Methodology

METHODOLOGY

III

The methodology pertaining to the study entitled **'Development and Evaluation of Functional Food Mixes on Adults with Cardiovascular Diseases and Impact of Diet Counselling'** is presented under the following headings

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B. Formulation of Food Mixes
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Phase I

A. Selection of Functional Food Sources

Today the world appears to be increasingly interested in the health benefits of foods and have begun to look beyond their basic nutritional benefits to disease prevention and health enhancement. Traditional systems of medicine owe their significance to the bioactive components that have their origin in plant sources and most of them were associated with routine food habits (Ullah and Khan, 2008). Currently, both the American Heart Association Science Advisory Council and American College of Cardiology do not recommend the use of single antioxidant vitamin supplement for cardiovascular disease prevention. Instead they recommend the consumption of a diet high in antioxidants and other nutrients such as fruits, vegetables, whole grains and nuts to reduce the risk of cardiovascular diseases (Gibbons *et al*, 2003).

After an extensive appraisal of earlier research for a holistic approach in the treatment and immunity enhancement through dietary approach, six functional foods - wheat germ, green gram, drumstick leaves, carrot, amla, and flax seed were selected for the formulation of food mixes for supplementation. Studies on management of cardiovascular disease using plant based foods are very important and more convenient to motivate people to accept. Additionally, practical dietary advice based on health effects of specific foods or food groups is much easier for the public to understand and implement than arbitrary numerical criteria for macronutrients (Hu, 2003).

Wheat germ is the embryo of the wheat kernel, which is relatively rich in protein, fat and several of the B vitamins. Wheat germ is sodium and cholesterol free, rich in vitamin E, magnesium, pantothenic acid, phosphorus, thiamine, niacin and zinc. It is also a source of coenzyme Q10 (ubiquinone)

and para-aminobenzoic acid (Shewry, 2009). Wheat germ is also rich in fibre and contains approximately one gram of fibre per tablespoon and can be useful in regulating bowel function and may be recommended for patients at risk for colon disease, heart disease, and diabetes (Kumar *et al* 2011).

Feeding different levels of wheat germ significantly decreased the serum levels of total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol, GOT, GPT and ALP and significantly increased the serum HDL cholesterol levels which represented a significantly decreased atherogenic index as compared to the positive control group (Rezq and Mahmoud, 2011).

Legumes, especially green gram occupy an important place in human nutrition. Dietary fibre, which is a heterogeneous mixture of several types of polysaccharides, is rich in legumes, especially in their husk fractions and contributes to beneficial therapeutic health effects (Tharanathan and Mahadevamma, 2003). Green gram has a phenol content of 327.64 mg per 100 g which is known for its antioxidant activity that protects human beings against harmful effects of oxygen radicals that underline the pathogenesis of diseases like atherosclerosis and carcinogenesis (Sreeramulu *et al,* 2009). Chung *et al* (2010) found green gram extracts to have a potent scavenging activity against reactive species and an inhibitory effect on low density lipoprotein oxidation.

Nandave *et al* (2009) reported that drumstick (*M. Oleifera*) extract possessed significant cardioprotective effect, which may be attributed to its antioxidant, antiperoxidative and myocardial preservative properties. The high antioxidant/radical scavenging effects observed in drumstick leaves appear to provide justification for their widespread therapeutic use in traditional medicine in different continents (Atawodi *et al.,* 2010). According to Nambiar *et al* (2010) dehydrated drumstick leaves tablet supplementation to hyperlipidimic adults had an overall positive impact on the lipid profile, with significant reduction in the non-HDL values.

Carrots contain predominant phytochemicals such as anthocyanins, phenolic acids and carotenoids. These phytochemicals could be useful in the treatment of metabolic syndrome since anthocyanins improve dyslipidaemia, glucose tolerance, hypertension and insulin resistance, the phenolic acids may also protect against cardiovascular disease and β carotene may protect against oxidative processes (Poudyal *et al.,* 2010). Phenolic acid of carrot provides protection against lipid peroxidation damages (Singh *et al.,* 2009).

The antioxidant activity of amla extract is associated with the presence of hydrolyzable tannins having ascorbic acid-like action (Pozharitskaya *et al.*, 2007). Studies by Kim *et al* (2010) suggest that fructose induced metabolic syndrome is attenuated by the polyphenol rich fraction of amla and intake of amla extract improves high fructose diet induced metabolic syndrome including hyperglycemia, hyperlipidemia and hypertension.

Flax seed is a rich source of three components with demonstrated cardioprotective effects: omega-3 fatty acid (α-linolenic acid), dietary fibre, and phytoestrogen lignans. Multiple clinical dietary intervention trials report that consuming flax seed daily can modestly reduce circulating total cholesterol and low density lipoprotein cholesterol in normolipidimic and hypercholesterolemic patients, as well as lower various markers associated with atherosclerotic cardiovascular disease in humans (Bassett *et al*, 2009). Daleprane *et al* (2010), observed that flax seed has a cardioprotective effect by improving the blood lipid profile and endothelial function. According to Moghaddasi (2011), it is much easier to digest ground flax seed when compared to unground flax seed and the medicinal properties of flax seed are on a higher scale if it is ground.

With this background, wheat germ (*Triticum aestivum*), green gram (*Phasleolus aureus*), drumstick leaves (*Moringa oleifera*), carrot (*Daucus*

carota), amla (*Emblica officinalis)* and flax seed (*Linum usitatissimum*) were selected as functional ingredients for the study.

B. Formulation of food mixes

For the formulation of food mixes, green gram was obtained in bulk from departmental stores. Drumstick leaves, carrot and amla were procured from the local market. Wheat germ and flax seed powder of superior quality were ordered in advance and obtained from 'Nuts and Spices' stores located at Chennai.

Among all the home scale techniques to increase nutritive value of foods, germination is a complex metabolic process during which the lipids, carbohydrates and storage proteins within the seed are broken down. Germination is induced by rehydration of the seed, which increases both respiration and metabolic activity thus allowing the mobilization of primary and secondary metabolites (Limami *et al.*, 2002). Germination induces the synthesis of hydrolytic enzymes. Significant changes occur in the seed during germination in biochemical and physical aspects and the total nutritive value is improved (Jingjun *et al.*, 2010). It is a simple and inexpensive treatment that naturally increases digestibility of the protein and decreases anti-nutritional factors such as phytates and trypsin inhibitors (Jezierny, 2010).

Hence, germination was selected for green gram which was soaked overnight and germinated till about two centimetres of rootlets developed. Drumstick leaves were cleaned free from foreign matters and thick stems. Carrot was shredded and amla was deseeded and shredded. All the ingredients were shade dried at room temperature on a clean dry muslin cloth till the moisture content significantly reduced to yield consistent weights. The ingredients were then pulverized using a pulveriser to obtain a homogenised powder. Equal proportions of green gram and carrot powders and half the amounts of amla and drumstick leaf powders in the ratio of 2:2:1:1 were blended homogenously to obtain the basic mix (BM) (Figure I and Plate I). This proportion was found to be acceptable from a number of trials.

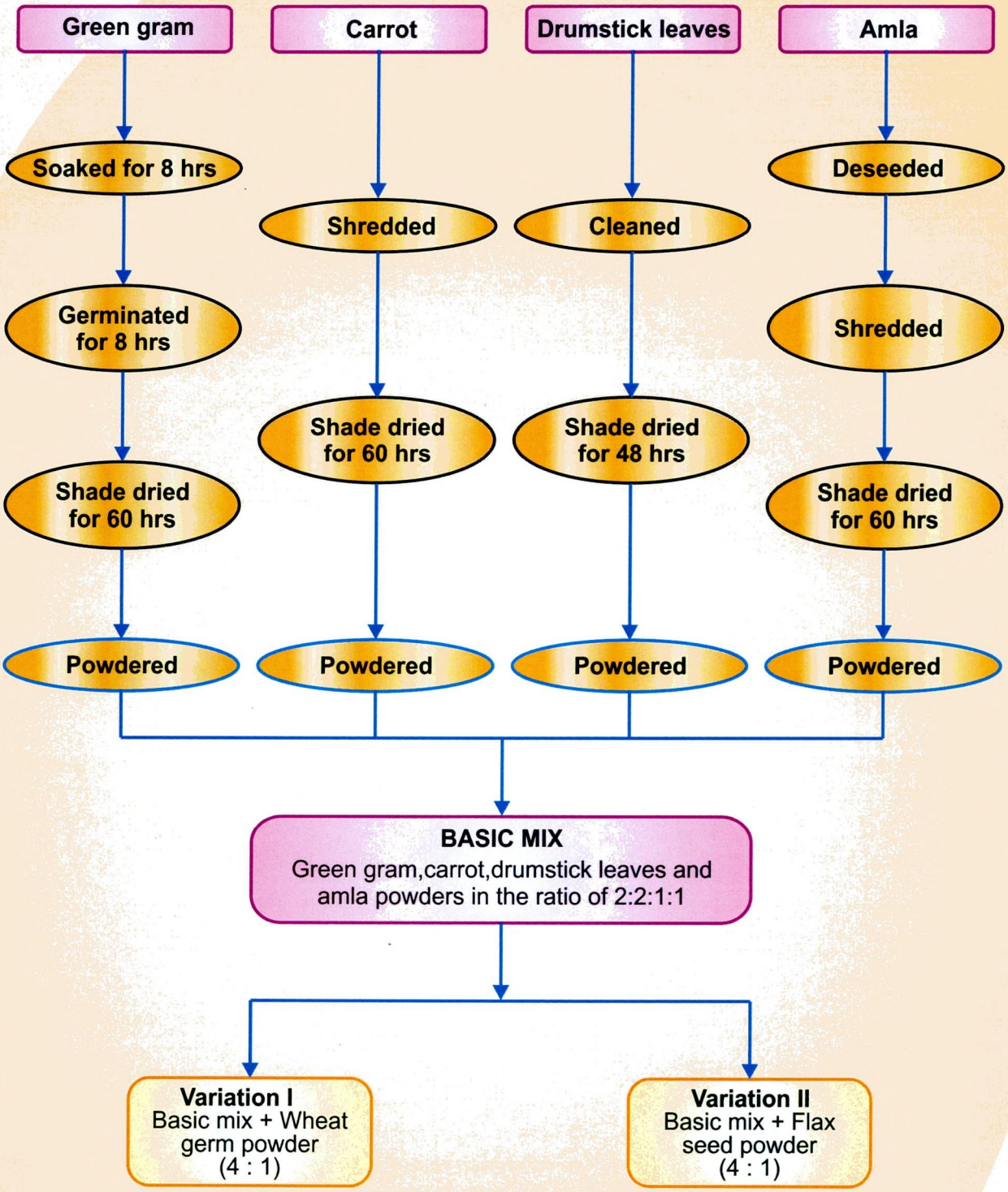


FIGURE 1

FORMULATION OF FUNCTIONAL FOOD MIXES

PLATE 1
FORMULATION OF FUNCTIONAL FOOD MIXES

With an interest to study and compare the combined effect of functional foods like wheat germ and flax seed powders on cardiovascular disease, they were added to the basic mix in the ratio of 4:1 (Basic mix: flax/wheat germ) in order to obtain the variation I with wheat germ and variation II with flax seed. The powders were prepared in bulk once in a fortnight and stored in air tight containers in a cool and dry place to protect from moisture, sunlight and insects.

C. Nutrient analysis and acceptability testing of food mixes

The basic mix and the two variations were analysed for their nutrient content. The proximate principles analysed included energy, protein, fat, carbohydrate, moisture and crude fibre and minerals analyzed included calcium, phosphorus, sodium, potassium, iron, zinc, magnesium, selenium, manganese and copper using the procedures recommended by NIN (Raghuramulu *et al.*, 2003). Vitamins such as vitamin A, C, and E were analysed using the AOAC (1995) procedures.

It was found that 30 g of food mix could meet one-third of the requirements of antioxidant vitamins such as vitamin A and C and minerals such as calcium and zinc. Further, the amount was within the safety limits of daily intake. This amount was also found to be convenient for consumption by the adults in one day and hence 30 g per adult per day was selected for study.

Various food preparations including idli, dosa, paruppu powder, chappathi, upma, sambar, dal, paniyaram, soup, porridge, curry and idiappam (Plate 2) were prepared in the laboratatory using standardised procedures as given by NIN (1982) incorporating 30 g of food mix to one serving of the food item. The prepared recipes were evaluated organoleptically by a panel of 30 trained members using a score card (Appendix I) with five point scale for their quality attributes.

D. Microbiological testing of food mixes

All the food mixes were analysed initially and after a storage period of three months for their microbial content (bacteria, yeast and mould) to evaluate the keeping quality of the mixes. Samples were analyzed to study

Plate 2
Recipes incorporating food mixes

the density of micro organisms by standard plate count, yeast and mould count (AOAC, 1995) and the results were compared with the permissible limits (The PFA Act, 1954).

E. Food costing

The total cost incurred in the preparation of the food mixes based on the market prices prevalent during the specific time, average cost was found out if purchased more than once, cost was computed and the economic feasibility was assessed and interpreted.

Phase II

A. Selection of area and adults for general study

Madurai city in Tamil Nadu state was selected for the conduct of the present study since the investigator is familiar with that area. Moreover, the city had a population of 10,74,149 of which males and females were 5,43,008 and 5,31,141 respectively (Madurai Corporation, 2006) at the time of the study and hence availability of adults for the study was easier.

The following six hospitals specializing in cardiovascular disease in Madurai city were selected for the study.

1. BGM Hospital (K.K.Nagar)
2. BGM Multispeciality Hospital (Iyer Bungalow)
3. GS Clinic (Theppakulam)
4. Hanna Joseph Clinic (K.K. Nagar)
5. Preethi Hospital (Mattuthavani), and
6. TVS Hospital (Chokkikulam)

These hospitals had expert cardiologists and a good number of cardiovascular patients regularly visit these hospitals for treatment or consultation and hence they could be approached for the study. Prior consent and cooperation were obtained from the doctors and hospital authorities before commencement of the study.

Both men and women aged 40 to 65 years visiting the hospitals with cardiovascular disease were recruited for the study. Informed consent was obtained from each participant. The identification of cardiovascular disease among the participants was based on physician's reports and baseline examination data. Diseases affecting the participants include hypertension, myocardial infarction, ischemic heart disease and a condition of post coronary artery byepass graft. Individuals were classified as hypertensive following the Joint Nutrition Committee VII Report (JNC, 2004) guidelines defining hypertension which is any patient above 18 years with systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg in the absence of acute illness, confirmed by at least two recordings on different days. Exclusion criteria included that those suffering from kidney diseases and those who were recently hospitalized.

The study protocol was presented before the Avinashilingam University's Ethical Committee members and got approved by them (HEC:2010:19).

B. Eliciting background information

The cardiovascular disease burden and disparities within the general population are largely determined by environmental, dietary, and other lifestyle factors. In general, social and environmental factors seem to be more explanatory and influential than genetic factors in explaining the population disease burden of chronic cardiovascular disease (Flack *et al.,* 2010). Interviewing is one of the major methods of data collection, defined as two-way systematic conversation between an investigator and an informant, initiated for obtaining information relevant to a specific study. It is useful for collecting a wide range of data from factual demographic details to highly personal and intimate information from a person (Maheshwari, 2011).

An interview schedule was evolved in order to elicit information on the socioeconomic background of the adults such as age, sex, education, occupation, type, size and income of the families. Results of many large prospective cohort studies are available which reveal that healthy diet and lifestyle are associated with low risk of cardiovascular disease or primary and secondary prevention of cardiovascular disease. Hence, questions on lifestyle

associated practices such as yoga, exercise, smoking and alcoholism were also included. Dietary pattern comprising of questions on type of diet, meal pattern, beverage consumption and diet modifications and health status in terms of clinical signs and symptoms were also included. Family history and medications were other essential aspects collected through the interview schedule. The schedule was pre-tested, modified, finalised and used for the study (Appendix II).

The selected adults were explained regarding the importance of the study and their full cooperation was obtained. Prior to interview, the adults were assured regarding participant anonymity, confidentiality, and the voluntary nature of participation. A total of 874 men and 766 women who visited the hospitals were interviewed by the investigator in the hospital premises over a period of eight months (Plate 3).

C. Assessment of nutritional status

An optimal scheme of nutrition assessment enables the clinicians to quickly detect the presence of disease condition and to provide guidelines for nutritional therapy. Nutrition assessment leads to a plan of care or intervention, designed to help the individual either to maintain the assessed status or attain a healthier status (Chirstie, 2010). The ABCDs of nutrition assessment comprises **A**nthropometric measurements, **B**iochemical tests, **C**linical/physical observations and **D**ietary intake.

1. Anthropometric measurements

Anthropometry is the science of making surface measurements on the human body based on anatomical landmarks. It is the single most universally applicable, inexpensive and non –invasive method available to assess the size, proportions, and composition of the human body. Anthropometric indicators for body fat are widely used to predict increased chronic disease risk at the individual and at population levels. Anthropometric measurements like waist circumference and waist hip ratio provide additional information about central fat distribution (Klein *et al.*, 2007).

The following anthropometric measurements were taken for all the adults participating in the present study according to the procedures recommended by the International Society for the Advancement of Kinanthropometry (Marfell-Jones *et al.,* 2001).

a) Height

Height is an anthropometric measurement used frequently for nutritional assessment (Vij, 2011). Standing height of all the adults was measured according to the procedures recommended by the International Society for the Advancement of Kinanthropometry (Marfell-Jones *et al.,* 2001) using a fixed stadiometer calibrated in centimetres. Each adult was made to stand without slippers with heels, buttocks and shoulders resting lightly against the backrest so that the Frankfort plane was horizontal. The head was held straight and arms hanging to the sides. A wooden scale was then placed close to the top of the head, crushing the hair and height was measured to the nearest 0.1 cm and recorded (Plate 4).

b) Weight

Weight is an important criteria in cardiovascular disease because of its maladaptive effects on various cardiovascular disease risk factors and its adverse effects on cardiovascular structure and function. Obesity has a major impact on cardiovascular disease, and is associated with reduced overall survival (Lavie *et al.,* 2009). Weight of all the adults was taken according to the procedures recommended by the International Society for the Advancement of Kinanthropometry (Marfell-Jones *et al.,* 2001) using a bathroom scale balance available at the hospital premises. The scale was first zeroed and the calibration was checked periodically. The adults were made to stand erect with light clothing, barefoot, and without touching any other object. Weight was measured in kilograms to the nearest 0.1 kg and recorded (Plate 5).

Plate 3
Collection of background information

Plate 4
Measurement of height

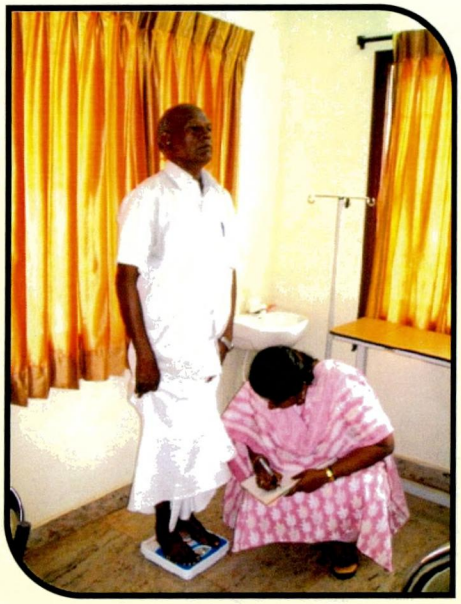


Plate 5
Measurement of weight

c) Body Mass Index

Body Mass Index (BMI) or Quetelet's index is a key index relating a person's body weight to his height and represents the most useful anthropometric index and frequently used to evaluate an individual's nutritional status with a special focus on obesity (Gallagher and Javed, 2009). The validity of BMI is based on the good correlation it presents with corporal fat and mainly internal fat, which is associated to the risk factor for developing chronic diseases (Ferra, 2005). BMI was calculated using the metric imperial formula (WHO, 2004).

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height } (m^2)}$$

World Health Organization (2004) has given the following guidelines for BMI interpretation.

Category	BMI range
Underweight	<18.5
Normal	18.5 to 24.9
Pre obese	25.0 to 29.9
Obese class I	30.0 to 34.9
Obese class II	35.0 to 39.9
Obese class III	> 40.0

All the adults in the present study were categorized according to BMI classification.

d) Waist and hip circumference

Waist circumference is a good marker of visceral fat and correlates strongly with cardiovascular disease risk factors (Janssen *et al.*, 2004). Waist circumference was measured to the nearest centimetre by the investigator with the help of an attendant with a fibre glass tape while the adults were in the standing position at the end of gentle expiration. Measurements were made halfway between the lower border of the ribs and the iliac crest in a horizontal plane.

Hip circumference was at the widest point over the gluteal (buttock) muscles over minimal clothing. The adults were made to stand erect with the body weight evenly distributed on both feet and legs slightly apart. For each of waist and hip circumference, two measurements to the nearest 0.1 cm were recorded. If the variation between the measurements was greater than 2 cm a third measurement was taken. The mean of the two closest measurements was calculated. The waist and hip circumferences were measured according to the International Society for the Advancement of Kinanthropometry (Marfell-Jones *et al.,* 2001).

The ACCF/AHA (Redberg *et al.*, 2009) has suggested that men with waist circumference more than 102 cm and women with a waist circumference more than 88 cm are to be classified as obese and men with a waist circumference of 94 to 101.9 cm and women with a waist circumference of 80 to 87.9 cm are to be classified as overweight.

e) Waist Hip Ratio

Waist Hip Ratio (WHR) is widely used as an indicator of abdominal or central adiposity in epidemiologic studies. Excess visceral fat indicated by the WHR, is important in the aetiology of chronic diseases due to its association with circulating free fatty acids, insulin resistance, hyper insulinemia, dyslipidemia and atherosclerotic inflammation markers (Parker *et al.*, 2009). Visceral fat is supplied by the portal blood system, excess fat in this area can lead to the release of fatty deposits to the blood stream. This excess fat is associated with adverse health effects such as cardiovascular disease, type 2 diabetes, and cancer (Herrera and Lindgren, 2010).

f) Waist Height Ratio

Recent studies have documented that the ratio of waist circumference to height is even superior to waist circumference and BMI to predict cardiovascular risk factors (Hsieh and Muto, 2010). Waist Height Ratio (WHtR) has several advantages over other indices as it combines the advantages of both BMI and waist to hip ratio by taking not only the height, but also the abdominal adiposity into account. Secondly it is less correlated

with age than BMI, which makes it possible to propose a non age dependant cut-off point, which is easy to manipulate by both professionals and common people. In addition, WHtR as a diagnostic test is very simple, easy, inexpensive and an accurate index (Yan *et al.*, 2007).

The potential independent effect of height on coronary heart disease, apart from age and other risk factors, may make WHtR a better index for prospective study that may be applied to both men and women, enabling busy physicians and other professionals to screen and counsel patients who face higher metabolic risks during physical examinations. This index serves as a 'second stethoscope' (Hsieh *et al.*, 2003). The value of 0.5 is suggested as an appropriate cut-off point for both adults and children (Mc Carthy *et al.*, 2006). The WHtR was calculated for all the adults and compared with the cut-off values.

2. Clinical parameters

By becoming familiar with the signs and symptoms of a condition, and by recognizing its prevalence and importance, a clinician is more likely to diagnose a patient with that condition (Ziegelstein *et al.*, 2009). Various clinical parameters that are indicative of signs and symptoms of cardiovascular disease such as breathlessness, chest pain, fatigue, giddiness, inability to work, heart burn and nausea were diagnosed among the adults with the help of a physician and recorded in the developed interview schedule.

3. Biochemical parameters

Biochemical assessment measures the levels of nutrients in biological tissues or fluids or evaluates biochemical functions dependant on an adequate supply of essential nutrients, reflecting subclinical nutritional status. Biochemical assessment is considered to be a more objective and precise methodology due to its consistency and accuracy. For assessing the biochemical profile, the adults were informed to consume an early dinner on the night prior to drawing blood for biochemical assessments (Plate 6). The next morning, following 12 hours of fasting, blood was drawn from the adults

with the help of a trained technician. The biochemical tests performed were fasting blood glucose, lipid profile (total cholesterol, triglycerides, LDL, HDL and VLDL cholesterol), glycosylated haemoglobin and serum vitamin antioxidants (Vitamin A, C, and E) (Plate 7).

a. Fasting blood glucose

Increased fasting blood glucose level is a well known risk factor for cardiovascular disease (Kadowaki *et al.*, 2008). Elevated fasting blood glucose levels are associated with an increased cardiovascular disease risk (Perez *et al.*, 2010). Hence, fasting blood glucose levels were estimated for all the adults using one Touch R Horizon^{TM} blood glucose monitoring apparatus.

b. Lipid profile

Lipid profile also known as coronary risk panel or lipid panel is a blood test done to assess the status of fat metabolism in the body and is important in heart disease. This includes measurement of lipids (fats) and its derivatives known as lipoproteins. All the parameters pertaining to the lipid profile were analysed using Toshiba (Japan) auto analyzer present in the sophisticated laboratory attached to the hospital using Roche kits.

i) Total cholesterol

Total cholesterol is one of the most commonly considered measurements on a lipid profile. It is defined as the sum of HDL, LDL and VLDL cholesterol circulating in the blood. High circulating levels of cholesterol in the blood can lead to fat being deposited in the walls of the arteries and narrowing them and development of plaques which can lead to various cardiovascular disorders (Cimmino *et al.*, 2010).

The National Cholesterol Education Programme (NCEP, 2002) classifies the total cholesterol levels as - desirable (<200 mg/dl), borderline high (200-239 mg/dl) and high (>240 mg/dl).

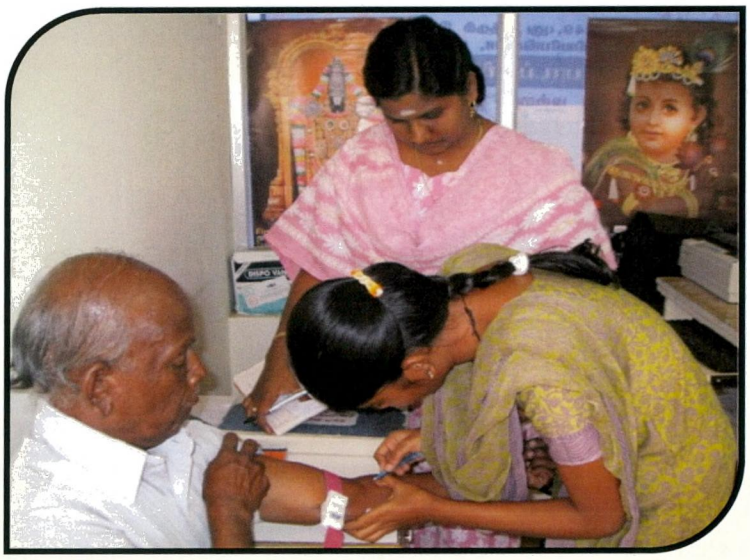


Plate 6
Drawing of blood sample

Plate 7
Biochemical estimation

ii) Triglycerides

Triglycerides are chains of high energy fatty acids that provide much of the energy needed for tissues to function. Elevated triglyceride levels in blood termed as triglyceridemia acts similar to cholesterol in causing plaque formation and can increase the risk of cardiovascular disease.

The classification of serum triglyceride levels according to the NCEP (2002) guidelines is as follows.

Category	Levels (mg/dl)
Normal	<150
Borderline	150 - 199
High	200 - 499
Very high	> 500

iii) High Density Lipoprotein (HDL) cholesterol

Cholesterol is carried around the body in the cells of mixed fat and protein called lipoproteins. The High Density Lipoproteins (HDL) contain more proteins and carry damaging fat deposits away from the arteries to the liver for recycling and are hence called as 'good cholesterol' packages. HDL cholesterol makes up 20 to 30 per cent of the total cholesterol and is inversely correlated with the risk of cardiovascular disease. Serum HDL levels below 40 mg per dl is categorised as low, 40-60 mg per dl as desirable and >60 mg per dl as high by NCEP (2002).

iv) Low Density Lipoprotein (LDL) Cholesterol

The Low Density Lipoproteins (LDL) have more fat and cholesterol and are referred to as 'bad cholesterol'. These packages deposit artery-clogging plaque in the vessels, which can lead to heart attack and stroke. LDL cholesterol typically makes up 60 to 70 per cent of the total serum cholesterol. It is the major atherogenic lipoprotein and has long been identified as the primary target of cholesterol lowering therapy. NCEP (2002) classifies the LDL cholesterol levels into 5 categories - optimal (100 mg/dl), above optimal

(100-129 mg/dl), borderline high (130-159 mg/dl), high (160-189 mg/dl) and very high (>189 mg/dl).

LDL cholesterol was calculated using the Friedwald's formula (Friedwald *et al.*, 1972).

$$LDL = TC - (HDL + VLDL)$$

Where, TC=Total Cholesterol,
HDL=High Density Lipoprotein and
VLDL=Very Low Density Lipoprotein.

v) Very Low Density Lipoprotein (VLDL) cholesterol

The Very Low Density Lipoproteins (VLDL) are triglyceride rich lipoproteins and contain 10 to 15 per cent of the total serum cholesterol. VLDL are produced by the liver and are precursors of LDL, some forms of which, particularly VLDL remnants appear to promote atherosclerosis, similar to LDL (Alagona, 2009). The NCEP (2002) has declared VLDL levels between 5 and 40 mg per dl as normal.

From the serum triglyceride levels, VLDL levels were calculated using the formula given below

$$VLDL = \frac{\text{Triglycerides}}{5}$$

Where, 5 is a constant factor.

c. Glycosylated Haemoglobin

Glycosylated haemoglobin (HbA1C) is the result of simple chemical reactions between haemoglobin and sugars after the synthesis of haemoglobin is complete in the post – translational modifications. Glycosylation begins during erythropoiesis and continues slowly throughout the life of haemoglobin and are consistent with their known lifespan of about 120 days. The level of HbA1C depends on the long – term level of glucose present in the blood and is proportional to average blood glucose concentration over the previous two to four weeks (Sultanpur and Kumar,

2010). The American Diabetes Association (2009) proposes HbA1C cut of point of 6.5 per cent as a diagnostic test for diabetes. All the 476 diabetic adults participating in the present study were assessed for glycosylated haemoglobin levels using the colorimetry method (Goldstein *et al.*, 1986).

d. Antioxidant vitamins

Free radical mediated oxidative damage to lipoproteins is known to be an important factor predisposing them to uptake into the vascular wall (Pasupathy *et al.*, 2009). Antioxidants terminate these chain reactions by removing the free radical intermediaries. Serum vitamin antioxidants that include vitamin A, C and E play a leading role in cardiovascular maintenance. Vitamin C plays a role in the conversion of cholesterol into bile acids and is needed for the normal metabolism of body fats. Vitamin C has been found to reduce adhesion of monocytes to the lining of blood vessels and thereby reduce the risk of atherosclerosis (Weber *et al.*, 1996). As one of the many parameters applied to evaluate the impact of supplementation, the following antioxidant vitamin levels were evaluated for the 180 adults participating in the supplementation study.

i. Vitamin A

Apart from its very significant role in vision and growth, vitamin A is also a potent antioxidant similar to vitamins C and E. Serum vitamin A levels were estimated for the adults using the Carr-price method (Raghuramulu *et al.*, 2003).

ii. Vitamin C

Vitamin C has the ability to reverse the vasomotor dysfunction often found in patients with atherosclerosis (Levine *et al.*, 1996). The serum vitamin C levels of the adults were estimated by the DNP method (Tietz, 1986). Ascorbic acid is oxidized by cuprous oxide to form dehydro ascorbic acid, which reacts with acidic 2, 4 dinitro phenylhydrazine to form a red bishydrazone which was measured spectrophotometrically at 520 nm.

iii. Vitamin E

Vitamin E refers to a group of compounds that include both tocopherols and tocotrienols which are important lipid soluble antioxidants being the most important function of vitamin E. Vitamin E levels in the blood samples were

estimated by Baker and Frank method (1988) which is based on the reduction of ferrous ions which forms a red coloured complex with α - α_1 dipiridyl.

D. Energy balance

American Heart Association (AHA) suggests that in order to avoid weight gain after childhood and maintain healthy body weight, energy balance should be achieved (Lichtenstein, 2006). Storage of triglycerides in the adipose tissue is dependent upon the balance between energy expenditure and energy intake. Preferential accumulation of adipose tissue in the abdominal area has been associated with dyslipidemia, a risk factor of cardiovascular disease (Despres and Lamarche, 1993).

Energy balance was calculated for all the 1640 adults chosen for the study. Difference between the energy intake and expenditure was calculated to find out the energy balance of each individual.

1. Food and nutrient intake

Dietary assessment, the first step in identifying nutritional status may confirm a lack or excess of one or more dietary constituents. Before developing and implementing effective intervention programmes to improve nutrition at the population level, it is important to know the nutritional situation of the target group (Elmadfa and Meyer, 2010).

A 24 hour dietary recall schedule (Appendix III) was administered to assess the dietary intake of the adults. Standard cups and measures were shown and the adults were asked to recall their exact food intake during the previous 24 hour period. Quantities of foods consumed were estimated in standard measurements and entered on the data sheet to determine the dietary intake of various foods. Nutrient intake of the adults was computed using the Food Composition Tables (Gopalan *et al.,* 2007) and compared with the Recommended Allowances (ICMR, 2009).

2. Energy Expenditure

FAO (2003) defines Total Energy Expenditure (TEE) as the energy spent, on an average in a 24-hour period by an individual or a group of individuals. The energy expenditure of all the selected adults was determined using the time activity record (Appendix IV). This involved self recording of various types of activities by the adults for 1440 minutes (24 hours) of the day. Schedules were distributed to the adults and they were instructed to carefully record their activities for the whole day. The filled schedules were then collected from the adults. The specific activities were then coded following the procedure recommended by ICMR (2002). The total energy expenditure for the whole day was calculated.

3. Assessment of energy balance

The total daily energy intake of the adults was calculated from the data obtained from 24 hour dietary intake schedule as explained earlier for each of the 1640 adults selected for the study. The total daily energy expenditure of each adult was also computed from the 24 hour activity schedule. Employing the factorial approach and the computed BMR from body weight and the recommended BMR factors for Indians for different levels of physical activity, the energy expenditure was computed and the difference between energy intake and expenditure was calculated to determine the energy balance for each individual using the procedures recommended by ICMR (2002).

E. Assessment of cardiovascular parameters

Assessment of cardiovascular parameters is an important step in knowing the severity of cardiovascular disease and the choice of treatment. The various cardiovascular parameters assessed in the present study based on the cardiologists' advice included Resting Heart Rate (RHR), Blood Pressure (BP), Electrocardiogram (ECG), Carotid Intima Media Thickness (CIMT) and Ruffier Functional Test.

1) Resting Heart Rate

The total number of heart beats in a lifetime remains fairly constant across species and there exists an inverse relationship between resting heart rate and life expectancy (Levine, 1997). There is strong evidence linking an increase in resting heart rate to increased risk of cardiovascular morbidity and mortality in the general population (Ferrari, 2003). A high heart rate leads to both greater myocardial oxygen consumption and decreased myocardial perfusion, the latter by shortening the duration of diastole, can induce or exacerbate myocardial ischemia (Diaz *et al.*, 2005). Studies have found a continuous increase in the risk of cardiovascular disease with heart rates above 60 beats per min (Fox *et al.*, 2007).

RHR more than 90 beats per min compared to less than 60 beats per min was associated with an almost two fold increased risk of cardiovascular disease mortality in men and a threefold increased risk in women (Cooney *et al.*, 2010). All the 1640 adults chosen for the study as and when they visited the hospital were examined for RHR in a quiet room after a brief resting period. The adults were seated in an upright position in a straight back chair and heart rate was measured for one minute using a stethoscope and expressed in terms of beats per minute.

2) Blood pressure

Blood pressure is defined as the pressure exerted by the blood upon the walls of the blood vessels, generally arteries, usually measured on the radial artery by means of a sphygmomanometer, and expressed in millimetres of mercury. It is intrinsically related to the heart's ability to pump, and the mean arterial blood pressure is proportional to the cardiac output. Therefore, physiologically, there is a strong cause for blood pressure as a marker of prognosis in heart failure (Raphael *et al.*, 2009). It is a powerful, consistent and independent risk factor for cardiovascular disease (Pickering *et al.*, 2005).

Blood pressure was measured for all the adults by the technician by auscultatory method using a sphygmomanometer as recommended by the Joint National Committee (2004). The adults were seated quietly in a chair with feet on the floor and arm supported at heart level. The cuff was tied

around the upper arm, pressure was raised to 200 mm Hg and then gradually released. Systolic blood pressure (SBP) was the point at which the first two Korotkoff sounds were heard and Diastolic Blood Pressure (DBP) was the point of disappearance of the Korotkoff sound. Blood pressure was measured for all the 1640 adults chosen for the study during their visit to the hospital with the help of a trained technician in the hospital premises (Plate 8).

The classification of blood pressure according to the Joint National Committee (2004) criteria is as follows.

Classification	Blood pressure (mm Hg)	
	Systolic	Diastolic
Normal	< 120	< 80
Pre Hypertension	120 - 139	80 – 89
Stage I Hypertension	140 - 159	90 - 99
Stage II Hypertension	> 160	> 100

3) Electrocardiogram

The electrocardiogram (ECG) is a diagnostic tool that measures and records the electrical activity of the heart in an exquisite detail. The ECG is a very commonly performed cardiac test because it is a useful screening tool for a variety of cardiac abnormalities and can be used to determine various details such as heart rate, heart rhythm, presence of conduction abnormalities, prior history of heart attack, coronary artery disease and abnormal thickening of heart muscles (Norris and Clark, 2009).

Accordingly a conventional 12-lead resting ECG was recorded for all the 1640 adults in the hospital premises by trained technicians over a period of eight months (Plate 9). The adults were asked to lie down and areas of arms and chest, of the adult were cleaned and the conducting jelly was applied. Electrodes were attached to specific areas. The adults were instructed to remain still and relaxed. The electrodes were connected by wires to a machine that converts the electrical signals from the heart into wavy lines, which were printed on paper and reviewed by the doctor. The entire procedure was done according to the Minnesota code (Prineas *et al.*, 1982).

4) Carotid Intima Media Thickness test

Carotid Intima Media Thickness (CIMT) is a useful, noninvasive, reproducible but operator-dependent, predictive, radiation-free, office-based technique (Kones, 2011). It is a new non invasive ultra sound and a well validated marker of subclinical atherosclerosis that provides a direct, non-invasive assessment of atherosclerotic burden and provides incremental predictive information for traditional risk assessment (Greenland *et al.*, 2000).

This test is performed with a high resolution B- mode ultra sound transducer. The hand held transducer when applied to image the carotid arteries, the sonographer measures the combined thickness of the intimal and medial layers of the carotid artery walls which provides a 'window' to the coronary arteries. In addition to its association with known cardiovascular risk factors, the rate of carotid intima media thickness progression is directly related to the risk for future cardiovascular events including heart attacks, cardiac death, and stroke. Subsequently, CIMT has been a valuable research tool in clinical trials in the assessment of therapeutic agents directed against atherosclerosis (Devine *et al.*, 2006).

In research and clinical settings, CIMT measurements are used to evaluate cardiovascular risk and to follow progression or regression of atherosclerosis in response to interventions (Tzou *et al.*, 2007).

This test is safe, painless and takes about 20 minutes. After applying conducting jelly to the skin over the neck, a small hand-held transducer is applied to image the carotid arteries. The CIMT test was done for a sub sample of eight adults that included two adults from each group of basic mix, variation I, variation II and control group chosen on the basis of their willingness to cooperate for the test. Moreover, the test is very expensive to the tune of Rs. 1200 per test. The test was performed before and after the supplementation period of six months. It was performed in the electrocardiogram laboratory attached to the hospital premises by technicians specially trained for this purpose (Plate 10).

5) Ruffier Functional Test

Functional fitness is the capacity to perform normal daily activities in a safe and independent fashion without undue fatigue or pain (Rikli and Jones, 2001). The hallmark symptom of chronic heart failure is exercise intolerance, often exhibited by early fatigue or shortness of breath with a minimal degree of exertion (Myers, 2008).

The Ruffier Functional Test (Epifanov and Apanasenko, 1990) is a performance test used to evaluate the efficiency of the cardiovascular system. It is a simple test which measures the functional state of the cardiovascular system and readiness of the individual for load. This test was conducted for all the 180 adults chosen for the supplementation study before and after the six months supplementation period (Plate 11). The steps involved in the test are as follows.

i. The individual was made to relax for 5 minutes and heart rate was measured in sitting position (S1).
ii. The individual was made to do 30 squats in 45 seconds and the heart rate was measured immediately (S2).
iii. The individual was made to calm down in sitting position for 1 minute and heart rate was measured (S3).

The index value was calculated using the formula

$$\textbf{Ruffier Index} = \frac{(S1 + S2 + S3) - 200}{10}$$

The results were interpreted according to the following classification (Epifanov and Apanasenko, 1990).

Calculated index value	Functional condition
Less than 3.0	Excellent
3.1 – 7.0	Good
7.1- 12.0	Average
12.1 – 15.0	Poor
More than 15.1	Very poor

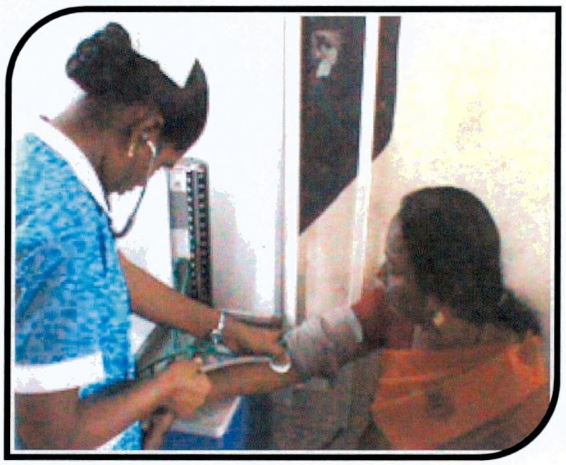


Plate 8
Measurement of blood pressure

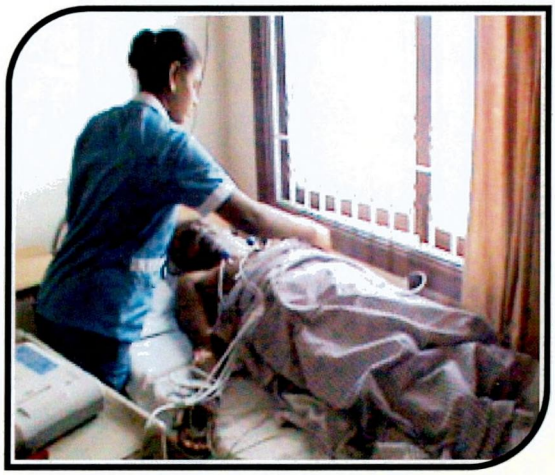


Plate 9
Measurement of ECG

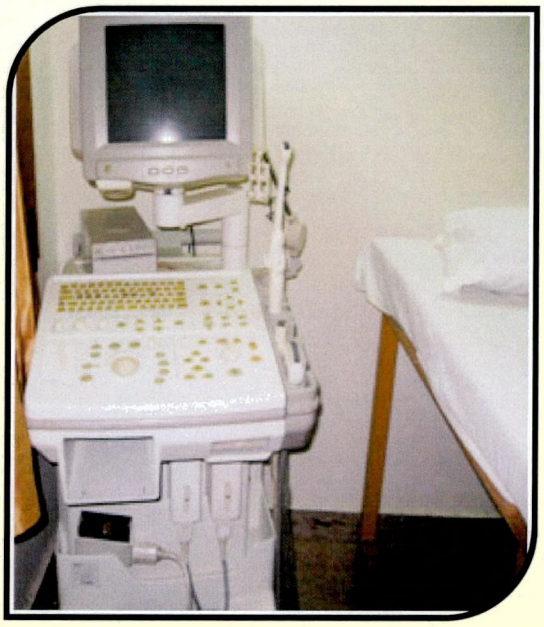


Plate 10
Ultrasound transducer for CIMT test

Plate 11
Ruffier functional test

Phase III

A. Selection of adults for supplementation study

Among the 1640 adults enrolled initially for the survey, a total of 180 individuals with cardiovascular disease excluding 18 dropouts were selected for supplementation study. The selection criteria included that these adults were living at close proximity to the hospital premises and therefore it was convenient for them to participate in the supplementation study and undergo biochemical estimation. Moreover they also had a controlled disease history. All the selected participants were well informed regarding the objective of the study and agreed to participate willingly and co-operate throughout the six months feeding trial and therefore ideally suited for the present study. Informed consent was obtained from all the 180 adults and prior permission was obtained from the hospital authorities for conducting the supplementation study.

All the selected adults were within the age group of 40 to 65 years having clinical signs of hypertension with high lipid profile and free from any other chronic illness. Diabetes and heart disease often occur together in patients, with each condition influencing the treatment of the other. A study by Bell (2003) revealed that about 12 to 25 per cent of patients with diabetes also had heart disease and up to 30 per cent of patients with heart failure also had diabetes. The two diseases have many common patho-physiologic links and each disease increases the risk of developing the other which when present together in the same patient, the risk of morbidity and mortality increases substantially (Mac Donald *et al.*, 2009). Hence, it was decided to include cardiovascular patients having a co-occurrence of type 2 diabetes mellitus in the study. But, in order to avoid the influence of diabetes mellitus from masking the results of the study, they were taken as a separate group.

The content of the functional food mixes was explained to the adults and highlighted about the functional properties of the ingredients. It was assured to the adults that they were going to consume only food based supplements which are free from any side effects. The adults were also informed to follow

their regular diet and medications and not to make any major changes in their dietary or lifestyle pattern throughout the period of study.

One hundred and twenty adults chosen for supplementation were divided into four groups (EG1, EG2, EG3 and CG1) of 30 each. Sixty adults suffering from cardiovascular complications along with type 2 diabetes mellitus were chosen and divided into two groups (EG4 and CG2) of 30 each. The different groups supplemented with different mixes are given below.

EG1: Experimental Group 1 supplemented with basic mix

EG2: Experimental Group 2 supplemented with variation 1 (basic mix + wheat germ)

EG3: Experimental Group 3 supplemented with variation 2 (basic mix + flax seed)

CG1: Control Group 1 with no supplementation

EG4: Experimental Group 4 supplemented with basic mix

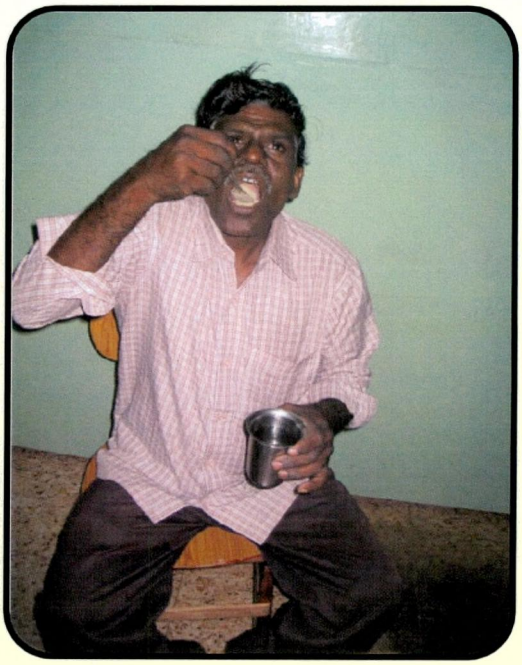
CG2: Control Group 2 with no supplementation

B. Conduct of supplementation study

Food mixes weighing 30 g each were filled into small pouches. Fifteen small pouches were put into a larger ziplock bag. The adults were distributed with one such large pack once in 15 days for a period of six months (Plate 12). They were asked to consume one small pouch (30 g) each day either separately or along with their food by mixing it in various preparations such as idli, dosa, sambar or rice (Plate 13).

During their fortnightly visits to the hospital, they were checked and interviewed by the investigator about any inconvenience or discomfort they faced in consuming the supplement. It was also made sure that they consumed the food mixes daily. For those adults who could not visit the hospital in person within fifteen days, the food pouches were sent through courier service in order to ensure continuous consumption of the food mixes. Follow up in between the visits was also done by regular telephonic enquiries.

Plate 12
Distribution of food mixes to the adults



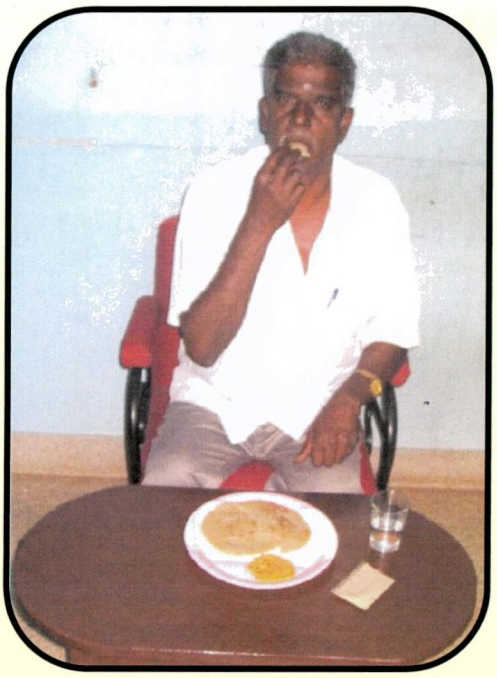


Plate 13
Consumption of food mix by the adults

C. Evaluation of the impact of supplementation

After a period of six months supplementation, the impact of the supplementation was assessed by comparing the following parameters before and after supplementation.

1. Anthropometric measurements

Anthropometric parameters including body weight, BMI, waist circumference, hip circumference, waist hip ratio and waist height ratio were assessed both before and after supplementation and the results were compared.

2. Biochemical parameters

Biochemical parameters namely fasting blood sugar, lipid profile, HbA1c and antioxidant vitamins were assessed before and after supplementation and compared with initial values to evaluate the effect of supplementation.

3. Cardiovascular parameters and performance test

The resting heart rate, blood pressure and electrocardiogram were measured for all the 180 adults. The Carotid Intima Media Thickness (CIMT) test was done for a sub sample of eight adults before and after supplementation and compared. Ruffier functional test was done for all the 180 adults before and after the supplementation study and the changes were observed.

Phase IV

A. Selection of adults for diet counselling

Self care is fundamental to achieve optimal outcome in patients with chronic disorders. Self care in heart disease is an intricate process that is the result of several decisions made at many levels and requires a good base of information and encouragement among the patients. A widely prevalent ignorance on cardiovascular disease among the adults and a demand for healthy diet practices was observed by the investigator right from the start of the study. This inspired the investigator to include diet counselling as one of the research activity for the study. The American college of Cardiology

Foundation and American Heart Association (2009) strongly believe that diet and physical activity counselling are the foundations for primary prevention and have the potential to either reduce or prevent the development of risk factors. In order to study any relationship between diet counselling and supplementation, a separate group that was counselled as well as supplemented with the basic mix was included along with control group for effective comparison.

A total of 60 adults from the initial survey who were not participating in the supplementation study were selected for diet counselling. All the 60 adults were willing to attend the counselling sessions regularly, located at accessible points of the city and were hence considered ideal for selection. The selected adults were categorized randomly into two groups of 30 members each. The first group (**DCG**) was given diet counselling alone while the second group (**DCSG**) was given supplementation of the formulated basic mix prepared as described in phase III along with diet counselling. The third group (**CG**) constituted the control group which was included in the supplementation study (Phase III) who were neither given counselling nor supplementation. This group was however given diet counselling after collecting the required data for the study owing to ethical reasons.

B. Development of counselling modules

A counselling programme was developed in order to teach the adults various aspects of health and cardiovascular disease such as general health status and its indicators, causes and consequences of cardiovascular disease, do's and don'ts of the same and some of the common metabolic disorders such as hypertension and diabetes mellitus, diet choices, foods to be included, restricted and avoided and also lifestyle modifications.

Teaching aids help to impart and emphasize information, stimulate interest, and facilitate the learning process. Teaching aids, properly chosen and skilfully presented provide a variety of instructional methods and enhance the amount of actual learning (Reddy, 1987). The following audio visual aids were developed to educate the adults. Care was taken to make all the aids

self explanatory and easily understandable by individuals of different levels of literacy.

1. Charts

A chart is something that shows a group of facts in the form of diagram, table, graph or text. Charts can be large enough to mount on a wall to facilitate group learning. Ten charts depicting symptoms and causes of heart disease, complications of cardiovascular disease, healthy lifestyle practices, healthy eating pyramid and food choices were developed (Plate 14).

2. Flash cards

Flash cards are a set of cards with brief visual message flashed one after the other in a logical sequence and is especially suited for step by step teaching. Five flash cards covering various aspects of diet and foods to be included, restricted and avoided were developed and used for counselling the participants (Plate 15).

3. Pamphlets

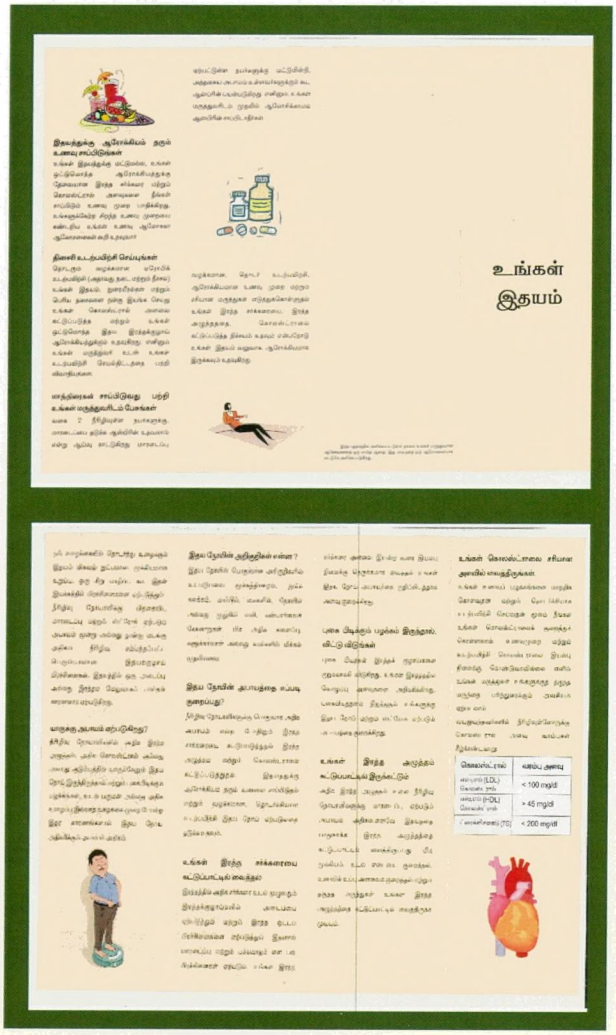
A pamphlet is an unbound booklet consisting of a single sheet folded into half or fourths or few pages that are pinned to make a simple book. A pamphlet containing foods that are to be included, restricted and avoided by cardiovascular patients and a sample menu were included in one pamphlet. Two more pamphlets with the checklist for cardiovascular disease patients and importance of diet in cardiovascular disease were also developed (Plate 16).

4. Booklets

Booklets are similar to books, except that they are smaller in size. Two booklets on healthy recipes for the heart and simple ways for a healthy life were developed to be distributed among the adults. These provided written examples of diet plans that met the recommended dietary guidelines and diet low in saturated fat and rich in whole grains, vegetables, and fruits as suggested by ICMR (Plate 17).

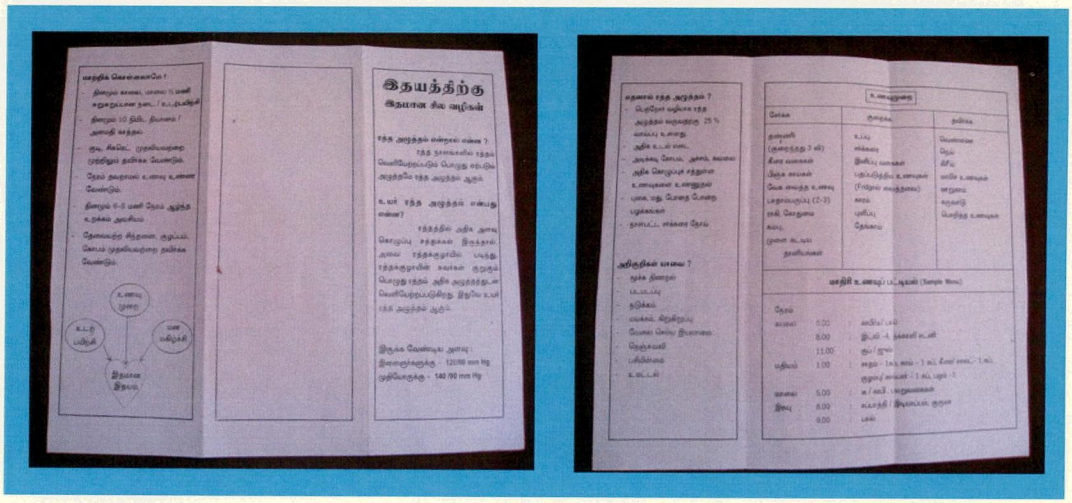
Plate 14
Charts developed for diet counselling

Plate 15
Flash cards developed for diet counselling



Pamphlet 1

Pamphlet 2



Pamphlet 3

Plate 16
Pamphlets developed for diet counselling

5. Displays

Display of various foods and heart healthy meal patterns can be more easily remembered and reproduced than by oral communications. Various displays of healthy meal food exchange and heart healthy foods were done which could be easily registered and remembered by the adults (Plate 18).

6. Power point presentation

A power point presentation for five minutes (14 slides) covering all aspects of diet in relation to cardiovascular disease was developed to be played during the counselling sessions (Plate 19).

C. Conduct of diet counselling programme

The entire diet counselling programme was scheduled for ten sessions over a period of five months. All the 60 adults chosen for counselling were first oriented as to what they will be learning in the counselling sessions. The first eight sessions were done by group contact method (Plate 20). The classes were scheduled on weekends, once in a fortnight for a duration of one hour each. All the participants were informed previously about the dates and were subsequently reminded by telephone calls, a couple of days prior to each session.

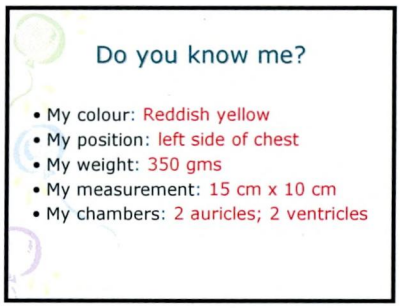
The adults were educated about the importance of diet change and its impact on cardiovascular disease. They were also exposed to latest scientific data on various diet modification schedules and its impact on health status and healthy life span. All the teaching aids developed were used during the sessions. Power point presentation was included in one of the sessions to emphasize the importance of a heart healthy life (Plate 21). The adults were provided with a list of resources such as pamphlets and booklets to enhance their adherence to the dietary intervention. They were encouraged to have individual food diaries and their scores were compared with each other in the consecutive sessions in order to encourage them psychologically. The last fifteen minutes of each session was made interactive. The adults were asked to feel free to ask any doubts regarding cardiovascular disease management.

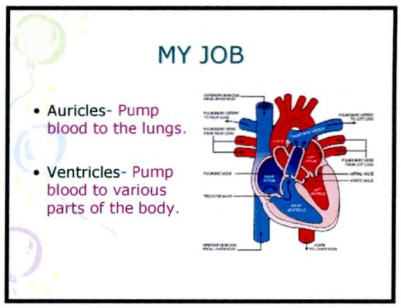
Plate 17

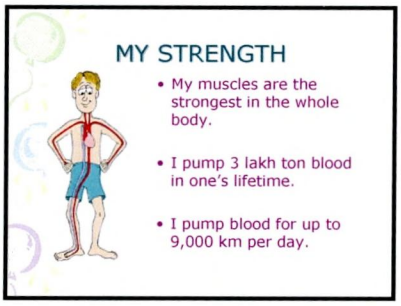
Booklets developed

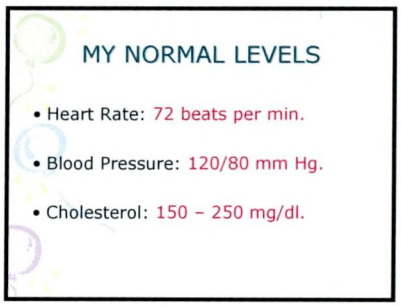
Plate 18

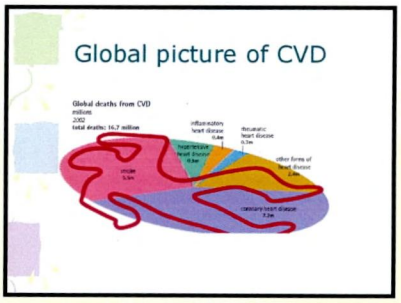
Displays of healthy meal

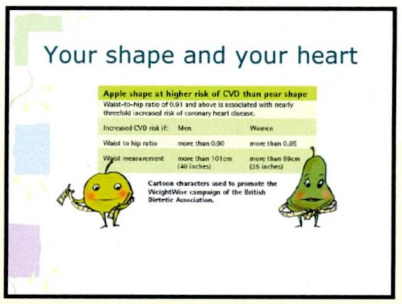


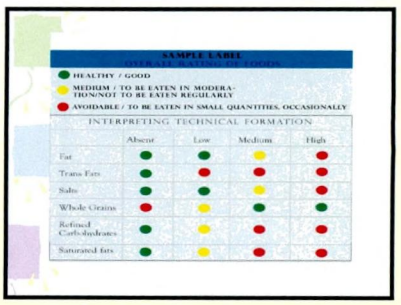


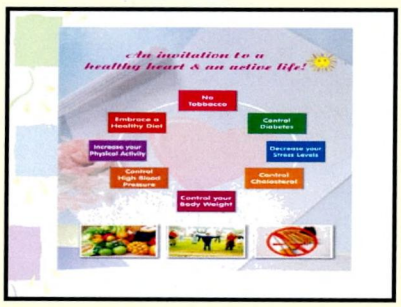


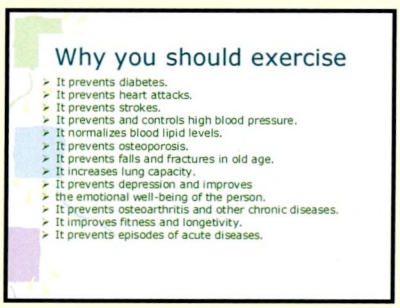


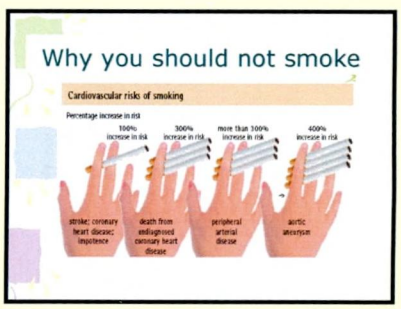












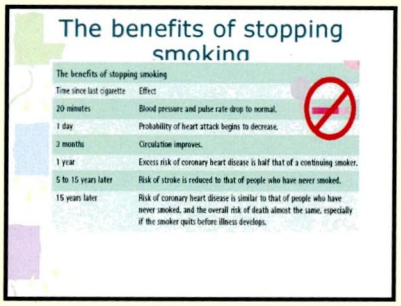


Plate 19

Slides prepared for power point presentation

All their doubts were cleared and myths were corrected. The last two sittings were individual contact sessions lasting for 30 minutes each (Plate 22). The aspects covered in the previous sessions were stressed upon and doubts were cleared. The patients were encouraged to follow the modified diet regimen along with lifestyle modifications.

D. Evaluation of the effect of diet counselling

Evaluation of the diet counselling was done in the following aspects.

1. Knowledge scores

A questionnaire was formulated specifically for this purpose (Appendix V) which comprised of fifty multiple choice questions developed based on the aspects covered in the counselling sessions. Each correct response was assigned score one and each incorrect answer was assigned zero. The questionnaire was administered before and after the counselling sessions. Completion time was on an average of 30 minutes. The pre and post test scores were computed. The percentage score of each participant was then calculated and was categorized as low (<25), average (26-50), good (51-75) and excellent (>75).

2. Healthy Eating Index

Indices are composite tools aiming to measure and quantify a variety of clinical conditions, behaviours, attitudes and beliefs that are difficult to be measured quantitatively and accurately. These indices are adequate tools concerning the evaluation of diet quality, while they have also shown moderate predictive ability in relation to chronic diseases and health determinants (Kourlaba *et al.*, 2009). Based on these guidelines, a food frequency questionnaire (Appendix VI) containing foods that are of nutritional significance in cardiovascular disease management was developed to reflect the various food groups as Healthy Eating Index. The frequency of consumption of each item indicated by the participants were transferred to a 5 point score system ranging from never (score-0) to 3 times per day (score-5) for heart healthy foods and from 3 times per day (score-0) to never (score-5)

Plate 20
Conduct of group diet counselling

Plate 21
Conduct of power point presentation

Plate 22
Conduct of individual diet counselling

for the foods to be avoided and restricted. The total scores were then converted to percentages.

3. Practice Index

According to the American Heart Association (Gidding *et al.*, 2009) effective counselling should generally reward behaviours that are considered satisfactory and ignore behaviours that are unsatisfactory. A ten point Practice Index (Appendix VII) with common practices related to cardiovascular disease like restriction of visible oil, consumption of fruits, consumption of vegetables, restriction of salt, restriction of sugar and sweets, restriction of ghee, restriction of pickle and papad consumption, consumption of low fat milk, restriction of fleshy foods, and regular moderate physical exercise was formulated. For each good practice followed regularly, a score of '10' was assigned and for an occasional practice, a score of '5' was assigned. A practice never followed was assigned '0' score. The total score was calculated before and after the counselling session. The scores were categorized as low (<25), average (26-50), good (51-75) and excellent (>75).

4. Anthropometry

Body weight, BMI, waist hip ratio and waist height ratio were assessed both before and after the diet counselling and the results were compared.

5. Biochemical estimation

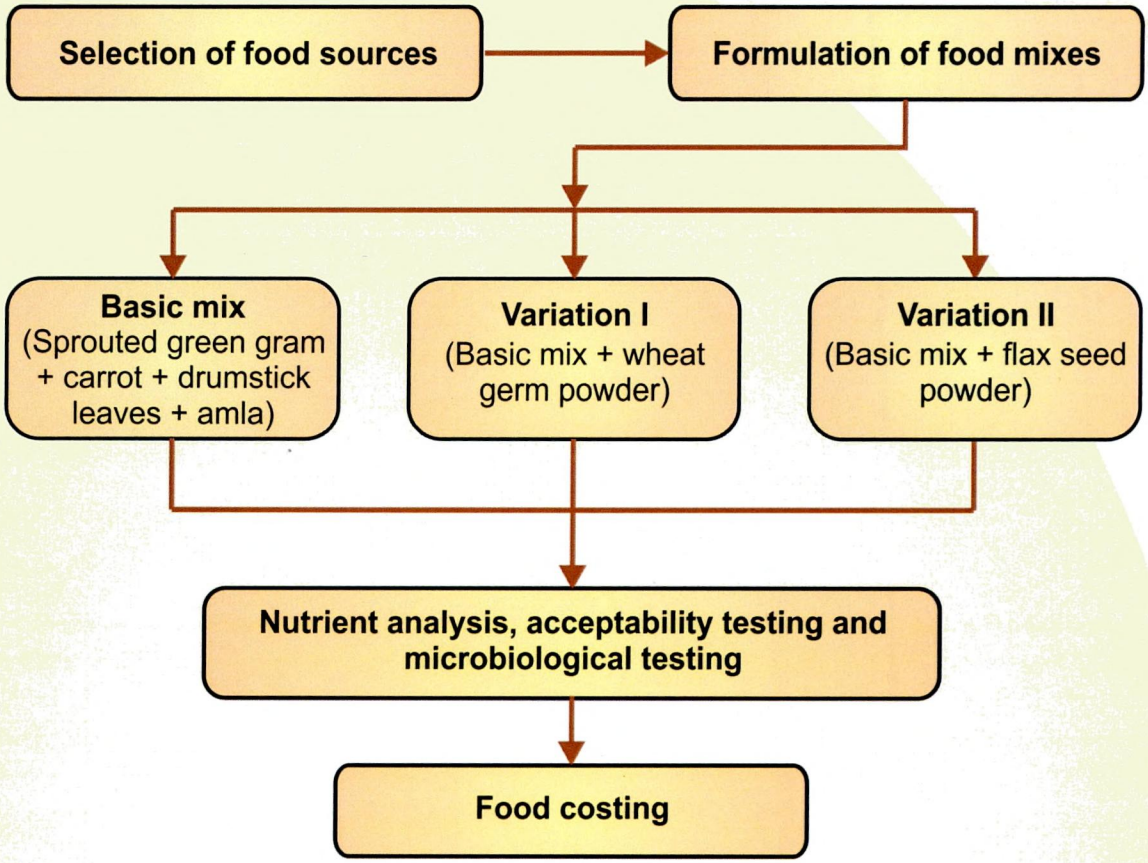
Biochemical estimation of lipid profile were done before and after counselling and compared to evaluate the effect of diet counselling.

Phase V

Data interpretation

The data that were obtained related to general, dietary and lifestyle background, functional food mix supplementation and diet counselling were entered in excel data sheet. Mean and standard deviation (SD) were calculated. Statistical significance was found out by performing student's 't' test wherever changes in parameters were observed.

Phase I



Phase II

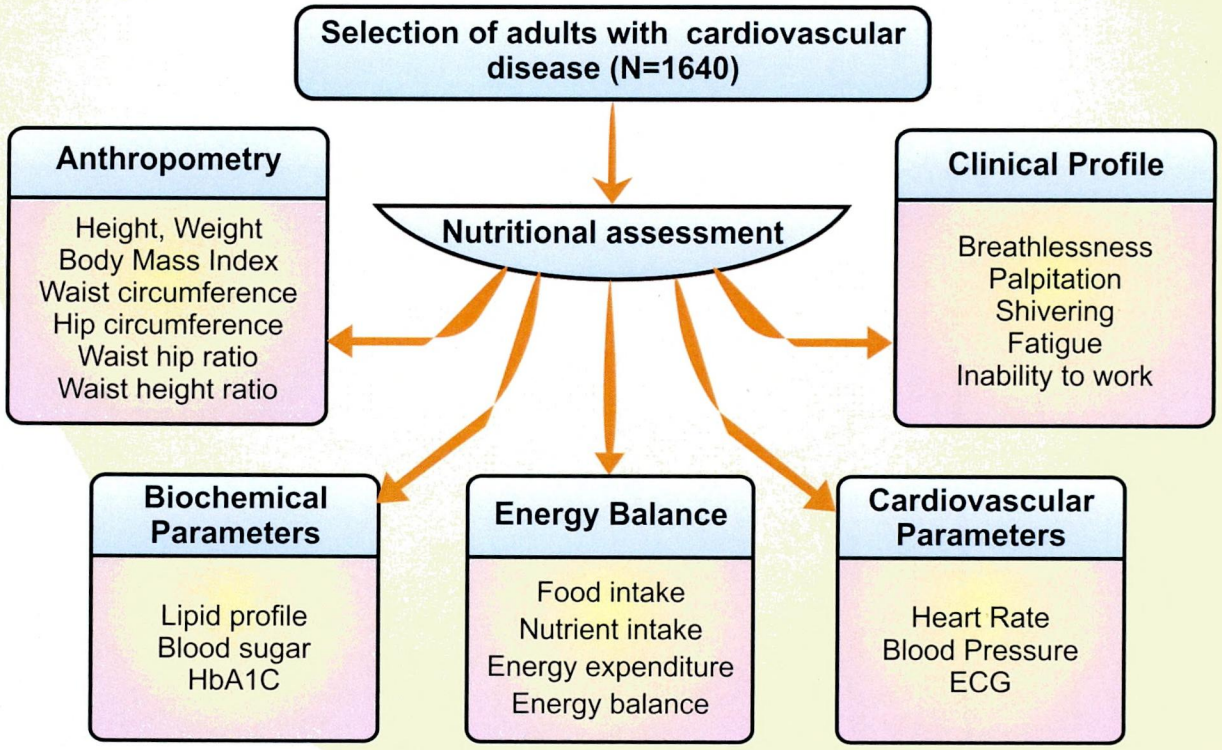


FIGURE 2
RESEARCH DESIGN

Phase III

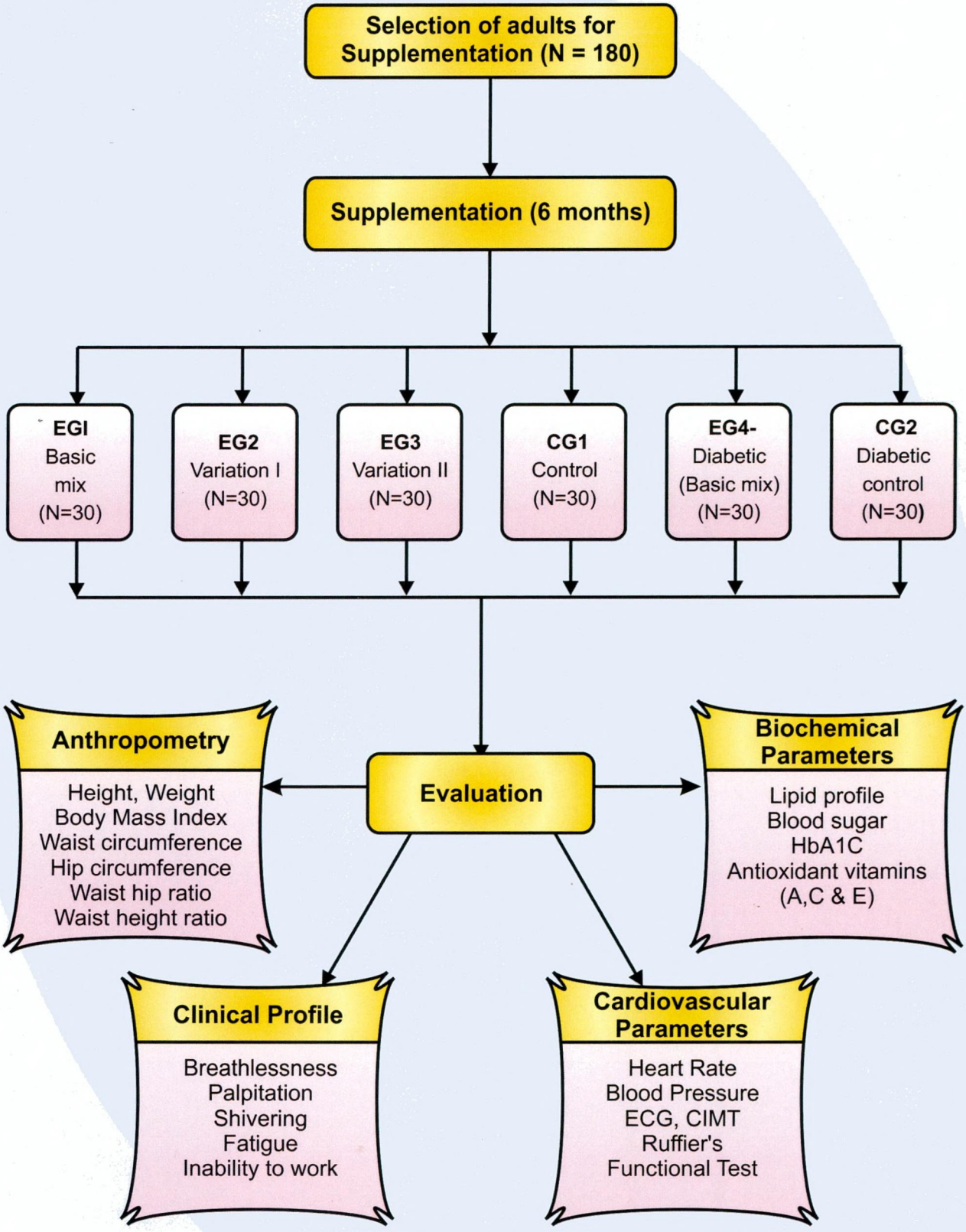


FIGURE 3
SUPPLEMENTATION STUDY

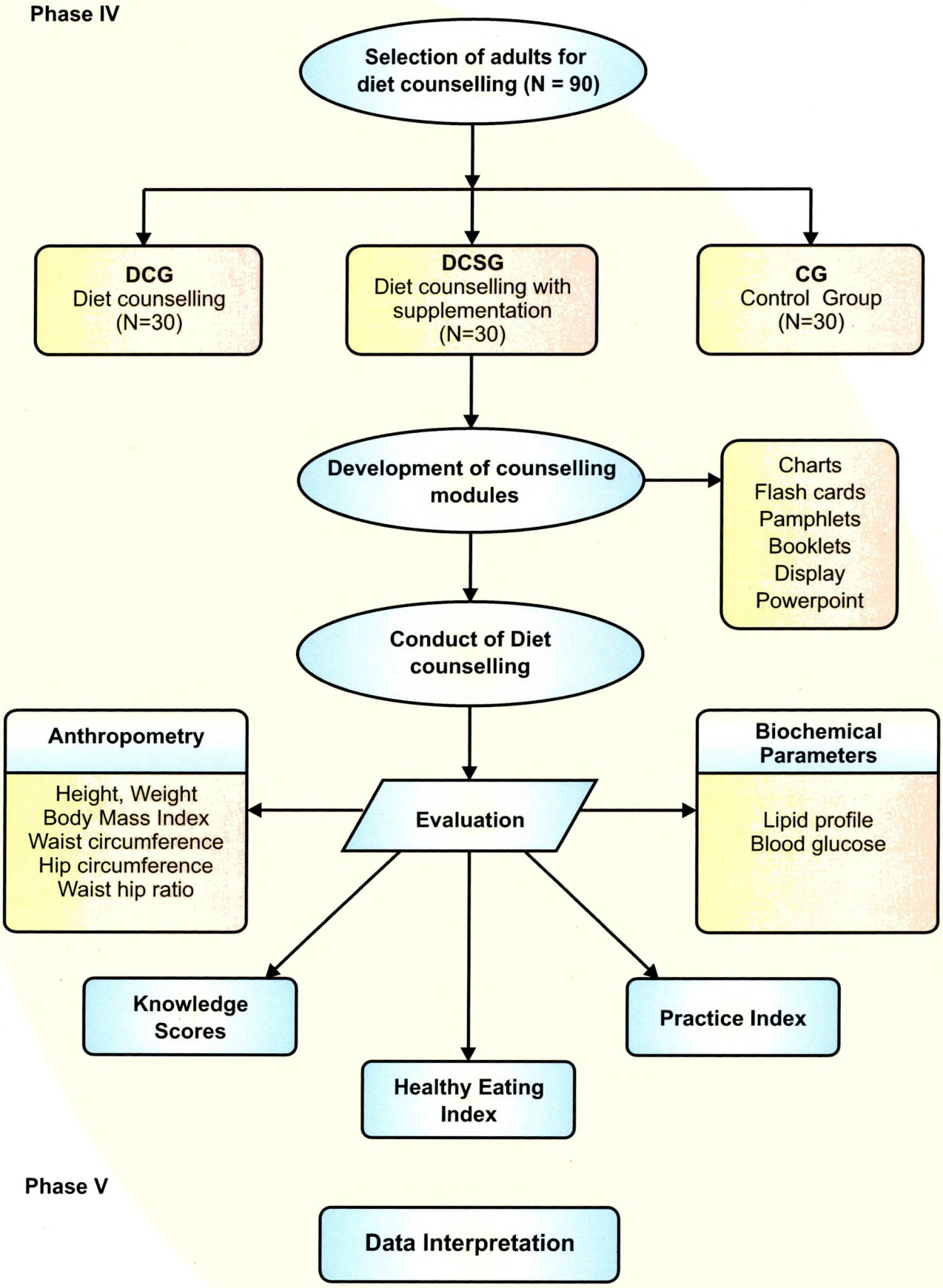


FIGURE 4
DIET COUNSELLING