Methods and Materials

NCDs are increasingly becoming a major cause of morbidity, disability, and mortality in the WHO South-East Asia Region. These diseases, which in the past were largely confined to industrialized countries, now account for over one-half of all deaths in low and middle income countries like India. The rapid changes in the economic, social, and demographic determinants of health as well as adoption of unhealthy lifestyles (e.g. consumption of tobacco and alcohol, unhealthy diet, physical inactivity) by large segments of population are contributing to the observed shift in the pattern of prevailing diseases and causes of death in this region. Planning of prevention and management strategies to control NCDs requires a systematic approach which involves surveillance of prevalence and risk factors of the various diseases. The second step would be to identify novel, cost effective and efficient therapeutic and alternate approaches and to assess their efficacy. The next step would be to involve public and private stake holders to promote these strategies in the community set up.

Keeping these aspects in mind we planned a study to look into the prevention of NCDs along with management of one of the NCDs (type 2 diabetes) using different approaches (Figure 28).

**Phase 1:** To study the Influence of Physical Activity on the Pathobiochemistry and prevalence of Secondary Complications in Diabetes Mellitus.

**Phase 2:** To study the efficacy of supplementation of Gymnema Sylvestre in Type 2 Diabetics.

**Phase 3:** To study the effectiveness of a Nutrition Health Promotion program in an industrial set up (DEAR study) for the non executive staff (Stage 1) and executive staff (Stage 2).
Figure 28: EXPERIMENTAL DESIGN OF THE STUDY

NUTRITION HEALTH PROMOTION PROGRAM (3) PATHOBIOCHEMISTRY OF DIABETES (1)

SUPPLEMENTATION (2)

Stage 1
(Non Executive)

Stage 2
(Executive)

Enrolment of subjects

Enrolment of subjects

Collection of Baseline data
(SES, Medical history, Family history, Personal habits, Dietary pattern & Physical Activity pattern)

Collection of Baseline data
(Ses, Medical history, Family history, Personal habits, Dietary pattern & Physical Activity pattern)

Assessment (One time)
(Anthropometry, Biophysical & Biochemical)

Assessment
(Anthropometry, Biophysical & Biochemical)

Intervention (3 months)

Experimental Controls
(3 months)

Supplementation No intervention

Collection of data (2nd month)

Collection of end data

STATISTICAL ANALYSIS
Phase I: Influence of Physical activity on the Pathobiochemistry of Diabetes Mellitus

This phase assesses the role of physical activity in the pathobiochemistry of diabetes by studying the clinical and metabolic profile of type 2 diabetics, and also its influence on the development of secondary complications. It is a retrospective, cross-sectional self-reported study.

Patient's Selection

A total of 212, type 2 diabetics (134 males; 78 females), were enrolled for the study from the out patient department of Swasthya Diabetic Clinic, All India Institute of Diabetes and Research at Ahmedabad, Gujarat, India with the consent of the consulting physician. The participants of the study were type 2 diabetics (as per WHO criteria), aged between 30 – 65 yrs and the duration of disease ranged from recently detected to a maximum of 35 years. Only diabetics who had come for a routine check up to the clinic were enrolled. Severely ill or disabled diabetics who were incapable of performing physical activity were excluded from the study. Also, diabetics who had come for consultation due to any severe or acute ailment or admitted in the clinic's nursing home due to conditions like diabetic ketosis, etc. were excluded from the study. The consulting physicians and specialists were actively involved in the clinical assessment of the subjects. Information regarding General Habits, Diet, Physical Activity, Clinical and Medical History of the subjects were obtained using a pretested, semi-structured questionnaire. Subjects underwent a one time comprehensive anthropometric, biophysical and biochemical check up.

Patient's History

Information regarding age, gender, occupation, socio economic status, family history of diabetes and any other NCDs, symptoms of onset of disease, precipitating factors, clinician comments on signs and symptoms, clinical history, treatment/medications, general habits (Substance abuse, Bowel bladder status, sleeping patterns, etc.), physical activity profile and
dietary intake (24 hr Dietary Recall Method) was elicited using a pretested semi structured questionnaire.

**Anthropometric parameters**

Height and Weight measurements: Height was measured without shoes to the nearest 0.5 cm and the weight of the subjects was measured on a pre-standardised weighing scale to the nearest 100g. BMI was calculated using the formula weight (Kg) divided by height (m²).

Waist and Hip measurements: Waist (WC) and Hip circumference (HC) were measured to the nearest centimetre with a dressmaker's tape. Subjects were made to stand straight and with their stomach relaxed. The tape was positioned mid-way between the top of the hip bone and the bottom of the rib cage. The tape measure was wrapped completely around the waist to obtain measurement. Subject was made to stand straight, feet together and with arms by their side with palms facing inwards. Hip circumference was measured 2 to 4 inches below navel level, the tape measure was wrapped and the circumference measured.

**Biophysical Parameters**

Blood Pressure (BP) determinants: BP was measured with the patient in a seating position after a five minute rest with standard clinical mercury sphygomanometer.

Percent body fat (% fat) was estimated using a standard fat monitor (Omron Model No HBF-306). The body fat in kilograms was derived from the percent fat value obtained from the machine.

Physical and Systemic examination were conducted to assess the subjects’ clinical conditions and development of secondary complications. The diagnostic techniques used were ECG and ECHO (wherever necessary) for assessment of cardiovascular status, peripheral pulsations and sensations to assess nerve status, Fundoscopy to assess development of retinopathy and Sonography to assess general abdominal status. The criteria used for diagnosis of complications are given in Table 14.
Table 14: Criteria for Diagnosis of Diabetic Complications.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Diagnostic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>SBP ≥ 140mmHg; DBP ≥ 90mmHg</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>ATP III Guidelines</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>Abnormal Fundoscopy</td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>Abnormal ECG, ECHO, TMT</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>Abnormal peripheral pulsations and sensations</td>
</tr>
<tr>
<td></td>
<td>Parathesia</td>
</tr>
<tr>
<td>Nephropathy/Impaired renal status</td>
<td>Previously diagnosed condition</td>
</tr>
<tr>
<td></td>
<td>MAU &gt; 30 mg/gm</td>
</tr>
<tr>
<td>Stroke</td>
<td>Previously diagnosed condition</td>
</tr>
</tbody>
</table>

Biochemical Evaluations and Assay Methods

Venous blood samples (for whole blood and serum analyses) after an overnight fast (12 hrs) were collected in appropriate vaccutainers. Urine samples of the subjects were also collected. Details of the tests conducted and the principles of the various methods used to analyze them are given herewith.

Blood glucose:

It was estimated by GOD/POD method using an enzymatic kit procured from Bayer Diagnostics, India. Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of peroxide (POD) oxidizes the chromogen, 4-aminoantipyrine/phenolic compound to a red coloured compound. The
intensity of the colour is proportionate to the glucose concentration and is measure at 505nm (490 – 530nm). The final colour is stable for two hours.

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconate} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\text{H}_2\text{O}
\]

(Red coloured complex)

**Total Cholesterol (TC)**

Cholesterol was estimated using enzymatic kit (Randox laboratories, USA) by the enzymatic end-point method. Cholesterol esters are hydrolysed to free cholesterol and fatty acids by cholesterol esterase (CHE). The free cholesterol is then oxidised by cholesterol oxidase (CHOD) to cholest-4-en-one and hydrogen peroxide. Liberated hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to produce quinonimine, a red coloured complex. The intensity of the colour is directly proportional to the total cholesterol in the sample, which is measured at 500nm (Hg 546nm). The final colour is stable for 60 minutes.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{CHE}} \text{Free cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-one} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine} + \text{H}_2\text{O}
\]

(Red coloured complex)

**High Density Lipoprotein Cholesterol (HDL – C)**

Enzymatic kit procured from Randox laboratories, USA was used for Direct HDL – C cholesterol determinations. The assay consists of two distinct reaction steps. Firstly, cholesterol esterase, cholesterol oxidase and subsequently catalase eliminate the VLDL – C, LDL – C and chylomicrons. Secondly, cholesterol eater is hydrolysed by cholesterol esterase
to cholesterol and fatty acid. The cholesterol is then oxidised to cholestenone and hydrogen peroxide by cholesterol oxidase. Hydrogen peroxide in the presence of peroxides reacts with 4-aminoantipyrine and HDAOS to produce a quinine pigment. The intensity of the quinonimine produced is directly proportional to the cholesterol concentration when measured at 600nm.

\[
\text{Serum} + \text{Precipitating agent} \rightarrow \text{Precipitate (VLDL + LDL)} + \text{Supernatant (HDL)}
\]

Cholesterol ester + H2O \(\xrightarrow{\text{CHE}}\) Free cholesterol + fatty acids

Cholesterol + O2 \(\xrightarrow{\text{CHOD}}\) Cholest-4-en-one + H2O2

\(H_2O_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O\) (Red coloured complex)

**Low Density Lipoprotein Cholesterol (LDL - C)**

The serum LDL-C concentration was calculated by the Friedwald’s formula (1972).

\[
\text{LDL-C} = \text{TC} - [\text{HDL} + (\text{TG}/5)]
\]

**Very Low Density Lipoprotein Cholesterol (VLDL)**

The serum LDL-C concentration was calculated by the Friedwald’s formula (1972).

\[
\text{VLDL - C} = \text{TG} / 5
\]

**Non High Density Lipoprotein Cholesterol (Non – HDL)**

Non – HDL was calculated by formula.

\[
\text{Non - HDL} = \text{TC} - \text{HDL} - \text{C}
\]

**Apolipoproteins A1 (Apo A1)**
Apolipoproteins were measured using antigen – antibody reaction by the end – point reaction. 10µL sample and controls are mixed with 900 µL buffer. The optical density is read at 340nm. To this 80 µL of the anti-serum is added. The solution is mixed and incubated for 5 minutes at room temperature. The optical density of the samples is re-read at 340nm.

**Apolipoprotein B (Apo B)**

Apolipoproteins were measured using antigen – antibody reaction by the end – point reaction. 40µL sample and controls are mixed with 900 µL buffer. The optical density is read at 340nm. To this 80 µL of the anti-serum is added. The solution is mixed and incubated for 5 minutes at room temperature. The optical density of the samples is re-read at 340nm.

**Total Lipids (TL)**

The method of Frings *et al* (1972) was employed for total lipid estimations. Lipids in the presence of sulphuric acid and phosphoric acid react with vanillin to form a chromophore, which is read at 540nm.

**Triglycerides (TG)**

Enzymatic kit using GPO – PAP method (Randox Laboratories, USA) was used for triglyceride estimation. Triglycerides are hydrolysed by lipase (LPL) to glycerol and FFA. Glycerol is phosphorylated by ATP in the presence of glycerol kinase (GK) to glycerol -3-phosphate which is oxidised by the enzyme, glycerol-3-phosphate oxidase (GPO) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4 aminoantipyrine and p-chlorophenol in the presence of peroxidise (POD) to produce quinonimine, a red coloured complex, which is measured at 500nm (Hg 546nm). The final colour is stable for 60 minutes.

\[
\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{FFA} \\
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}
\]
Creatinine was estimated using standard reagent kits from where the alkaline picrate method is used for Creatinine determination. Creatinine present in serum or urine reacts with alkaline picrate to form a coloured complex. The intensity of the colour developed is proportional to Creatinine concentration and is measured photometrically at 510nm (505 – 530nm) or with a green filter.

Glycated Haemoglobin (HbA1c)

The VARIENT Haemoglobin A1c estimation utilises the principle of ion exchange high performance liquid chromatography (HPLC) for the separation of HbA1c without interference from Schiff base, lipemia and temperature fluctuations. The VARIENT’s two dual piston pumps deliver a programmed buffer gradient of increasing ionic strength to the system. Samples for analysis are diluted with hemolysis reagent (citrate solution, pH 5.0) and incubated at 18 – 28 °C for 15 minutes for Schiff base removal. The samples are then placed in the sample compartment of the instrument, automatically injected into the analytical flow path and applied to the cation exchange column, where the haemoglobin is separated based upon the attraction of the haemoglobin to the column material. The separated haemoglobin is then passed through the flow cell of the filter photometer, where changes in the absorbance are measured at 415nm; background variations are corrected at 690nm. A calibrator (lyophilised human red blood cell hemolysate containing gentamicin, tobramycin and EDTA preservatives) is analysed with each run for the determination of HbA1c. A chromogram of the changes in the absorbance is plotted versus the retention time (Bio-Rad Laboratories 2000).
Phase 2: Supplementation of Gymnema sylvestre among type 2 diabetics

This phase assesses the efficacy of Gymnema sylvestre (Gurmar, Meshashringi) in the management of type 2 diabetes. The study design was a quasi-experiment.

Patient selection

A total of 58 type 2 diabetics were enrolled for the study from the free-living population in Vadodara, Gujarat. They were counselled about the benefits of GS and requisites of the study that the diet, physical activity pattern and drug usage during the study period should remain unaltered. The diabetic patients were then divided purposively into two groups: control (n=19) and the experimental (n=39) group. The selection criterion of the group in this quasi-experiment was based on the willingness to participate in the study. Information regarding general habits, dietary intake, physical activity pattern, clinical and medical history of the subject was collected using a pre-tested, semi-structured questionnaire. The questionnaire was also used to elicit information and qualitatively assess the commonly occurring symptoms in diabetic patients (like appetite, polyphagia, polyuria, fatigue, bowel-bladder status, etc.) before and after intervention.

Intervention

The experimental group was given 250 mg of Gymnema sylvestre, in the form of capsules containing extracts of the leaves to be consumed twice a day before meals for a period of three months. Their anthropometric, biophysical and biochemical parameters were assessed thrice - at the beginning of the study (baseline), after 2 months (60 days) and after three months (90 days). The control group received no supplementation or placebo and their anthropometric, biochemical and biophysical parameters were assessed twice - at baseline and three months after enrolment (Figure 29). An approval from the departmental medical ethical committee was obtained before conducting the clinical trial.
Figure 29: Experimental Design of Phase 2

Enrolment of Diabetics (n=58)
(Counselling about GS and necessity of unaltered diet & drug during study period)

Collection of Baseline data
(SES, Medical history, Family history, Personal habits, Dietary pattern & Physical Activity pattern)

Baseline Assessment
(Anthropometry, Biophysical & Biochemical)

Subjects purposively divided into 2 groups.
(Selection bias: Willingness to participate in the study)

Experimental group
(n = 39)
Supplementation (Duration 3 months)
Collection of data (2nd month)
Supplementation

Control group
(n = 19)
No intervention

Collection of end data
(Anthropometry, Biophysical & Biochemical)

Statistical analysis
Anthropometric parameters

Height and Weight measurements: Height was measured without shoes to the nearest 0.5 cm and the weight of the subjects was measured on a pre-standardised weighing scale to the nearest 100g. BMI was calculated using the formula weight (Kg) divided by height (m²).

Waist and Hip measurements: Waist (WC) and Hip circumference (HC) were measured to the nearest centimetre with a dressmaker’s tape using the methodology described in Phase 1.

Biophysical Parameters

Blood Pressure (BP) determinants: BP was measured with the patient in a seating position after a five minute rest with standard electronic sphygmomanometer. Percent body fat was estimated using a standard fat monitor (Omron Model No HBF-306). The body fat in kilograms was derived from the percent fat value obtained from the machine.

Biochemical Evaluations and Assay Methods

Venous blood samples (for whole blood and serum analyses) after an overnight fast (12 hrs) and 2 hrs after consumption of a meal (lunch) were collected in appropriate vacutainers. Urine samples of the subjects were also collected. Details of the tests conducted and the principles of the various methods used to analyze them are given herewith.

Fasting Blood Glucose (FBS)

FBS was estimated using enzymatic reference method with hexokinase. Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by NAD⁺ to form NADH. The concentration of the NADH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm.
D-Glucose + ATP $\xrightarrow{\text{HP}}$ D-glucose-6-phosphate + ADP

D-Glucose-6-phosphate + NADH$^+$ $\xrightarrow{\text{G6DH}}$ D-6-phosphogluconate + NADH

**Fasting insulin**

Insulin levels were determined by chemiluminescence methodology using the sandwich immunoassay principle. The estimation was carried out in Immulite 2000.

**Total Cholesterol (TC)**

Total cholesterol was estimated using end point enzymatic colorimetric technique. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye. The colour intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

Cholesterol esters + H$_2$O $\xrightarrow{\text{CE}}$ Cholesterol + RCOOH

Cholesterol + O$_2$ $\xrightarrow{\text{CHOD}}$ cholest-4-ene-3-one + H$_2$O$_2$

2 H$_2$O$_2$ + 4-AAP + phenol $\xrightarrow{\text{POD}}$ quinone-imine dye + 4 H$_2$O
HDL Cholesterol (HDL – C)

HDL fraction of Cholesterol was determined using enzymatic, colorimetric method (CHOD/PAP) without sample pre-treatment. The principle of HDL-Cholesterol Direct is based on the adsorption of synthetic polyanions to the surface of lipoproteins. LDL, VLDL, and chylomicrons are thereby transformed into a detergent-resistant form, whereas HDL is not. Combined action of polyanions and detergent solubilises cholesterol from HDL, but not from LDL, VLDL, and chylomicrons. Solubilised cholesterol is oxidized by the sequential enzymatic action of cholesterol esterase (CE) and cholesterol oxidase (CHOD). The hydrogen peroxide formed reacts with N, N-bis (4-sulfobutyl)-m-toluidine (DSBmT) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) and forms a red quinoneimine dye. The colour intensity of the red quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 552 nm.

LDL, VLDL, chylomicrons + polyanions → lipoprotein-polyanion

HDL + detergent → micelle complexes

Micelle complexes → oxidized cholesterol + H₂O₂

H₂O₂ + 4-aminoantipyrine + DSBmT → quinoneimine dye

LDL Cholesterol (LDL – C)

Enzymatic, colorimetric method (CHOD/PAP) was used for the direct estimation of LDL – C. HDL, VLDL, and chylomicrons are specifically hydrolyzed by a detergent. The released cholesterol contained in these lipoproteins reacts immediately in the enzymatic action of cholesterol esterase (CE) and cholesterol oxidase (CHOD) generating hydrogen peroxide.
The latter is consumed by a peroxidase (POD) in the presence of 4-aminoantipyrine to generate a colourless product. During this first step, LDL particles remain intact. The reaction of LDL cholesterol is initiated by the addition of another detergent together with a coupler, N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT). The second detergent releases cholesterol in the LDL particles which are subjected to the enzymatic reaction in the presence of coupler to produce a coloured product. The colour intensity of the red quinoneimine dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 552 nm.

**Step 1**

\[
\text{HDL, VLDL, chylomicrons + detergent} \rightarrow \text{released cholesterol}
\]

\[
\text{Cholesterol} \xrightarrow{\text{CE/CHOD}} \text{oxidized cholesterol + H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{colorless product}
\]

**Step 2**

\[
\text{LDL + detergent} \rightarrow \text{released cholesterol}
\]

\[
\text{Cholesterol} \xrightarrow{\text{CE/CHOD}} \text{oxidized cholesterol + H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine + DSBmT} \xrightarrow{\text{POD}} \text{quinoneimine dye}
\]

**Triglycerides (TG)**

Enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone was used to assess triglycerides. Triglycerides are hydrolyzed by
lipoprotein lipase (LPL) to glycerol and fatty acids. Glycerol is then phosphorylated to
glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK). The oxidation
of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form
dihydroxyacetone phosphate and hydrogen peroxide ($H_2O_2$). In the presence of peroxidase
(POD), hydrogen peroxide effects the oxidative coupling of 4-chlorophenol and 4-
aminophenazone to form a red-coloured quinoneimine dye, which is measured at 512 nm.
The increase in absorbance is directly proportional to the concentration of triglycerides in the
sample.

$$\text{Triglycerides} \xrightarrow{LPL} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{GK} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + O}_2 \xrightarrow{GPO} \text{dihydroxyacetone phosphate + H}_2\text{O}_2$$

$$2\text{H}_2\text{O}_2 + 4\text{-aminophenazone + 4-chlorophenol} \xrightarrow{POD} \text{quinoneimine + 4 H}_2\text{O}$$

**Apolipoprotein A\(_1\) (Apo A\(_1\))**

Apolipoproteins are the protein constituents of the lipoproteins. The lipoproteins are
classified according to their ultracentrifugal flotation density. Apolipoprotein A-1 is the
major protein constituent of high-density lipoproteins (HDL). The methodology used to
assess Apo A was rate nephelometry where, the rate of increase in light scattered from
particles suspended in solution, as a result of complexes formed during an antigen –antibody
reaction is measured. In the performance of the APA test antibody to human apolipoproteins
A-1 is brought in to contact with human apolipoprotein A-1 in a sample. The increase in light
scatter resulting from the antigen antibody reaction is converted to a peak rate signal, which
is a function of the sample apolipoprotein A-1 concentration. Following calibration of a
particular analyte, the peak rate signal for that particular assay is automatically converted to
concentration units by the analyzer. The APA test measures the rate of increase in light
scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

Apolipoprotein A₁ (sample) + Antibody $\rightarrow$ Apolipoprotein A₁ (sample) - Antibody (aggregates)

Apolipoprotein B (Apo B)

Apolipoprotein B is the major constituent of LDL. The combined determination of apolipoprotein A-1 and apolipoprotein B and the calculation of the apolipoprotein B/apolipoprotein A-1 ratio can reflect a disorder of lipid metabolism and the risk of developing atherosclerosis and coronary heart disease particularly well providing an excellent addition to the classical HDL/LDL cholesterol determination. Apolipoprotein was measured by rate nephelometry technique, the rate of increase in light scattered from particles suspended in solution, as a result of complexes formed during an antigen-antibody reaction is measured. In the performance of the APB test antibody to human apolipoprotein B is brought in to contact with human apolipoprotein B in a sample. The increase in light scatter resulting from the antigen antibody reaction is converted to a peak rate signal, which is a function of the sample apolipoprotein B concentration. Following calibration of a particular analyte, the peak rate signal for that particular assay is automatically converted to concentration units by the analyzer. The APB test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

Apolipoprotein B (sample) + Antibody $\rightarrow$ Apolipoprotein B (sample) - Antibody (aggregates)

Glycated Hemoglobin (HbA₁c)

HbA₁c was quantitatively assayed using IFFC and FDA approved automated dedicated high performance liquid chromatography (HPLC) method using the NGSP certified dedicated kit. The principle used ion exchange HPLC. The samples are automatically diluted on the d-10,
injected into the analytical flow path, and applied to the analytical cartridge. The d-10 deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the haemoglobin is separated based on their ionic interactions, then pass through the flow cell of the filter photometer, where change in the absorbance at 415 nm are measured. The d-10 software performs reduction of raw data collected from each analysis; two level calibrations are used for quantization of the HbA1c values. A sample report and a chromatogram are generated for each sample. The A1c area is calculated using an Exponentially Modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area. HbA1c covers all fractions, this includes labile HbA1a, HbA1b and pre HbA1c. These fractions are labile and hence do not represent the stable or long term change. HbA1c represents the true long term glycemic control.

SGOT

This test is carried out for the quantitative determination of Aspartate aminotransference (AST) in blood. It is helpful in the evaluating the diseases status related to Cardiac, skeletal muscle & liver. It uses Kinetic method, according to International Federation of Clinical Chemistry (IFCC), without pyridoxal-5'-phosphate. AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD⁺. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm.

\[
\text{L-Aspartate} + 2\text{-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+
\]

SGPT

This test is carried out for the quantitative determination of Alanine aminotransferase (ALT) in blood. Its determination is helpful in the evaluating the diseases status related to liver. It
uses Kinetic method, according to the International Federation of Clinical Chemistry (IFCC), without pyridoxal-5'-phosphate. ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD⁺. The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm.

$$L\text{-Alanine} + 2\text{-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + L\text{-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

**C - Reactive Protein (CRP)**

CRP is an acute phase reactant, beta globulin in nature. Ultra sensitive CRP is an important aid in the detection and evaluation of Infection, Tissue Injury, Inflammatory disorders and associated diseases. It has high sensitivity and high negative predictive value. It rises and decreases rapidly within 4 to 8 hrs according to inflammatory activity, thus is suitable to detect current inflammation. Elevated values are associated with pathological changes and provide information for diagnosis, therapy and monitoring of inflammatory conditions and associated diseases. High sensitivity CRP adds to the predictive value of other markers used to assess the risk of cardiovascular and peripheral vascular diseases. The estimation was carried out in an Immulite 2000 using Sandwich Immunoassay, Chemiluminescence methodology.

**Microalbumin (MAU)**

Microalbumin is the most important marker for glomerular dysfunction, diagnosis of kidney diseases and to monitor incipient diabetic nephropathy. It is determined using Immunoturbidimetry. Human albumin present in urine forms precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.
Phase 3 Nutrition Health Promotion program in an industrial set up
(DEAR study)

This section assesses the role of a unique multifaceted Nutrition Health Promotion Program for the executive and non-executive staff. Both the programs were customised for their target population and conducted in a single industry that had two mutually exclusive. The program was conducted in two stages each of which involved the following;

Step 1: Health Assessment
- Introduction to Wellness program
- Enrolment of the subjects
- Lifestyle factors
- Blood Profile, Anthropometric, Clinical assessment
- Assessment of Knowledge, attitude and practices
- Food Consumption pattern and dietary habits study

Step 2: Results Evaluation Session
- Evaluation of Metabolic Risk Factors

Step 3: Intervention opportunities
- Health and wellness counselling for prevention and follow up
- Health and wellness counselling for management
- Nutrition Health Education

Step 4: Health and Nutrition Tracking
- Monitoring Exercise patterns
- Complete Blood Profile, Anthropometric, Clinical and Fitness Tests
- Questionnaires to assess knowledge, attitude and practices

Step 5: Health outcomes
- Participation rates and Feedback
- Lifestyle and Behavioural changes
- Desirable Biometric changes
- Healthier Factory kitchen practices (if present)
Stage 1: Prevention of non-communicable diseases through dietary counselling and focused physical activity in an industrial set up – (DEAR study)

Subject Selection

Employees of an industry (Halol, Gujarat, India) and their spouses were purposively enrolled (n=39). Data on their socio economic status, anthropometry, medical history, dietary history, physical activity pattern and was elicited. Knowledge, Attitude and Practices were assessed using a comprehensive KAP questionnaire. A comprehensive biochemical and biophysical analysis was conducted at baseline, post intervention for employees and their spouses. The effectiveness of the intervention was determined by carrying out anthropometric and biochemical assessments for the employee's a year after NHE counselling sessions were withdrawn.

Subjects's History

Information regarding age, gender, occupation, socio economic status, family history of diabetes and any other NCDs, symptoms of onset of disease, precipitating factors, clinician comments on signs and symptoms, clinical history, treatment/ medications, general habits (Substance abuse, Bowel bladder status, sleeping patterns, etc.), physical activity pattern (7 day activity recall) and dietary intake (3 day dietary recall and food frequency method) was elicited using a pretested semi structured questionnaire.

Anthropometric parameters

Height and Weight measurements: Height was measured without shoes to the nearest 0.5 cm and the weight of the subjects was measured on a pre – standardised weighing scale to the nearest 100g. BMI was calculated using formula that is weight (Kg) divided by height (m²).

Waist and Hip measurements: Waist and hip circumferences were measured to the nearest centimetre with a dressmaker’s tape.
Biophysical Parameters

Blood Pressure (BP) determinants: BP was measured with the patient in a seating position after a five minute rest with standard electronic sphygmomanometer. Percent body fat was estimated using a standard fat monitor (Omron Model No HBF-306). The body fat in kilograms was derived from the percent fat value obtained from the machine. ECG of the subjects was monitored using a standardized ECG machine.

Physical Activity Pattern

The subjects were given a diary to maintain their physical activity pattern for a week. The pattern was then used to identify sedentary behaviour and calculate their average Energy Expenditure using The FAO report series on Human Energy Requirements (2001).

Assessment of Dietary intake

The dietary intake of the subjects was assessed using the 3 day recall method and the pattern of food consumption was assessed using a food frequency questionnaire. The nutritive values of the foods consumed were calculated from the raw ingredients using the values given in “Nutritive Value of Indian Foods” by Gopalan.

Biochemical evaluation and assays

Venous blood samples (for whole blood and serum analyses) after an overnight fast (12 hrs) were collected in appropriate vaccutainers. The sample was used to analyse FBS, Fasting Insulin, TC, TG, LDL – C, HDL – C and HbA1c. The methodologies of these assays have been described in Phase 2.

Intervention:

A series of Nutrition Health Education (NHE) lectures were conducted along with focused physical activity for the employees for duration of three months (Figure 30).
Nutrition Health Education (NHA) was then imparted using the following media: (a) Seminars (b) Focused physical activity (c) Practical sessions (d) Educational material.

The purpose of conducting these multiple approach nutrition advocacy sessions was to create awareness among the people that would lead to sustained behavioural changes. The program conducted was as follows; Nutrition health education (NHE) seminars were held once a week using PowerPoint presentations for employees and their spouses in the industrial premises. The group counselling sessions conducted in the local dialect or Hindi covered a wide variety of subjects like Non-communicable diseases (NCDs) – its Causes, Effects, Prevention and Management, Lifestyle practices, Nutrients – their Role, Requirement and Sources, Energy Intake and Expenditure, Good eating practices, Meal and Menu planning. Practical sessions were given on Aerobic exercises, Yoga, Stress management. Education imparted was constantly reinforced using NHE posters placed at key decision making points, NHE booklets and verbal reminders. The employees and their spouses also received practical group counselling sessions on aerobic exercises, yoga and stress management where personal health queries were also addressed.

Physical activity was promoted among the employees by educating them about the ways and means to increase energy expenditure. In an attempt to promote physical activity among them, a walking track was made to facilitate a safe and healthy environment for exercise where the motivated employees indulged in brisk walking for 30 min. This focused and supervised physical activity was carried out 6 times a week.

A situational analysis of the canteen food was also performed. Then the canteen staff was given special education on hygiene and nutrients that derived from various food sources, their importance and requirements. Sessions of meal preparations were conducted to practically show methods of safe and effective cooking and a new menu was also planned and implemented with consensus with the representatives of the factory’s canteen committee.
Figure 30: Experimental Design of DEAR study Stage 1

**Enrollment of subjects**

<table>
<thead>
<tr>
<th>Data Collected:</th>
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</thead>
<tbody>
<tr>
<td>• Socio Economic Status</td>
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<tr>
<td>• KAP</td>
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<tr>
<td>• Anthropometry (Ht, Wt, WC, HC)</td>
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<td>• Clinical History</td>
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<tr>
<td>• Biophysical (BP, Pulse, % fat)</td>
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<td>• Biochemical (FBS, Insulin assay, Lipid Profile, HbA1c)</td>
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<tr>
<td>• Dietary intake (3-day recall, Food frequency)</td>
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<tr>
<td>• Physical activity pattern (7 day activity recall)</td>
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</tbody>
</table>

**INTERVENTION (DURATION 3 MONTHS)**

- Nutrition Health Education
  - Non Communicable Diseases
  - Causes & effects of NCDs
  - Prevention and Mgmt of NCDs
  - Nutrients - role, importance, sources & requirements
  - Meal Planning
  - Aerobic Exercises, Stretches & Area specific reduction exercise
  - Yoga
  - Stress Management

- Focused Physical activity
  - Brisk walking for 30 mins, 6 days/week

- Capacity Building
  - Organization:
    - Provision of safe, convenient and accessible physical activity area
    - Posters on nutrition education
    - Canteen situation analysis
    - Nutritious and hygienic meal preparation and canteen menu change
  - Individual:
    - Nutrition education material (Posters & booklet in the local dialect)
    - Knowledge Attitude & Practices

**Post Data Collection**

<table>
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<tr>
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<tbody>
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<td>• Physical activity pattern (7 day activity recall)</td>
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**1 year after intervention**

<table>
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<tr>
<td>• Dietary intake (Food frequency)</td>
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<td>• Physical activity pattern</td>
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</table>
Stage 2 Nutrition Health Promotion Program in an Industrial office set up using electronic media (DEAR study)

Subject Selection

Employees of an industry (Vadodara, Gujarat, India) were purposively enrolled (n=21). Data on their socio economic status, anthropometry, medical history, dietary history, physical activity pattern and was elicited. Knowledge, Attitude and Practices were assessed using a comprehensive KAP questionnaire. A comprehensive biochemical and biophysical analysis was conducted at baseline, post intervention.

Subject's History

Information regarding age, gender, occupation, socio economic status, family history of diabetes and any other NCDs, symptoms of onset of disease, precipitating factors, clinician comments on signs and symptoms, clinical history, treatment/ medications, general habits (Substance abuse, Bowel bladder status, sleeping patterns, etc.), physical activity pattern (7 day activity recall) and dietary intake (3 day dietary recall and food frequency method) was elicited using a pretested semi structured questionnaire.

Anthropometric parameters

Height and Weight measurements: Height was measured without shoes to the nearest 0.5 cm and the weight of the subjects was measured on a pre – standardised weighing scale to the nearest 100g. BMI was calculated using formula that is weight (Kg) divided by height (m²).

Waist and Hip measurements: Waist and hip circumferences were measured to the nearest centimetre with a dressmaker’s tape.

Biophysical Parameters

Blood Pressure (BP) determinants: BP was measured with the patient in a seating position after a five minute rest with standard electronic sphygmomanometer. Percent body fat was
estimated using a standard fat monitor (Omron Model No HBF-306). The body fat in kilograms was derived from the percent fat value obtained from the machine. ECG of the subjects was monitored using a standardized ECG machine.

**Biochemical evaluation and assays**

Venous blood samples (for whole blood and serum analyses) after an overnight fast (12 hrs) were collected in appropriate vacutainers. The sample was used to analyse FBS, Fasting Insulin, TC, TG, LDL – C, HDL – C and HbA1c. The methodologies of these assays have been described in Phase 2.

**Intervention**

A custom made, user friendly, interactive website ([www.e-sanjeevani.com](http://www.e-sanjeevani.com)) was designed to promote Nutrition Health education in an office set up (Figure 31). This was considered appropriate as the employees could access the information at a time convenient to them. The employee’s were supposed to access the site for a minimum duration of 15 mins/day for a period of three months. The site provided them information of lifestyle practices, food, physical activity and NCDs. It also equipped them to calculate simple anthropometric parameters and calculate energy intake and expenditure. The site also has a section where their personal queries could be addressed and feedbacks registered.

**Statistical Analysis**

Data collected in each of the phases conducted were analyzed appropriately using Microsoft Excel and SPSS 11.5 software’s to derive meaningful results. Results are expressed as Mean ± SD. Analysis of differences between means was performed using students “t” test. Other statistical analysis techniques used in the different phases were Chi square test, Fisher’s exact test, Pearson’s correlation, ANOVA and Multivariate analysis in general linear model [95% Confidence Interval (CI)]. All the tests were considered significant at $p < 0.05$ level.
**Figure 31: Experimental Design of DEAR study Stage 2**

<table>
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<td><strong>Data Collected:</strong></td>
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</tbody>
</table>

**INTERVENTION (DURATION 3 MONTHS):**

- **Nutrition Health Education**
  - Non Communicable Diseases
  - Causes & effects of NCDs
  - Prevention and Mgmt of NCDs
  - Nutrients - role, importance, sources & requirements
  - Meal Planning
  - Aerobic Exercises, Stretches & Area specific reduction exercise
  - Yoga
  - Stress Management

- **Self Health assessment tools**

- **Capacity building**
  - Nutrition education in the form of website
  - Knowledge Attitude & Practices

- **Feedback/ interaction on demand**

- **Enrollment of subjects**

- **Post Data Collection**