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## Chapter III

### Methodology

The present study titled “**Development and Evaluation of Nutraceutical Energy Rich Nutribar on the Sprint Performance of Women Athletes During Covid- 19 Pandemic**” was carried out under the following stages:

#### PHASE I

##### 3.1 DEVELOPMENT OF THE NUTRACEUTICAL ENERGY-RICH NUTRIBAR FOR ATHLETES

- 3.1.1 Procurement and processing of raw materials
- 3.1.2 Formulation and Standardisation of Nutribar
- 3.1.3 Acceptability of the Product

#### PHASE II

##### 3.2 EVALUATION OF NUTRIENT AND NUTRACEUTICAL POTENTIALS AND SHELF-LIFE STUDIES

- 3.2.1 Macronutrients and Micronutrients analysis
- 3.2.2 Evaluation for nutraceutical potentials and total antioxidant capacity
- 3.2.3 Shelf-life analysis of the developed Nutribar

#### PHASE III

##### 3.3 SELECTION OF ATHLETES AND COLLECTION OF BACKGROUND INFORMATION

- 3.3.1 Selection of Area and Athletes
- 3.3.2 Development of Methods and Tools for Data Collection
- 3.3.3 Assessment of Socioeconomic Status

#### PHASE IV

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### **3.4 ASSESSMENT OF NUTRITIONAL STATUS AND PHYSICAL PERFORMANCE**

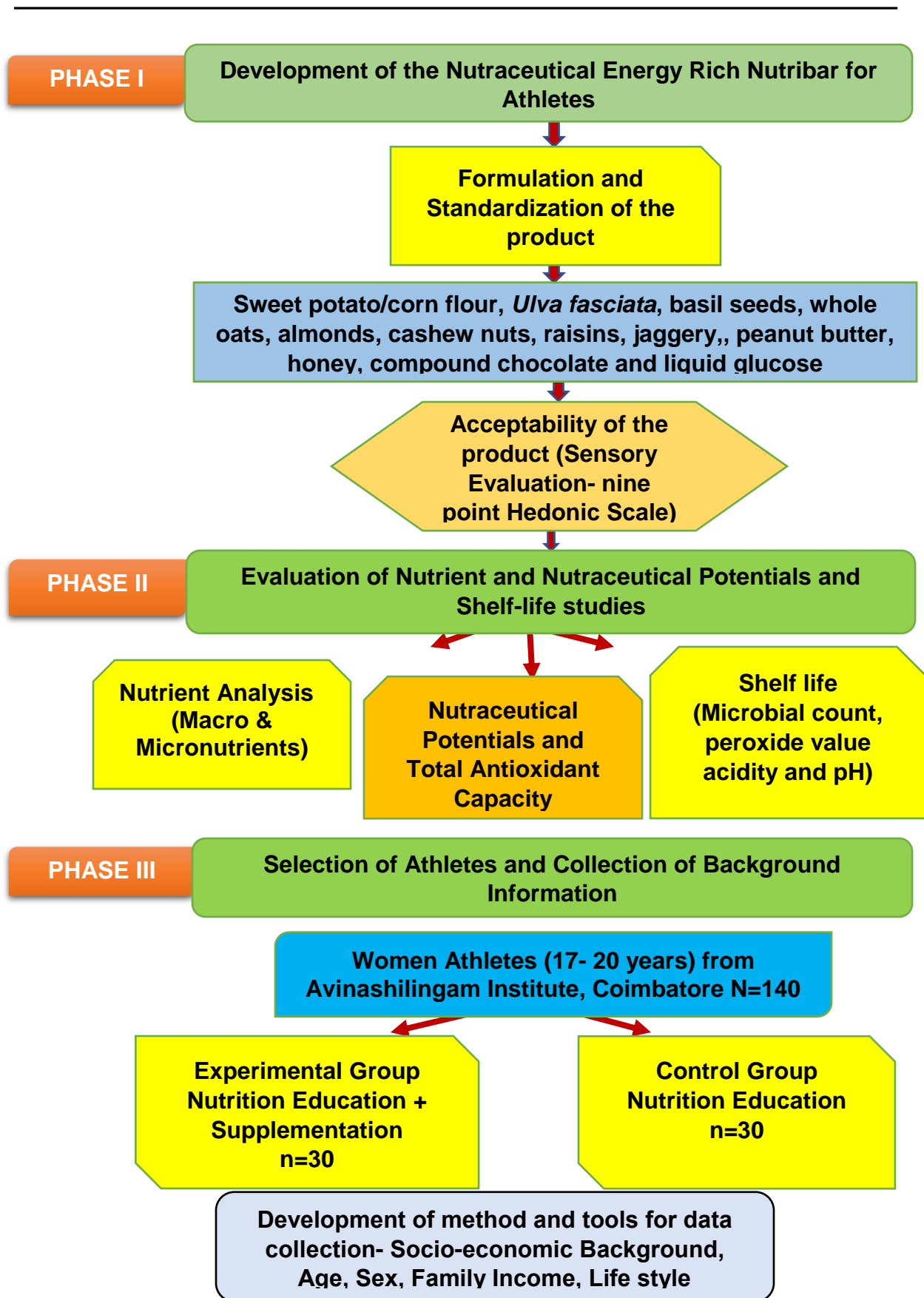
- 3.4.1 Assessment of Nutritional Anthropometry
- 3.4.2 Body Composition Analysis
- 3.4.3 Assessment of Biochemical Parameters
- 3.4.4 Clinical Examination
- 3.4.5 Dietary Survey
- 3.4.6 Assessment of Physical Performance

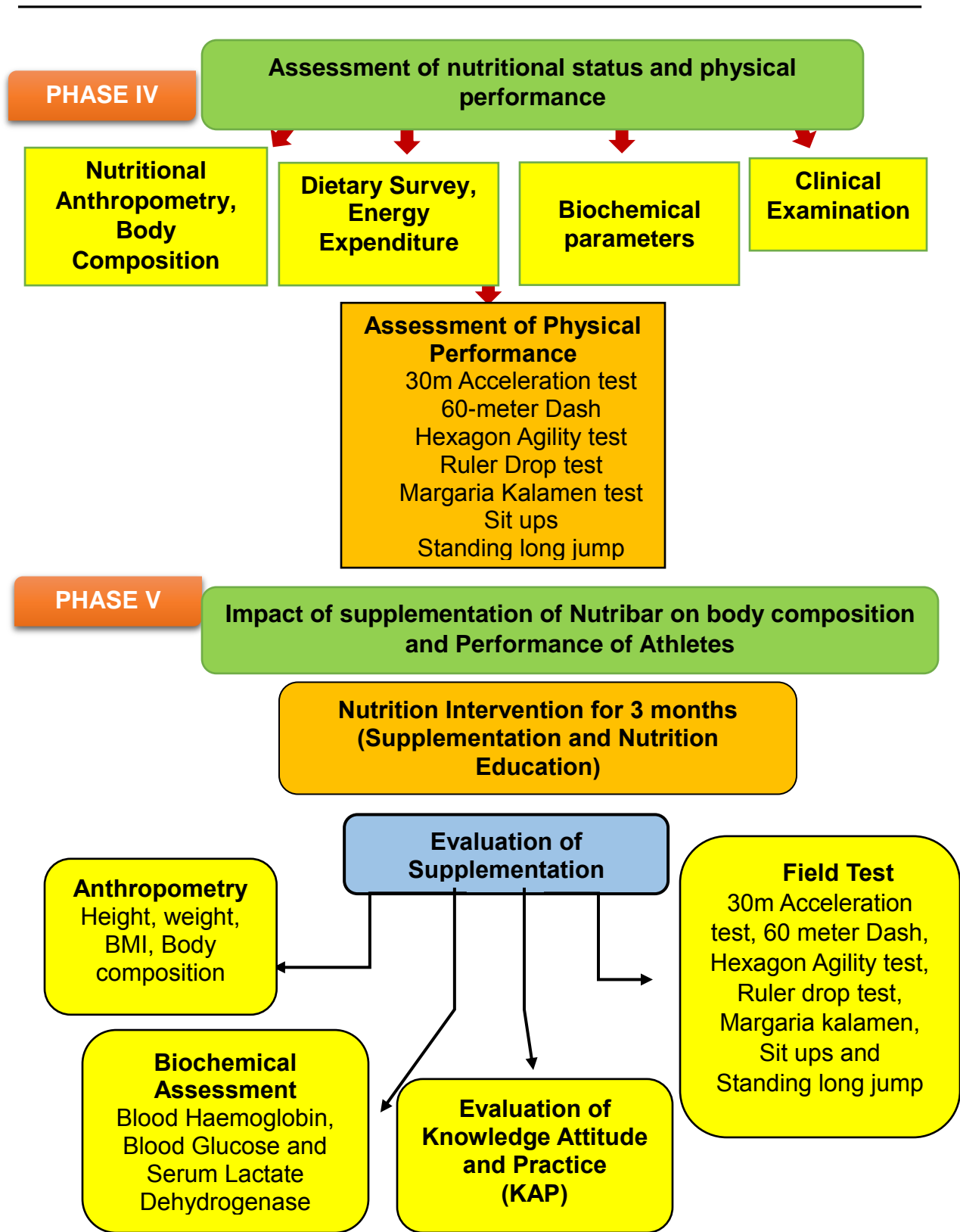
### **PHASE V**

### **3.5 IMPACT OF SUPPLEMENTATION OF NUTRIBAR ON BODY COMPOSITION AND PERFORMANCE OF ATHLETES**

- 3.5.1 Supplementation of NutriBar to selected Athletes
- 3.5.2 Formulation of Nutrition Education Material, Imparting Nutrition Education and Impact Evaluation
- 3.5.3 Evaluation of the Effect of Supplementation on the sprint performance
- 3.5.4 Validation and Interpretation of Data

The Research Design of the Methodology followed for the study is given in Figure 2.





**Figure 2**  
**Research Design**

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The research study was approved by the Institutional Human Ethical Committee of the Avinashilingam Institute (AUW/IHEC/FSN- 19-20/XPD-29) (Appendix I). The clinical trial is registered in the Clinical Trial Registry of India (CTRI) of ICMR and the trial registration number was obtained (CTRI/2021/08/035705) (Appendix II). The investigator took permission from the authorities of the selected Institute (Appendix III).

An information sheet clearly explaining the objective, nature, and duration of the study was prepared in both English and Tamil and was distributed to all the participants. Oral, as well as written consent was obtained from all the participants (Appendix IV). The subjects were instructed to follow the procedures as per the protocol of the project. It explained that no incentives or monetary benefits will be provided for participating in the study. The subjects were given full freedom to withdraw at any point in time. The respondents were given confidentiality assurance about the personal data collected.

## **PHASE I**

### **3.1 DEVELOPMENT OF THE NUTRACEUTICAL ENERGY-RICH NUTRIBAR FOR ATHLETES**

#### **3.1.1 Procurement and processing of raw materials**

The main ingredients in the NutriBar formulation included sweet potato (*Ipomoea batatas*), *Ulva fasciata* (green seaweed), and basil seeds (*Ocimum basilicum* seeds). The corn flour was used as the binding agent for the standard product. Several studies have revealed that Potato starch is a great replacement for cornstarch because it tastes bland and is gluten-free. Hence the variations were developed by the addition of sweet potato. The NutriBar also contained whole oats (*Avena sativa*), almonds (*Prunus dulcis*), cashew nuts (*Anacardium occidentale*), raisins, nattu sakkarai (jaggery powder), peanut butter (*Arachis hypogaea*), honey (*Apis*), compound chocolate and liquid glucose (glucose syrup). Glucose syrup, popularly known as confectioner's glucose, is a syrup formed from the hydrolysis of starch. It is used as a thickening, sweetener, and humectant in commercially

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prepared food items. Glucose syrup is also used in the manufacture of a variety of sweet treats.

The ingredients for the preparation of the Nutribar were obtained from the local supermarket, Coimbatore. *Ulva fasciata* seaweed was collected from the sea coast Gulf of Mannar and Palk Bay of Mandapam, Ramanathapuram District. The ingredients were cleaned using water to remove any debris and stones. Sweet potatoes were washed, peeled, grated, and lined in a clean tray with uniform thickness. A cabinet tray drier was used for drying. Drier was pre-heated before the drying process. The tray drier was set initially at 120°C and sequentially temperature was reduced to 60°C and dried within 8 hours. The cleaned *Ulva fasciata* was placed in a Cabinet tray drier which was pre-heated before the drying



process. The tray drier was set initially at 60°C and sequentially temperature was reduced to 50°C the ingredients dried within 4 hours to obtain powdered form. In a food processor, roasted peanuts were ground into peanut butter. Turn on the food processor and process for 4-5 minutes, or until you have a smooth, creamy "liquid" peanut butter. Basil seeds, cashew nuts, oats, and almonds were all roasted individually in a small frying pan over low heat until the raw scent was and a golden brown colour emerged. It was important not to overcook the components. In a mechanical pulverised mill, the roasted components were crushed into a powder individually. It was then distributed uniformly on a clean sheet to allow for rapid heat emission and brought to room temperature. To maintain shelf life and prevent product quality deterioration, the ground components were kept in zip lock bags and placed in airtight containers. The major components

used in the Nutribar's production are listed below.

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Sweet potato also known as *Ipomoea batatas* L. Lamarck syn. *Convolvulus batatas* L. is a prominent leguminous crop which has a widespread application & economic value in the worldwide market. Sweet potato is a tropical American native. In tropical and sub-tropical regions like as Africa, China, and India, it is a significant tuber crop. The primary producers in India include Andhra Pradesh, Assam, Bihar, Tamil Nadu, and Orissa. For the present study, a pink variety of sweet potatoes was selected due to its availability for athletes, sweet potato is a great option after a long training session. The body's glycogen levels diminish after intensive training. Glycogen levels can be restored with sweet potatoes. Athletes enjoy this superfood, which offers slow-digesting carbohydrates to keep them full, just like oatmeal. This superfood also contains a lot of potassium, which helps to avoid muscle fatigue. Since it contains dietary fibre with functional qualities such as pectin, cellulose, hemicellulose, and lignin, sweet potato ranks seventh among crops in terms of the prevalence of which uses in the nutritive diet. Furthermore, sweet potatoes are high in biologically active chemicals such as flavonoids, phenolic compounds, and carotenoids, all of which are antioxidants.



***U. fasciata* powder**

When compared to higher plants, all marine algae are rich in nutrients and new components (Fleurence, 1999). Seaweeds' nutritional value derives mostly from their polysaccharide, protein, micronutrient content. Seaweeds are a good source of dietary fibre because their cell walls contain a lot of non-

*Ulva fasciata* green seaweed was collected from Ramanathapuram District's sea shore, including the Gulf of Mannar and Palk Bay. The sea coasts of the Gulf of Mannar and Palk Bay in Ramanathapuram District have abundant marine algal growth. Scientists from the Central Marine Fisheries Research Institute (CFMRI) in Mandapam, Ramanathapuram District, verified the seaweed.



***Ulva fasciata***

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digestible polysaccharides (Rupérez and Saura-Calixto, 2001). Seaweeds have been used in enormous quantities as food in Asia since ancient times, although they are largely utilised as food additives in the western world (Glickman, 1987). However, there has been an increasing interest in edible seaweeds throughout Europe and Latin America (Acleto, 1996; Aguilera-Morales *et al.*, 2005).



Basil seeds (*Ocimum basilicum* seeds), also known as Sabja seeds, falooda seeds, or tukmaria seeds, are nutrient-dense superfoods with immunity-boosting effects. Basil seeds have the power to promote digestive health, aid in weight loss, control blood sugar, cool the body, relieve stress, reduce inflammation, and prevent certain infections. Various studies have found that the dietary fibre found in sabja seeds aids with blood sugar regulation. Sabja seeds are an anti-inflammatory substance that aids in the alleviation of pains, aches, and swelling. These seeds have been used in Ayurveda to treat arthritic swelling. Sabja seeds are said to have a relaxing impact on the psyche, reducing stress and anxiety. Basil seeds are high in antioxidants, which aid to protect against free radical damage, while also protecting against free radical damage. Basil seeds may also contain significant amounts of iron and calcium (Melina *et al.*, 2016).

Several organisations and authorities define functional foods in different ways. According to The International Food Technologists Expert Panel, Functional Foods Opportunities, and Challenges, “functional foods” foods are the dietary components which give health benefits in addition to daily nutrition. The ingredients chosen for the present study are known to deliver essential nutrients to extents that are often more than those required for balance growth, and development, as well as other biologically active substances that have health or physiological effects that are

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sought. As a result, Nutribar was made by combining the above ingredients in various proportions.

### **3.1.2 Formulation and Standardisation of Nutribar**

The complete composition and the process of preparation of Nutribar are not revealed since the product has been applied for patent (under examination) and published (202141013795A/28/03/2021), at Indian Patent Office, Chennai (Appendix V). The Bar was formulated using nutrient-dense ingredients in various proportions and the best product was chosen for various studies. The product was standardized by trials in clean and sterile instrumentation within the food process laboratory. The Nutribars were prepared in three different variations along with the standard. The ingredients were mixed in varied proportions to achieve three variations apart from the standard preparation. Corn flour was used as the binding agent for the standard product whereas sweet potato flour was used in the variations with the addition of basil seed powder and seaweed powder in different percentages.

The ingredients used in the development of Nutribar are presented in the Table III

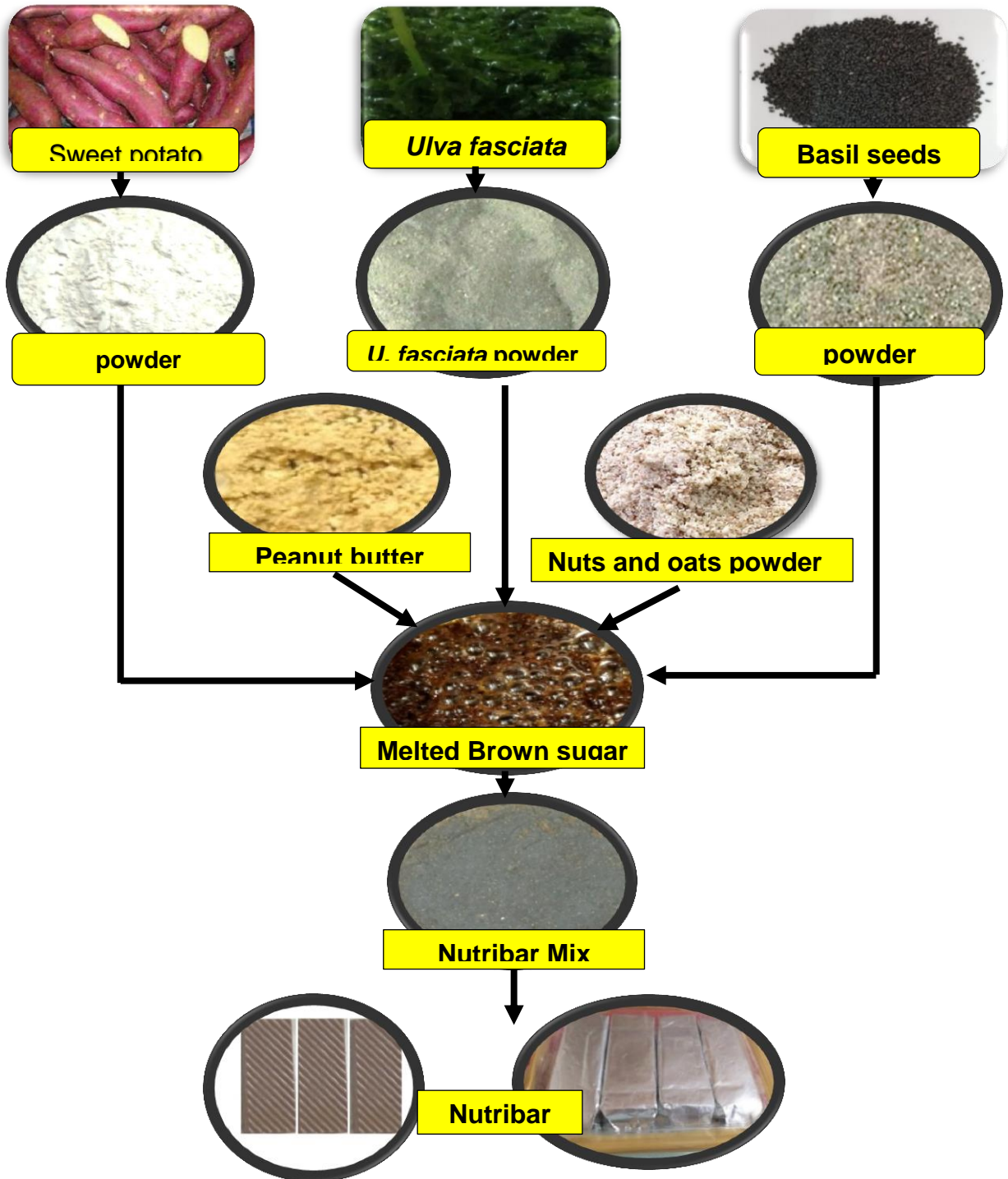
Figure 3 presents the steps involved in developing Nutraceutical energy rich Nutribar

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**Table III**

**Ingredients used for the development of Nutribar**

<b>Ingredients (g)</b>	<b>Standard</b>	<b>Variation I</b>	<b>Variation II</b>	<b>Variation III</b>
Corn flour	+	-	-	-
Sweet potato flour	-	+	+	+
<i>Ulva fasciata</i>	-	+	+	+
Basil Seeds powder	-	+	+	+
Jaggery powder	+	+	+	+
Peanut Butter	+	+	+	+
Liquid Glucose	+	+	+	+
Cashew nut	+	+	+	+
Almond powder	+	+	+	+
Raisins	+	+	+	+
Oats	+	+	+	+
Honey	+	+	+	+



Patent Filed and Published, Indian Patent Office, Chennai  
(202141013795A/28/03/2021)

Figure 3

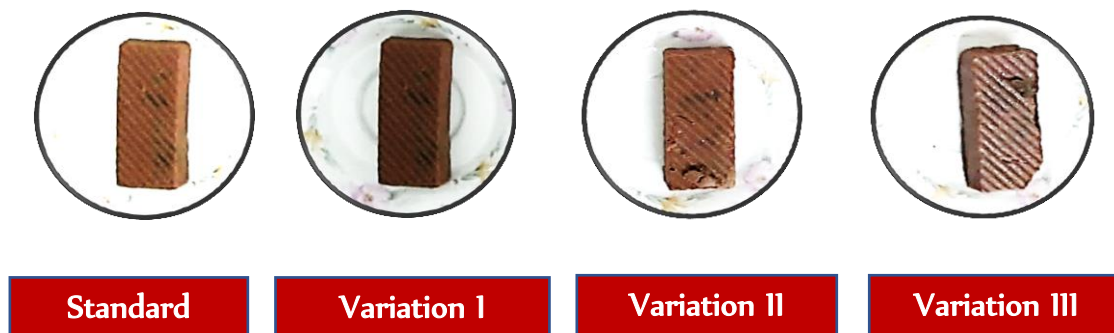
Development of the Nutribar

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### 3.1.3 Acceptability of the Product

The organoleptic study was carried out to determine the organoleptic properties of the developed product. The sensory examination was conducted with a total of 52 qualified panel members. The panellists were shown the newly prepared products. To eliminate prejudice in taste, the panellists were given a glass of water. They were asked not to consume any other highly flavoured meal for at least 1 hour before to the test in order to avoid taste interaction that might impact the evaluation choice. The variations were labeled as V1, V2, V3, and standard. The indecared quality parameters such as colour, taste, appearance, flavor, and overall acceptability were assessed using a 9-point hedonicscale. The nine- point hedonic scale ranging from 1= Dislike extreme and 9 = Like extreme (Lawless & Heymann, 2013). It is also known as the grade or taste scale. The components used, the proportions, and the cause of variation were not given to the panelists in order to reduce any taste expectation. All panelists were given separate sheets with instructions for evaluating the product. The variation with the highest overall score was considered the best-accepted variation and it was chosen for further analysis and supplementation.

Figure 4 represents the Standardisation of Nutribar



**Figure 4**  
**Standardisation of the Nutribar**

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## PHASE II

### 3.2 EVALUATION OF NUTRIENT AND NUTRACEUTICAL POTENTIALS AND SHELF-LIFE STUDIES

#### 3.2.1 Macronutrients and Micronutrients analysis

The highly accepted variation based on sensory evaluation was further subjected to nutrient analysis. Proximate analyses for example energy, protein, fat, CHO, and fibre were analysed. Carbohydrate was analysed by the Anthrone method (Hedge and Hofreiter, 1962), The protein content of the food was analyzed by estimating organic nitrogen by the Kjeldahl method. Fat was analysed by the Soxhlet method (Sadasivam and Manikam, 1996). The micronutrients namely beta carotene, zinc, magnesium, calcium, iron, moisture, and total Ash were also analyzed by the standard AOAC Official Methods (Raghuramulu *et al.*, 2003) (Appendix VI).

#### 3.2.2 Evaluation for nutraceutical potentials and total antioxidant capacity

Phytochemicals such as flavonoids, alkaloids, terpenoids, glycosides, tannins, phenolic, and saponins were analyzed by both qualitative and quantitative methods. The tests were done in sterilized environments in triplicates. Total Antioxidant Capacity [TAC] calculation is a recognized procedure of simultaneous measurement of different elements of the antioxidant defense system. The total antioxidant capacity of the Methanol Extract of the sample was determined by the AOAC official method according to the procedure (Appendix VI).

#### 3.2.3 Shelf life analysis of the developed nutribar

The pH, acidity, peroxide value, Total Bacterial Count (TBC), and total fungal count of the Nutribar stored from the 0-7th, 14th, 21st, and 28th days of storage studies for the Standard and developed product at room temperature as well as at refrigerator temperature (4° C) was determine to know the shelf life of the product. The shelf life was analyzed by the Standard AOAC method. TBC was analyzed by the Standard operating procedure for the enumeration of micro-organisms, by

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Colony Count Technique at 35°C (TBC)IS 5402:2012/ISO 4833:2003, BAM, DGHS Manual (Appendix VI).

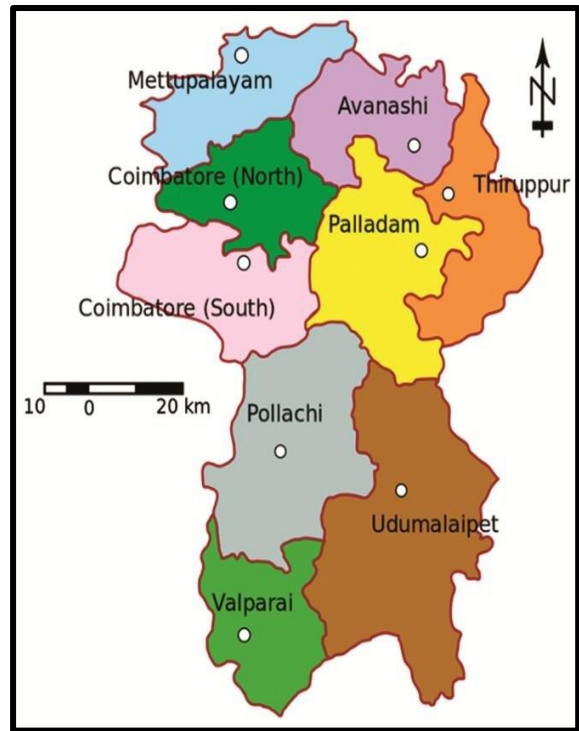
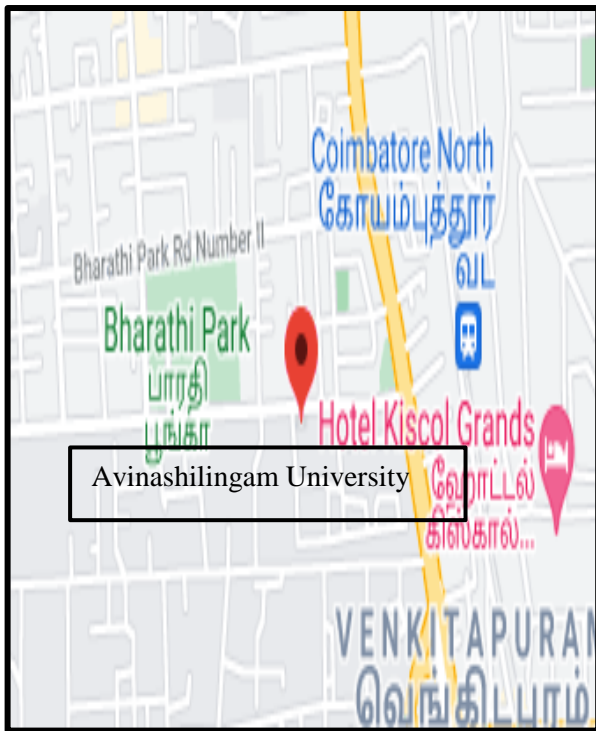
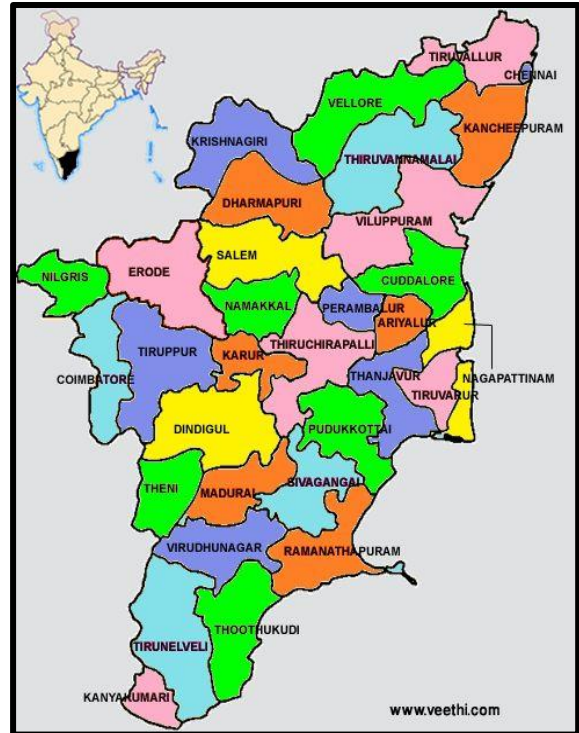
### **PHASE III**

## **3.3 SELECTION OF ATHLETES AND COLLECTION OF BACKGROUND INFORMATION**

### **3.3.1 Selection of Area and Athletes**

The research was carried out at the Avinashilingam Institute for Home Science and Higher Education for Women in Coimbatore, Tamil Nadu, India. With a metropolitan population of over two million, Coimbatore is the 2<sup>nd</sup> biggest city in Tamil Nadu and the fifteenth largest urban group in India. It is a prominent commercial town and has been labeled the "Manchester of South India" on several occasions. Coimbatore is known for its sports and recreational activities. The Avinashilingam Institute for Home Science and Higher Education for Women has grown to become one of the country's major institutes for providing quality education to women at all levels. Avinashilingam Institute promotes female sport and provides an opportunity for inspiring and engaging women and girls with the vision of sport that can change the world with active and winning women. Avinashilingam Institute has 16.8 acres of Sports grounds that include indoor and outdoor sports. Students are active members of cultural and sports committees at both institutional and University levels and are encouraged to participate in intra and inter-institutional sports competitions and cultural activities. All women will have more possibilities to participate in sports and physical leisure as a result of this provision, which will improve their health, well-being, and quality of life. Avinashilingam University teams include basketball, Handball, volleyball, football, Badminton, Table Tennis, Hockey, Kabaddi, Kho-Kho, Chess, Yoga, Mallakhamb, Silambam, Taekwondo, fencing, Judo, cross-country and track, and field like sprints, middle and long distance events .

The Coimbatore map of the selected areas is shown in Figure 5



**Figure 5**  
**Coimbatore District Map and Areas Selected for the Study**

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A randomised, controlled trial procedure was used to choose the sprinters. A Randomized Controlled Trial (RCT) is an experimental type of impact evaluation in which the population receiving the programme or policy intervention was randomly selected from the eligible population, and a control group was also randomly selected from the same eligible population. (White *et al.*, 2014). The study was ethically permitted by the Institutional Human Ethical Committee of the Avinashilingam University (AUW/IHEC/FSN- 19-20/XPD-29) (Appendix I). The clinical trial was also registered in the Clinical Trial Registry of India (CTRI) of ICMR and the trial registration number was obtained (CTRI/2021/08/035705) (Appendix II).

Total of 140 women sprinters constituted within the age group of 17-20 years from Avinashilingam Institute for Home Science and Higher Education who are participating in the sprinting competition were selected. Among the 140 sprinters, 60 sprinters were identified based on the inclusion and exclusion criteria for the supplementation study. Out of 60 subjects, one sprinter had discontinued during the study due to covid 19. Hence, the experimental group constituted 29 subjects and control group constituted of 30 subjects.

Socio-economic status, clinical status, and dietary survey (24-hour food recall) were determined for all the sprinters, and nutritional anthropometry and physical fitness level and biochemical parameters were assessed on 60 willing sprinters before the supplementation and 59 sprinters after the supplementation.

### **3.3.1.1 Selection of Athletes for Nutrition Intervention**

Fifty-nine sprinters who were involved in regular practice and were willing to take part were included into the supplementation studies. After that, the athletes were divided into two groups.; the experimental group (Supplementation and Nutrition education) and the control group (Nutrition education).

#### **Inclusion Criteria:**

- All Young Adult women Sprinters both indigenous and non-indigenous

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- Age <17 to 20 years
  - Both married and unmarried women
  - Willing to participate in the study

**Exclusion Criteria:**

- Subjects who were below 17 years and above 20 years
- Pregnant and lactating subjects
- Subjects who could not comply with the intervention more than 3 days a week
- Subjects enrolled in other clinical trials
- Subjects with complications of metabolic disorders

**Sample size calculation:** To Calculate the Sample Size without population and prevalence rate

Assuming Standard deviation of the population to be 0.05 and  $z = 1.645$  (90% confidence) and error = 5% we get,

$$N = \frac{z^2 \times SD^2 (1-D)}{e^2}$$

The total sample size calculated will be,  $N = 140$

It was very difficult to get the appropriate or large sample size for supplementation since the study was conducted during COVID 19 pandemic. Therefore, only 60 willing sprinters constituted the subjects for the supplementation study.

### **3.3.2 Nutrition Education**

Nutrition education was provided to all 59 athletes included in the study. The nutrition education questionnaire formulated was administered to the sprinters and evaluated to understand their initial nutrition knowledge. Based on their initial nutrition knowledge, education materials were prepared.

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Nutrition education was given online to all the sprinters using PowerPoint presentations, modules, and discussions by various nutrition education materials developed by referring Nutrition and Hydration in Sports Guidelines for the Excellence, ILSI, NIN, SAI, 2007; (Duvillard *et al.*,2004; Mcardle,1996).

### **3.3.2.1 Development of Methods and Tools for Data Collection**

Scheduled questionnaires are particularly useful in getting the correct information because the investigator can explain the questions thoroughly to the respondent through face-to-face contact and clarify doubts (if any) raised by the respondent in the course of collecting information (Kothari, 2005). Since the study was conducted during COVID 19 Pandemic, socio economic background, age, sex, family income, and lifestyle were collected from the subject by questionnaire method through google forms. Family medical history, details on ailments/morbidity, and dietary patterns were also collected from selected sprinters.

A questionnaire was formulated to assess the Socio-Economic Status (SES) of the sprinters which included detailed information on aspects such as income, type of family, and occupation (Appendix VII).

A separate questionnaire was modified and structured to measure the nutritional status. The aspects of nutritional status assessment included anthropometry, biochemical, clinical assessment, medical history, dietary pattern, and lifestyle pattern questions. Dietary intake was monitored by the 24-hour recall proforma developed by Sathyanarayana *et al* (1989).

The nutrition education questionnaire was formulated by referring to Nutrition and Hydration Guidelines for Excellence in Sports, ISLI, NIN, and SAI (2007) on aspects regarding basic nutrition and sports nutrition Knowledge, Attitude, and Practice (Appendix VIII).

### **3.3.3 Assessment of Socio economic Status**

The socio economic status of all the 140 athletes was evaluated in terms of age, sex, type of family, religion, area of residence, family income, and occupation using the google forms questionnaire to assess socio-economic status.

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## PHASE IV

### 3.4 ASSESSMENT OF NUTRITIONAL STATUS AND PHYSICAL PERFORMANCE

#### 3.4.1 Assessment of nutritional status

The study of variations in physical dimensions and gross composition of the human body at various ages and levels of nutrition is known as nutritional anthropometry (Madden, 2016). Diet and nutrition significantly impact the pattern of growth and the body's physical state. As a result, anthropometric measures may be used to determine nutritional status.

#### 3.4.2 Anthropometric parameters

Height and weight; skinfold thickness such as biceps, triceps, subscapular, supra iliac; Body circumference – chest, waist, hip were measured based on Sport Kinanthropometric Assessment Guidelines for Athletes (<http://homepages.ihug.co.nz/~rip/Anthropometry/>). The anthropometric sites and descriptions provided by Ross and Jones (1991) and the standard specifications of the International Society for the Advancement of Kinanthropometry (ISAK) were taken into consideration for the measurement of anthropometry using the appropriate equipment and standard procedures.

**The instrument used in the measurement:** The FLOVEIN Skin Fold Caliper (India) is used for the measurement of skinfold thickness. While the Tanita WB-3000 Digital Physician's Model 660 lbs. was used to determine the anthropometric measurements.

**Technical error measurement:** Each anthropometric parameter was measured thrice and the average of the three measurements was calculated and taken as the mean value. The instruments were calibrated with the known weight each day before taking the measurements.

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### **3.4.2.1 Standing Height**

A person's height is controlled by both hereditary and environmental factors. Only long-term deprivation affects height. It's being used as a measurement of chronic or long-term deprivation (Srilakshmi, 2010).

Standing height was measured using the MCP Healthcare Stature Meter wall mount height measurement scale for adult 2M/200CM Roll Ruler and Tanita's 660 pound capacity computerised physician's model WB-3000 made in Japan. The sprinters were made to stand erect on the stadiometer platform, without shoes, facing directly forward, feet together, and arms hanging by their sides. The measurement was obtained as the maximum vertical distance between the stadiometer's platform and the vertex of the head, which was firmly touched by the stadiometer's movable head pointer and read from the calibrated scale. (Plate 1).

### **3.4.2.2 Body weight**

The greatest extensively utilised and consistent anthropometric parameter for assessing nutritional status is body weight. It is made up of all of the body's elements. such as minerals, water, bone, protein, and so on (Bamji *et al.*, 2004).

For the present study, body weight was measured by using Inbody 720 Body Composition Analyser made in Seoul, Korea. The athletes were made to wear minimal clothing and should not hold any other object for support and without footwear while standing on the platform of the analyser. The body weight of the subject was automatically measured when the athletes stand on the footplate of the analyzer (Plate 2).

### **3.4.2.3 Skinfold Measurement**

FLOVEIN Skin Fold Caliper, made in India.” was used to measure the skinfold thickness of the sprinters.

#### **3.4.2.3.1 Biceps skinfold**

The biceps skinfold was calculated by measuring the distance between the inferior border of the Acromion process and the external superior border of the head of the radius in the centre of the upper arm.

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#### **3.4.2.3.2 Triceps**

The sprinters were instructed to stand erect with their hands by their sides. The skinfold was taken from the right arm's triceps muscles, Parallel to the olecranon process, midway between the acromion process and the radius's superior edge The fold was aligned with the length of the arm.

#### **3.4.2.3.3 Supra iliac**

The sprinters were made to stand, down at the sides, arms down, the skin folds right above the line crest on the side of the waist was grasped. The spring handle was entirely released after the caliper was fitted. When the scale stopped drifting, the skinfold thickness was measured to the nearest 1mm.

#### **3.4.2.3.4 Subscapular**

The fat fold was measured by lifting it up with the thumb and fingers along a line running about 45° to the spine, Right below and lateral to the angle of the left scapula, in the natural line of skin cleavage.

Athlete was instructed to stand with her arms down at his sides, and relaxed. When the scale's drift stopped, The crease at the lower part of the shoulder blade, where the skinfold was grasped, is around a 45-degree angle. The skinfold thickness was measured to the closest 1 mm once the spring handle was fully released. (Plate 3).



**Plate 1. Height Measurement**



**Plate 2. Weight Measurement**



**Plate 3. Skinfold Thickness**

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### **3.4.2.4 Circumference**

#### **3.4.2.4.1 Chest circumference**

The sprinter gently abducted her arms to allow the anthropometrist to wrap the tape around the subject's chest while the anthropometrist's left hand adjusted the tape at the athlete's back to the horizontal level of the specified mesosternale. The tape scale was aligned with the zero on the stub end of the tape using the cross-handed technique. At the end of a regular expiration, the reading was taken. (Plate 4).

#### **3.4.2.4.2 Waist circumference**

The sprinter instructed to stand straight. The appraiser used a cross-handed technique to position a non-stretchable fiberglass tape horizontally at the level of the noticeable waist narrowing. The tape was then placed in the recording position and the measurement was made (to the nearest millimeter) at the end of the normal expiration (Plate 5).

#### **3.4.2.4.3 Hip circumference**

The maximum circumference of the hips, including the maximum extension of the buttocks, was measured posteriorly in the horizontal plane. Sprinters was made to stand erect in front of the investigator with feet together, the tape was positioned around the hip, at the level of the symphysis pubis and the greatest gluteal protuberance, and the circumference was measured using fiberglass tape upto 0.1 accuracies at the fullest part of the hip (maximum posterior of the extension between the iliac crest and buttocks) keeping the tape parallel to the floor and recorded (Plate 6).



**Plate 4. Chest circumference**



**Plate 5. Hip circumference**



**Plate 6. Waist circumference**

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### **3.4.3 Assessment of Body Composition**

Body composition was measured using the 4-Compartment model analyser InBody720 (South Korea) whereby there are four separate elements namely the total body water, protein, minerals, and body fat. Cellular membranes split total body water into intracellular and extracellular water. The machine was able to provide various body compositional constituents.

The technique of bioelectrical impedance analysis (BIA) is commonly utilised for determining body composition. The technology is easy to use, quick to implement, and non-invasive. BIA measures the resistance of body tissues to the flow of a very small (less than 1 mA) alternating current. Impedance is made up of tissue resistance as well as additional opposition (reactance) caused by membrane capacitance, tissue interfaces, and non-ionic tissues. The resistance recorded is roughly similar to muscle tissue resistance (Plate 7).

#### **3.4.3.1 Intracellular water (ICW), Extracellular water (ECW), Total Body Water (TBW)**

Total Body Water (TBW) is measured using a multi-frequency approach that divides it into two categories: ICW and ECW. The amount of water within the cellular membrane is referred to as intracellular water (ICW). The total amount of water in the blood and interstitial fluid is referred to as extracellular water (ECW). The proportion of ICW and ECW in a healthy body should be around 3:2.

#### **3.4.3.2 Body Fat Mass**

Body fat mass determines the total lipids taken from other cells and fat. Under the skin, as well as between the abdominal muscles and the muscles themselves, body fat is stored. When body fat mass exceeds the usual range, obesity is identified.

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#### **3.4.3.3 Soft Lean Mass**

By deducting the mineral contained the bones from Fat Free Mass, Soft Lean Mass can be computed.

#### **3.4.3.4 Fat Free Mass**

After Body Fat Mass is subtracted from body weight, Fat Free Mass is the weight of the remaining components.

#### **3.4.3.5 Skeletal Muscle Mass(kg)**

For an examinee's regular weight, the recommended amount of Skeletal Muscle Mass is 100 percent. Muscles are divided into three categories: visceral muscle, heart muscle, and skeletal muscle. The amount of skeletal muscle, on other hand, alters as a result of the activity. As a result, InBody720 distinguishes between SMM and Soft Lean Mass. By comparing the % of Body Fat Mass and Skeletal Muscle Mass found in each bodily component, the amount of obesity can be determined in a more proactive and precise manner.

#### **3.4.3.6 Body Fat Mass(kg)**

The percentage of body fat should keep maintained at her normal weight is 100 percent standard Body Fat Mass. In general, males should have a Body Fat Mass of 15% and females should have a Body Fat Mass of 23%.

#### **3.4.3.7 Percent Body Fat (%)**

The ratio of body fat to total body weight is expressed in % body fat. Percent Body Fat (%) = Body Fat Mass ( kg ) / Body Weight ( kg ) × hundred, Males have a normal percent body fat of 15% and females have a standard percent body fat of 23%, whereas males have a standard range of 10%-20% of Percent Body Fat and ladies have a standard range of 18-28 percent of Percent Body Fat

#### **3.4.3.8 Waist-Hip Ratio**

It's a good way to Figure out where your body fat is distributed. However, it results in inconvenient body measurements and inaccuracy, as seen by differences in measurements recorded by different measurers. As a result, measuring it with measuring tapes for obesity treatment is difficult.

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INBody720 calculates a systematic estimate WHR of the subject using impedance index. The assessment of the abdominal fat ratio by InBody720 can be a helpful technique for treating obesity due to its high degree of reliability and accuracy. An adult diagnosed with abdominal obesity has excessive visceral fat mass as a result of elevated blood levels of free fatty acids compared to subcutaneous fat, which leads to heart disease, hypertension, diabetes, and other clinical conditions. Standard waist to The hip ratio for women is 0.8, whereas it is 1.0 for men. Formula for calculating WHR is as follows:

$$\text{WHR} = \frac{\text{Waist Circumference (cm)}}{\text{Hip Circumference (cm)}}$$

#### **3.4.3.9 BMR (Basal Metabolic Rate)**

Basal Metabolic Rate (BMR) specifies the minimum amount of energy essential to maintaining key functioning while at rest. BMR can be calculated using InBody720 by means of a FFM-based regression equation, which is well-known. FFM is quite well for having a close relationship with BMR. The most common method for calculating BMR is indirect calorimetry, which relies on oxygen demand. InBody720, on the other hand, calculates BMR using Fat-Free Mass as follows:

$$\text{REE} = 21.6 \text{ FFM ( kg )} + 370 \text{ ( FFM=Fat Free Mass, kg )}$$

#### **3.4.3.10 Precautions**

- The test was carried out prior to a meal. The test was administered two hours after the last meal in circumstances where the examinee had already eaten. This is due to the fact that the weight of the meal is based on its mass. which might lead to measuring inaccuracies.
- To avoid biological mistakes, the volunteers were told to empty their bladders before being measured.
- Volunteers were told to stay away from vigorous exercise because it can lead to momentary changes in body composition.

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- Because body water tends to increase during menstruation, measurements of female volunteers were not taken during this time.
  - The test was carried out at a temperature of 20<sup>0</sup>C–25 °C. The body composition may change in hot or cold weather.



**Plate 7. Body composition**

**Plate 1-7 Anthropometric measurements of selected sprinters**

**3.4.4 Assessment of Biochemical Parameters**

Parameters such as blood hemoglobin, random blood glucose, and serum Lactose Dehydrogenase (LDH) were estimated on 60 willing Sprinters before the conduct of the intervention study and for 59 Sprinters after the intervention study. Blood hemoglobin was estimated using the cyanmethaemoglobin method (Raghuramulu *et al.*, 2003). Lactose Dehydrogenase (LDH) was assessed by UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) (Young *et al.*, 2001). Blood glucose was measured by the Glucose kit (GOD-POD) method. The procedure is enclosed (Appendix IX). For the

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collection of blood samples, the subjects were instructed to assemble at the Institute by strictly abiding by COVID 19 protocols. Blood (3.0ml) was collected by a trained lab technician separately for each analysis in respective tubes. (Plate 8).



**Plate 8. Collection of Blood**

### **3.4.5 Clinical Examination**

Clinical examination adds an important component to the entire evaluation of the person (Robert and William, 2000). Clinical examination was performed in this study by examining the sprinters from head to foot for signs and symptoms using a clinical assessment proforma and asking the sprinters if they felt any signs of dietary inadequacy using classification of clinical signs and symptoms (Srilakshmi, 2010). The prevalence of diseases in families and athlete morbidity (in the previous 6 months) were investigated by the Physician and reported.

### **3.4.6 Dietary survey**

Dietary assessment is a set of methods for measuring and/or estimating food intake, which can then be converted to nutritional intake using food consumption tables (Dwyer, 2000). Assessment of dietary intake was carried out using a 24-hour food recall outlined by Kapil *et al.*, (2003). Food and nutrient consumption were compared using the ISLI, NIN, and SAI (2007) proposed food allowances for the specific event, as well as the ICMR guidelines (2020).

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### 3.4.7 Estimation of energy expenditure

Three key elements contribute to overall energy expenditure (number of calories required per day):

1. Resting metabolic rate or basal metabolic rate
2. Food's thermogenic impact
3. Activities of daily living (ADL) - Physical activity

The product of the basal metabolic rate (BMR) and the level of physical activity is the total daily energy expenditure (TEE) (PAL). The measurement of the TEE has added objectivity to the estimation of the energy needs of groups of people, especially when energy expenditure, rather than energy intake, was used to quantify energy requirements given by FAO/WHO/UNU, 1985, (Alfonzo *et al.*, 2004). The total energy expenditure of the sprinters was determined using the energy expenditure pattern calculated using a physical activity questionnaire (William, 2005). Each subject was asked to recall her physical activity for 24 hours before the measurement. In physical activity proforma, 24 hours have been divided into 48 slots of 30 min each. Time spent on various physical activities by the subjects was graded into various intensity zones namely light, moderate and heavy activity. For the whole day, the activities were summed up in each category of light, moderate and heavy. The energy cost of participating in the activity was calculated by multiplying the amount of time spent working out by the calorific value per minute. (Appendix X).

**Table IV**  
**Physical activity factors**

Exercise Level	Details	Calorie Calculation (Daily Needs)
Little to no exercise		BMR x 1.2
Light exercise	1-3 days per week	BMR x 1.375
Moderate exercise	3-5 days per week	BMR x 1.55
Heavy exercise	6-7 days per week	BMR x 1.725
Very heavy exercise	twice per day, extra heavy workouts	BMR x 1.9

### 3.4.8 Assessment of physical performance

Physical fitness is defined as the capacity to undertake everyday tasks, leisure activities, and vocational tasks without getting overly tired. Components of physical fitness i.e. speed (30m Acceleration test, 60 meter Dash); explosive power of leg muscles (Margaria Kalamen test); muscular endurance (Sit-ups); Reaction Time (Ruler drop test); agility (Hexagon Agility test) and power test (Standing long jump) were measured using the standard procedure adopted from the methodologies for Fitness Assessment Field Test (Ray *et al.*, 2011) (Appendix XI) (Plates 9a to 9f)



**a. 60 meter Dash**



**b. 30m Acceleration test**



**c. Standing long jump**



**d. Hexagon Agility test**



**e. Sit- Ups**



**f. Margaria Kalamen test**

**Plate 9. Performance test**

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## PHASE V

### 3.5 IMPACT OF SUPPLEMENTATION OF NUTRIBAR ON BODY COMPOSITION AND PERFORMANCE OF SPINTERS

#### 3.5.1 Supplementation of Nutribar to selected Athletes

Since Aluminum foil and its composite materials have moisture resistance, gas barrier properties, and light-blocking properties. More conducive to the long-term preservation of chocolate, the Nutribar was manufactured and packed in paper-aluminum foil polyethylene laminate (PFP) 50g each. Owing to the current situation of the Covid19, the developed product was supplemented to the subjects by couriering to their particular Address. The sprinters in the experimental group were instructed to consume the Nutribar 10- 15 minutes before the practice thrice a week for 90 days. The sprinters have the training session thrice a week as part of their curriculum (Plate 10).



Plate 10. Preparation and packaging of Nutribar

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### 3.5.2 Formulation of Nutrition Education Material, Imparting Nutrition Education and Impact Evaluation

#### 3.5.2.1 Formulation of Nutrition Education Materials

All 59 sprinters were included in nutrition awareness program. The nutrition education questionnaire formulated was administered to the sprinters and evaluated to understand their initial nutrition knowledge. Based on their initial nutrition knowledge, education materials were prepared.

Nutrition education was given online to all the sprinters using PowerPoint presentations, modules, and discussions by various nutrition education materials developed by referring Nutrition and Hydration in Sports Guidelines for the Excellence, ILSI, NIN, SAI, 2007; (Duvillard *et al.*,2004; Mcardle,1996).

PowerPoint Presentation prepared by the researcher was projected to the sprinters as it is a way of attracting the audience towards the expression of their views and arguments. Powerpoint presentations mix audio and visual elements, making them easier to understand the concepts. By simply employing slides in lectures, even ordinary instruction or training becomes engaging. (Paul, 2008) (Figure 6).

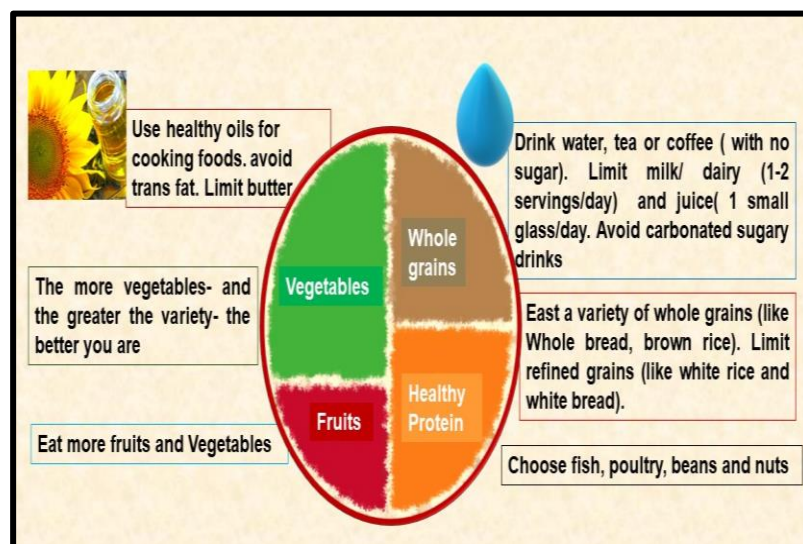


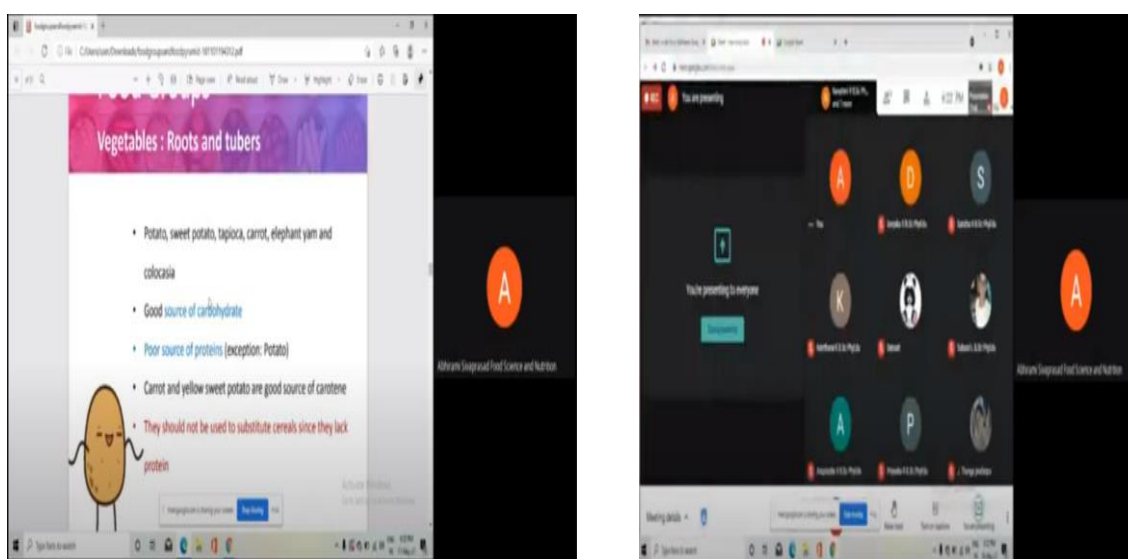
Figure 6

Material used for imparting nutrition education

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### 3.5.2.2 Imparting Nutrition Education

The investigator conducted the education program through online mode with the help of google meet. Selected sprinters were given an education program thrice a week, for 3 months to complete the education program for all the selected sprinters. Details on basic nutrition such as food groups, food pyramid, nutrient content of different foods, balanced diet, pre, during, and post-competition meals, do's and don'ts for sprinters, and women's hygiene was taught to the sprinters using PowerPoint (Plate 11).



**Plate 11. Nutrition education in progress**

### 3.5.2.3 Evaluation of the Impact of Nutrition Education

The influence of the nutrition education program was evaluated by administering the same nutrition education questionnaire which was provided initially to all the sprinters, again, and the improvement in nutrition knowledge, attitude, and practice (KAP) were assessed by comparing the information given by the sprinters in the proforma, before and after the nutrition education program.

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### **3.5.3 Evaluation of the Effect of Supplementation**

The impact of the intervention was evaluated based on the changes in anthropometric, biochemical, body composition, and physical fitness parameters after supplementation. Biochemical parameters namely hemoglobin, blood glucose, serum lactate dehydrogenase, and Physical Fitness parameters namely, speed; explosive power of leg muscles; muscular endurance; Reaction Time; agility, and power test were assessed before and after the intervention. The final scores obtained were compared with the initial scores and the impact of supplementation was assessed.

### **3.5.4 Validation and Interpretation of Data**

All the data were scrutinized by SPSS v.26.0 for Windows (SPSS Inc., Chicago, IL). The significance level was set to the level of  $P < 0.05$  and  $P < 0.001$ . The following were the statistical tests applied in the different phases.

#### **Phase I**

- The descriptive statistics ie, mean and standard deviation were used to evaluate the mean sensory characteristics of food are appearance, colour, taste, flavor, texture, and overall acceptability.
- One way ANOVA to interpret intra-group variations; is to compare significant differences in the mean of the sensory scores of Nutribar prepared with varying proportions.
- Post – Hoc Duncan’s test - analysing the multiple comparisons of the mean sensory score attained by Nutribar with Different Levels.

#### **Phase II**

- Paired t-test was utilized to analyse the significance of total antioxidant capacity between standard and selected Nutribar.

#### **Phase IV**

- Descriptive statistics; mean and standard deviation were used to illustrate the essential distribution of the anthropometric and body composition parameters.

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- The descriptive statistics ie, mean and standard deviation were used to to illustrate the essential distribution of the Biochemical and Physical performance of the sprinters

### **Phase V**

- The paired t-test is used to assess the significance of the impact of supplementation using pre test and post test intervention scores and changes in Anthropometric parameters, Body composition, Biochemical parameters, and Physical performance.
- Karl Pearson's chi-square correlation analysis was applied to assess the association between the Nutrient intake and Biochemical parameters

#### **3.5.5 Validation of Data**

Height and weight measurements for assessing the anthropometry analysis for 60 subjects was recorded by the Investigator. Further the data on body composition on 60 sprinters from Avinashilingam University were done by the investigator. With the help of an assistant trained earlier in the technique, body composition measurements were taken for sprinters. Biochemical analysis was carried out twice for pre analysis and post analysis with the help of laboratory assistant. Education was given for a three month period. Since the researcher herself took anthropometric and body composition measurements in subjects, there was no probability of bias in data collection. Data obtained from body composition analyser was validated for body composition measurements by comparing body fat obtained from body fat analyser and using skin fold calipers with 5 per cent variability and using standardized procedures.