measures the intensity of the activity and does the needful. It is much more precise than a pedometer.

Morbidity and Injury Profile (Appendix 6).

Injuries are a part of any sport and their nature varies across various sports. The injury profile captured the variety of injuries participants (cricketers) underwent and the time to recover from the same.

Morbidities are a part of everyone's life and food intake is compromised during this period. Morbidities of the last 30 days were recorded to assess whether there is any impact of the same on the food intake of the participants.

Both Morbidity and Injury profiles were assessed using a semi-structured questionnaire.

Fitness Assessment

Fitness tests vary from sport to sport. Several cricket-specific fitness tests were conducted for the participants. The procedure for execution and scoring of these tests are described below.

Vertical Jump Test

The vertical jump test is a test of lower body power. The test was first described nearly 100 years ago (Sargent, 1921).

Purpose: To measure the leg muscle power

Equipment required: measuring tape or a marked wall, chalk for marking wall (or jump mat).

Procedure: The participant stands side to a wall and reaches up with the hand closest to the wall. Keeping the feet flat on the ground, the point of the fingertips is marked or recorded. This is called the standing reach height. The participant then stands away from the wall, and leaps vertically as high as possible using both arms and legs to assist in projecting the body upwards and has to attempt to touch the wall at the highest point of the jump. The difference in distance between the standing reach height and the jump height is the score. The best of three attempts is recorded.

Results

The following chart depicted in table 3.5 is used for the purpose of scoring

Table 3.5: Scoring chart for Vertical jump test

Rating	Males (cm)	Females (cm)
Excellent	>70	>60
Very good	61-70	51-60
Above average	51-60	41-50
Average	41-50	31-40
Below average	31-40	21-30
Poor	21-30	11-20
Very poor	<21	<11

https://www.topendsports.com/testing/norms/vertical-jump.htm

Run-a-Three test

Running speed, acceleration, and agility are very important physical attributes for cricket players, particularly for moving between the wickets and when fielding. The Run-a-Three Test is a cricket fitness test that assesses both speed and agility in a very specific cricket situation. The run-a-three involves sprinting over the actual pitch distance three times, carrying a bat and incorporating two 180-degree turns. This test not only assesses speed, but also technique to turn and running the bat in at the end.

Purpose: The aim of this test is to determine the running speed up and down the cricket pitch while wearing cricket equipment.

Equipment required: Timing gates or stopwatch, non-slip running surface, tape for marking the ground and cone markers.

Procedure: The run-a-three test involves three repeated maximal sprint trials to be performed over the same distance as between the batting creases on a cricket pitch (58 feet or 17.68 meters). The players carry their cricket bat (and can also be wearing cricket kit such as pads and helmets). They start running from one end, run to the other crease where they turn and run back to the starting line, turn again then run through the final line. The starting position is with the foot over the starting line with the bat in hand. Players should slide the bat over the crease mark at each end when turning and at the finish. The players are required to keep the bat in their dominant hand throughout the test.

Results: Three trials are allowed and the time is recorded for each straight run (58 feet / 17.68m) and total time for the run-a-three. Turning time for the changes of direction between runs 1–2, and 2–3 can also be recorded (timed from when the participant passes through the timing gate 5 m away from the crease until they returned through the same timing gate). Table 3.6 depicts the cut offs for scoring. (https://www.topendsports.com/testing/tests/run-a-three.htm)

Table 3.6: Scoring chart for Run a three test

Rating	**Time**
Excellent	<9.2 sec
Very good	9.2- 9.69 sec
Good	9.7 – 10.19 sec
Fair	10.2-10.69 sec
Poor	>10.69 sec

(NCA)

Prone hold test

It is a simple fitness test of core muscle strength, and can also be used as a fitness exercise for improving core strength.

Purpose: The prone hold test measures the control and endurance of the back/core stabilizing muscles.

Equipment required: Flat and clean surface, stopwatch, recording sheets, pen.

Procedure: The aim of this test is to hold an elevated position for as long as possible. Start with the upper body supported off the ground by the elbows and forearms, and the legs straight with the weight taken by the toes. The hip is lifted off the floor creating a straight line from head to toe. As soon as the participant is in the correct position, the stopwatch is started. The head should be facing the ground. The test is over when the participant is unable to hold the back straight and the hip is lowered.

Scoring: The score is the total time completed. Table 3.7 gives the classification.

Table 3.7: Scoring chart for Prone hold test

Rating	Time
Excellent	>6 minutes
Very good	4-6 minutes
Above average	2-4 minutes
Average	1-2 minutes
Below average	30-60 seconds
Poor	15-30 seconds
Very poor	<15 seconds

(https://www.topendsports.com/testing/tests/plank.htm)

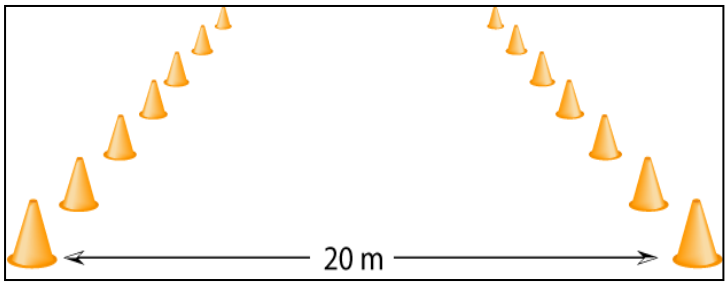
Yo- Yo Test

The Yo-Yo Endurance Test (continuous) is a variation of the beep test, part of the yo-yo test series developed by the Danish soccer physiologist Jens Bangsbo. There are two versions of this test: Level 1 and 2 (beginners and advanced level).

The level one test is effectively the same as the standard beep test. The Level 2 test starts at a higher running speed and has different increments in speed. There is also an intermittent version of the Yo-Yo test, which incorporates a recovery period after each 40m (2x20m) run.

Purpose: The test evaluates an individual's aerobic endurance fitness.

Equipment required: Flat, non-slip surface, marking cones, measuring tape, pre-recorded audio CD or MP3 (or Team Beep Test software), CD player, recording sheets.



Procedure: Use cones to mark out two lines 20 meters apart as per the diagram. The participants start with their foot behind one of the lines and begin running when instructed. They continue running between the two lines, turning when signalled by the recorded beeps. After each minute or so, the pace gets quicker. If the line is not reached in time the participant must run to the line, turn and try to catch up with the pace within 2 more 'beeps'. The test is stopped if the participant fails to catch up with the pace within the two ends.

Scoring: The athlete's score is the total distance covered before they were unable to keep up with the recording. The Yo-Yo intermittent test usually takes between 6-20 minutes for level 1 and between 2-10 minutes for level 2. (https://www.topendsports.com/testing/tests/yo-yo-endurance.htm)

Twenty metre Speed Test

Sprint or speed tests can be performed over varying distances, depending on the factors being tested and the relevance to the sport.

Purpose: The aim of this test is to determine acceleration, and it is also a reliable indicator of speed, agility and quickness.

Equipment required: measuring tape or marked track, stopwatch or timing gates, cone markers, flat and clear surface of at least 40 meters.

Procedure: The test involves running a single maximum sprint over 20 meters, with the time recorded. A thorough warm-up should be given, including some practice starts and accelerations. Start from a stationary position, with one foot in front of the other. The front foot must be on or behind the starting line. This starting position should be held for 2 seconds prior to starting, and no rocking movements are allowed. The tester should provide hints to maximizing speed (such as keeping low and driving hard with the arms and legs) and encouraged to continue running hard past the finish line.

Results: Two trials are allowed, and the best time is recorded to the nearest 2 decimal places. The timing starts from the first movement (if using a stopwatch) or when the timing system is triggered and finishes when the chest crosses the finish line and/or the finishing timing gate is triggered. *(https://www.topendsports.com/testing/tests/sprint-20meters.htm)*

Forty metre Speed Test

Purpose: The aim of this test is to determine acceleration and speed.

Equipment required: measuring tape or marked track, stopwatch or timing gates, cone markers, flat and clear surface of at least 60 meters.

Procedure: The test involves running a single maximum sprint over 40 meters, with the time recorded. A thorough warm up should be given, including some practice starts and accelerations. Start from a stationary position, with one foot in front of the other. The front foot must be on the starting line. The runner should be stationary prior to starting. The person timing should stand at the finish line with one arm held high, and call 'ready' followed by a sweep down their arm quickly to start the participant (do not call out 'go' due to the time delay in the participant hearing the call). As the arm sweeps down, the tester should start the stopwatch which is held in the downward sweeping arm, and finish the stopwatch as their chest passes through the finish line.

Results: Three trials are allowed, and the best time is recorded to the nearest two decimal places. Table 3.8 depicts the scoring guidelines for this test. (https://www.topendsports.com/testing/tests/sprint-20meters.htm)

Table 3.8: Scoring for 40 metre sprint test

Rating	Time
Excellent	<5.3 sec
Very good	5.3- 5.6 sec
Good	5.61-5.9 sec
Fair	5.91-6.20 sec
Poor	>6.2 sec

(NCA)

Repeated Sprint ability test

A repeat sprint test of anaerobic capacity, involving ten 30m sprints performed every 30 seconds.

Purpose: This is a test of anaerobic capacity, the ability to recover between sprints and produce the same level of power repeatedly.

Equipment required: 2 stopwatches, measuring tape, marker cones, and at least 50 meter track.

Procedure: Marker cones and lines are placed 30 meters apart to indicate the sprint distance. Two more cones are placed a further 10 meters apart on each end. At the instructions of the timer, the participant places his/her foot at the starting line, then on 'go' two stopwatches are started simultaneously, and the participant sprints maximally for 30 metres, ensuring that they do not slow down before reaching the finish line. One stopwatch is used to time the sprint, the other continues to run. The time of the first sprint is then recorded. The participants use the 10 meter cone to slow down and turn and return to the 30 metre finishing point, which then becomes the next start line. The next sprint will be in the opposite direction. Each 30 meter sprint starts 30 seconds after the previous run started. This cycle continues until ten sprints are completed, starting at 30 sec, 1 min, 1.5 min, 2 min and so on after the start of the first sprint.

Scoring: The fatigue index is calculated by taking the average time of the first three trials and dividing it by the average time of the last three trials. This will give a value of approximately between 75 and 95%. For example, if the times for the first three sprints were 6.9, 7.1, and 6.7 (average 6.9 seconds) and the last three times were 7.6, 8.2, and 7.9 (average 7.9 seconds), the fatigue index will be 6.9 ÷ 7.9 = 0.87 (Good). Table 3.9 depicts the cut offs for this test.

Table 3.9: Scoring chart for Repeated Sprint ability test

Rating	Fatigue Index
Excellent	>89%
Good	85-89%
Average	80-84%
Poor	<80%

(https://www.topendsports.com/testing/tests/sprint-fatigue.htm)

Cardiovascular Endurance test - 2 km

Purpose- To assess the cardiovascular endurance of an individual

Equipment required: stopwatch, marked 2 km track or path on level ground with firm and smooth surface, heart rate monitor (optional).

Procedure: The test involves walking as fast as possible over 2 km. The walking time and the heart rate are recorded at the end of the test. Heart rate can be measured using the manual method or using a heart rate monitor.

Scoring: The results are calculated from the time of the 2 km walk, heart rate (HR) at the end of the walk, body mass index (BMI) and age. Norms are available to place individual scores within a fitness category. VO_{2max} can be calculated by using the following formula (Oja&Tuxworth, 1995*):* VO_{2max} (ml/min/kg) = 116.2 – 2.98 × walking time (sec) – 0.11 × HR – 0.14 × age – 0.39 × BMI (https://www.topendsports.com/testing/tests/walk.htm)

PHASE 2

A- SURVEY ON THE COMPOSITION OF PROTEIN SUPPLEMENTS AND SPORTS DRINKS AND

B- DEVELOPMENT, SUPPLEMENTATION AND IMPACT EVALUATION OF A COCOA FLAVANOL RICH DRINK ON MUSCLE RECOVERY

Protein supplements and sports drinks are commonly consumed by cricketers in general. Due to this reason, a market survey of commercially available protein supplements and sports drinks was conducted.

PHASE 2-A: SURVEY ON COMPOSITION OF COMMERCIALLY AVAILABLE PROTEIN SUPPLEMENTS

The composition of the protein supplements was studied in terms of protein content, source and cost. For this, all (three) sports supplement stores in the city of Vadodara, Gujarat were identified. All protein supplements from the websites of these stores were listed. Further, the detailed composition of these products was accessed from the official websites of the respective brands. Products that did not provide Nutrition Facts Panel were excluded from the study. In all, sixty products were surveyed.

PHASE 2-A: SURVEY ON COMPOSITION OF COMMERCIALLY AVAILABLE SPORTS DRINKS

The composition of sports drinks was studied in terms of carbohydrate and electrolyte content, ingredients used and cost. Three sports supplement stores in the city of Vadodara, Gujarat were purposively selected. All sports drinks from the websites of these stores were listed. Further, the detailed composition of these products was accessed from the official websites of the respective brands. Products that did not provide Nutrition Facts Panel were excluded from the study. In all, fifty products were surveyed.

PHASE 2-B: DEVELOPMENT, SUPPLEMENTATION AND IMPACT EVALUATION OF A COCOA FLAVANOL RICH DRINK ON MUSCLE RECOVERY

The survey of Protein supplements and Sports drinks revealed that there was no Muscle Recovery drink available in the market. To address this gap, a muscle recovery drink was developed, standardised and supplemented to study the impact.

- Development and Standardization of a cocoa flavanol rich drink

- The experimental drink had 10 gm of natural unprocessed cocoa powder which contributes 350 mg of cocoa flavanols. The drink was made from cocoa powder, milk and sugar and the volume was made up to 250ml. The drink was standardized and sensory evaluation using 9 point hedonic scale (Appendix 7) was performed by a semi-trained panel.

- The placebo was milk (+sugar) with added colour and flavor to make it look and taste similar. Placebo was developed to match the nutrient composition of the experimental drink.

Figure 3.2: Sensory Evaluation of the Experimental and Placebo drink by the semi-trained panel members

Sample size estimation for supplementation

- Documentary evidences suggest that a minimum of 12 participants are required for a minimum detectable difference of 350U/L for Creatine Kinase with a β>0.80. (Lipsey et al, 1990 and Saunders et al, 2004)

Inclusion Criteria for supplementation

- Those who are not intolerant or allergic to any food ingredient in the supplement.
- Those who did not have abnormally high baseline Creatine Kinase levels

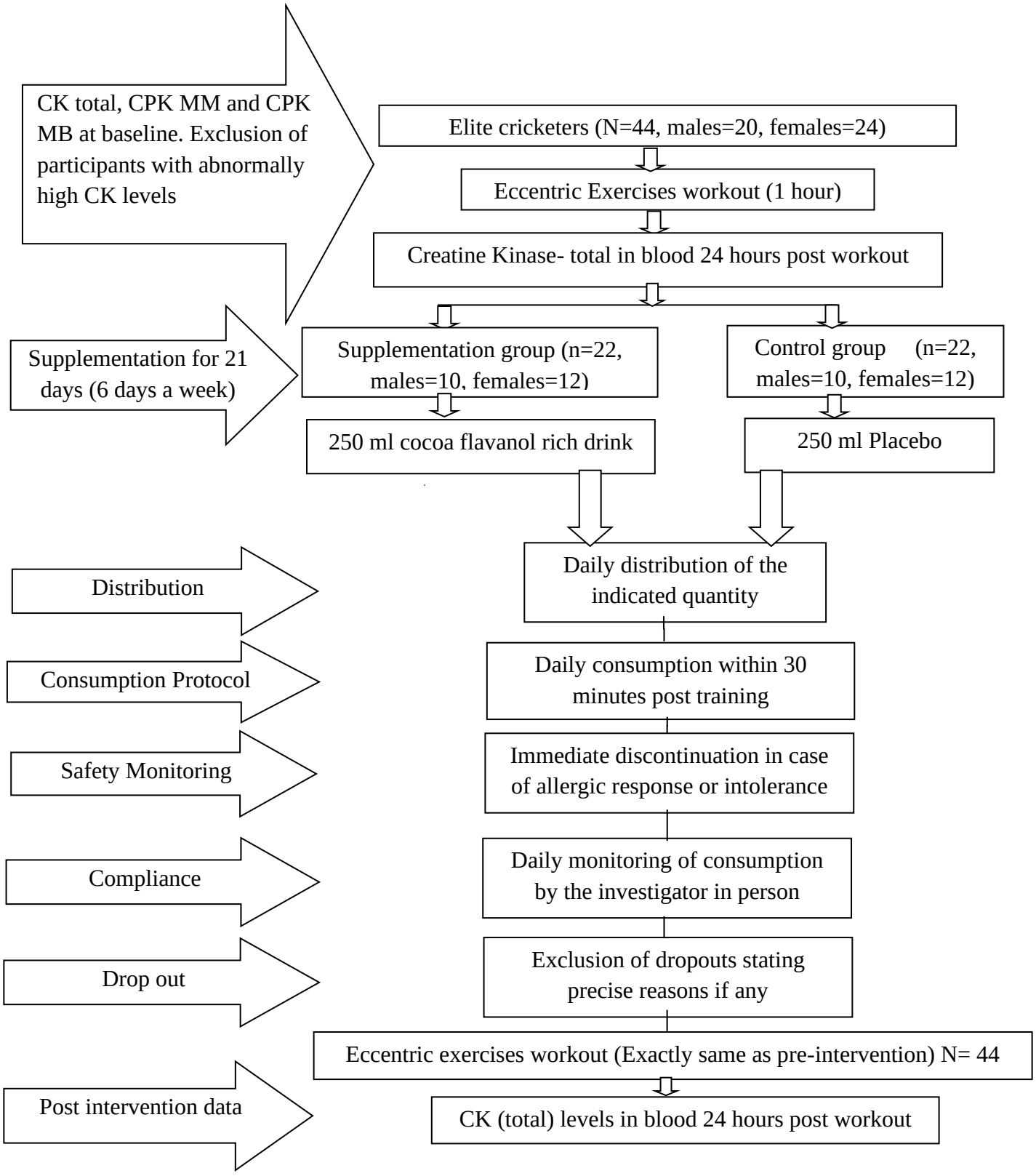
Exclusion criteria for supplementation

- Those who did not find the chocolate flavour acceptable.
- Those who had any gastrointestinal issues or any acute morbidity on the day of supplementation.

EXPERIMENTAL DESIGN/CONSORT- PHASE 2 – B

Figure 3.3 illustrates the experimental design of this phase.

Figure 3.3: Experimental design for phase 2(B)



- Creatine Kinase was assessed in blood to determine the recovery status. It was analysed using the enzymatic kit method.

- The Creatine Kinase total and CK MM and CK MB levels were assessed on day 1 after a resting phase of 10 days to capture the actual baseline values. This was assessed to rule out any muscle or cardiac abnormality.
- On day 2, all the participants had to perform a strenuous workout (Appendix 8 and 9) consisting of eccentric exercises which would naturally result in elevated Creatine Kinase total levels.
- Twenty four hours post this workout; the blood was again assessed for Creatine kinase total levels to capture the elevated levels post workout.
- The participants were randomly divided into 2 groups the supplementation group which received 250 ml of the experimental drink and the placebo which received 250 ml of the Placebo drink.
- The intervention was carried out for 21 days during which the participants were provided with the drinks on the ground itself and had to consume it within 30 minutes post training.
- At the end of 21 days, the participants again performed the exact same workout consisting of eccentric exercises as on day 2 and 24 hours later their blood was assessed to capture the post intervention creatine kinase total levels.
- The participants were given a list of foods having cocoa powder (Appendix 10) and asked not to consume any of those foods during the intervention period of 21 days so that it does not interfere with the results.

Considerations

The standardized drink was prepared by the Researcher under the supervision of the investigator.

- The drink was approved by the Anti doping Agency of the Board of Cricket Control in India (BCCI)
- The participants were neither rewarded nor had to bear any cost of the supplementation.

DESCRIPTION OF METHODS - PHASE 2- B

Estimation of Creatine Kinase through Enzymatic kit method

Summary of the test

Creatine kinase, also referred to as creatine phosphokinase is a cellular enzyme with a wide tissue distribution. CK is found mainly in skeletal and cardiac muscle. CK's physiological role is associated with ATP generation for contractile or transport systems. Serum CK is almost increased following acute myocardial infarction or skeletal muscle damage. The enzyme is commonly elevated in myocarditis of any cause, cerebrovascular accidents, rhabdomyolysis, polymyositis and acute physical exertion. CK is also increased in muscular dystrophies. In Duchenne's muscular dystrophy, CK

elevations of 20-200 times normal are common. Low CK may reflect decreased muscle mass or muscle wasting.

Principle of the procedure

The VITROS CK Slide method is performed using the VITROS CK Slides and the VITROS Chemistry products calibrator kit 3 on VITROS Chemistry systems.

- The VITROS CK Slide is a multi-layered, analytical element coated on a polyester support.
- A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. This layer also contains N-acetylcysteine (NAC) to activate CK without pretreating the sample.
- When the sample is deposited on the slide, creatine kinase catalyses the conversion of creatine phosphate and ADP to creatine and ATP. In the presence of glycerol kinase (GK), glycerol is phosphorylated to L- α- glycerophosphate by ATP. Oxidation of L- α- glycerophosphate to dihydroxyacetone phosphate and hydrogen peroxide occurs in the presence of L- α- glycerophosphate oxidase. (α-GPO). Finally, leuco dye is oxidised by hydrogen peroxide in the presence of peroxide to form a dye.
- Reflection densities are monitored during incubation. The rate of change in reflection density is then converted to enzyme activity.

Test Type and Conditions

Test type – Multiple point rate
Approximate incubation time- 5 minutes
Temperature - 37°C
Wavelength – 670 nm
Sample drop volume – 11 µL

The sample dilution procedure is mentioned in Appendix 11.

The upper limit of the reference interval is reported to be affected by population characteristics such as the degree of physical activity and race. Distributions of CK values from normal, healthy participants often demonstrate a positive skew which leads to variable upper reference limit estimates.

Expected Values and Reporting Units

Table 3.10 presents the reference range for CK in males and females.

Table 3.10: Reference interval for CK

	SI Units (U/L)
Females	30-135
Males	55-170

The Calibration process, Quality Control procedure, Precision and Specificity are described in Appendix 11.

CPK MB Estimation

The instrument used- Fujifilm Dri Chemistry Analyser

Method and principle

10 µl of plasma or serum is deposited on a FUJI DRI- CHEM SLIDE CKMB- P. while incubating at 37°C, the spotted specimen is diffused uniformly in the spreading layer, where CK-M activity is inhibited by the anti-CK M subunit antibody but activated by the N-acetyl cysteine (NAC) to promote the reaction of creatine phosphate with ADP, producing creatine and ATP.

The reaction between ATP and glucose is catalysed by hexokinase to produce glucose-6- phosphate and ADP. Glucose-6- phosphate is then oxidised by glucose 6 phosphatase dehydrogenase, yielding NADH. NADH reduces nitrotetrazolium blue by the action of diaphorase, finally producing formazan dye. The increase of absorbance by the generated dye is measured from 2.5 min to 5 min at 540 nm by reflective spectrophotometry and the CKMB activity is calculated according to the installed formula. (Manual of Fujifilm systems)

The detailed procedure is mentioned in Appendix 12.

CK MM calculation

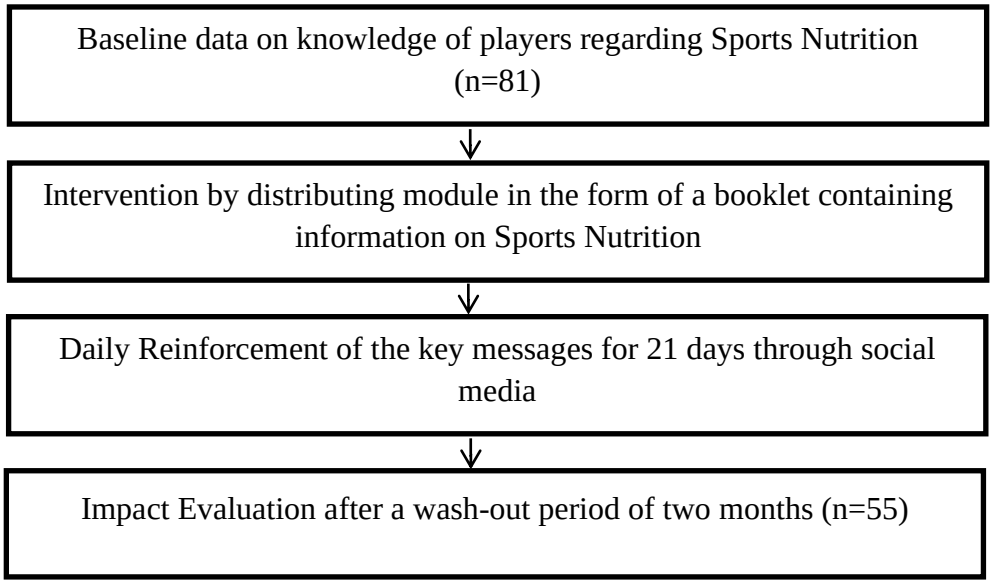
CK MM was calculated using the following formula

CK MM= CK total – (CK MB*1.5)

<u>PHASE-3</u>: ASSESSMENT OF NUTRITION KNOWLEDGE OF CRICKETERS AND IMPACT EVALUATION OF INTERVENTION WITH A NUTRITION EDUCATION TOOL

Figure 3.4 illustrates the study design of this phase.

Figure 3.4: Study design of phase 3



Nutrition awareness was assessed on the basis of knowledge scores derived from the administration of a semi-structured pretested questionnaire (Appendix 13). This questionnaire consisted of some very basic questions regarding nutrition and sports nutrition specifically from the angle of cricket. Based on the gaps in knowledge identified from administering this questionnaire, a module in the form of a booklet (Appendix 14) was developed. This module was designed to deliver the content in a very easy-to-understand way. Also, there was daily reinforcement of the key messages for 21 days after distributing the booklet sent on what's app group through the coaches. The impact evaluation was carried out after a wash-out period of two months. The same questionnaire was used at the baseline and for collecting post Nutrition education tool intervention responses.

DESCRIPTION OF METHODS (PHASE 3)

Knowledge Attitude Practices Questionnaire

This tool was administered to assess the knowledge and attitude of the participants regarding sports nutrition and the practices they follow. This was carried out through a semi-structured questionnaire. Based on the knowledge scores, the participants were classified into poor, average, good, very good and excellent. The questionnaire consisted of close-ended multiple choices questions or questions with only yes or no responses. For each correct response, a score of one was assigned and the wrong

answer was scored as zero. Only the responses related to the knowledge-based questionnaire were scored. Following were some of the components that were assessed under the KAP survey.

a. Functions of various nutrients in the body and sources of the same.
b. Consumption pattern of foods and fluids, before, during and after competitive events/matches, foods avoided in general by the participants and especially during the events and reasons for the same.
c. Knowledge regarding supplements and use of the same in terms of type, frequency and quantity.
d. Basic information pertaining to Carbohydrate loading, ergogenic aid

This same questionnaire was administered post Nutrition education tool intervention to assess the impact on the knowledge of the participants.

Components of the Nutrition Education Tool

Some key concepts incorporated in the booklet are as follows.

- Nutrients of significance to athletes
- Nutrition before, during and after the event
- Recovery Nutrition
- Hydration
- Electrolyte requirements

Data Monitoring

- Anthropometric measurements, body composition analysis, Nutrition education tool intervention and drink supplementation were monitored by the research guide.
- Blood collection for haemoglobin and creatine kinase was done by a technician and monitored by the investigator.

Considerations

The results of the research were made available to the participants in writing either personally or through their coaches (as mentioned in the consent form).

Ethical Clearance

The study has received clearance from Institutional Medical Ethics Committee with approval number IECHR/2015/18.