NUTRITIONAL STATUS OF PRE AND POST-MENOPAUSAL WOMEN OF VADODARA.
METABOLIC AND INFLAMMATORY RESPONSE TO SUPPLEMENTATION OF WHOLE ROASTED FLAXSEEDS IN PRE-MENOPAUSAL OVERWEIGHT/OBESE FEMALE SUBJECTS

SYNOPSIS OF DOCTORAL RESEARCH

A report submitted in partial fulfilment for the Degree of Doctor of Philosophy

(Ph.D. Registration No. 57 Dated 21-07-11)

Guide

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AUGUST 2014

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Introduction

For many decades, the term 'women's health' have been constrained to maternal and reproductive health in developing countries and many other dimensions of women's health have been overlooked. As a result of urbanization and nutrition transition, the burden of non-communicable diseases (NCDs) has shown a steep rise in both the genders. But the delusion of cardio vascular diseases (CVDs) being only men's health problem has led to underestimation of risk of these diseases for women. Around 1.2 million women aged between 20 and 59 years died due to CVD's in year 2008 worldwide. Out of these women 80 per cent were from low and middle income countries. The prevalence of breast and lung cancer is highest among all the cancers in women and around half of the cancer cases occurring among women are from developing countries (NCD Alliance, 2011). According to World Health Organization (WHO), NCDs are likely to account for 37% of the deaths in India. The estimated prevalence of four major metabolic risk factors i.e. high blood pressure (31.7%), raised glucose (10%), raised cholesterol (28.3%) and overweight/obesity (14.6%) is high among Indian females and is expected to rise in the next decade (WHO, 2011).

Menopause and risk of non- communicable diseases:

As per definition by WHO (1996), the term menopause refers to 'the permanent cessation of menstruation and fertility resulting from the loss of ovarian follicular activity'. In this condition ovaries reduce their production of the female sex hormone-oestrogen, which is known to have protective effect against various chronic illnesses. Menopause can affect the body weight, composition and abdominal fat deposition and if obesity is prevalent among pre-menopausal women it can also alter the age of natural menopause *vice versa* (Devis et al, 2012). Obesity is characteristically more prevalent in females and reason behind is stated as fluctuations of sex hormones at different stages of life, menopause being the predominant one. Due to the menopausal transition the gynoid type fat distribution among the females starts shifting to android type distribution. Obesity can also have an adverse effect on menopausal symptoms (Devis et al, 2012).

The Oestrogen hormone protects blood vessels by dilating the endothelium. The major effects of the decline in oestrogen levels on blood pressure are: relative

increase in androgen levels, activation of renin-angiotensin system leading to high renin levels, increase in plasma endothelin levels, higher salt sensitivity, increase in insulin resistance, higher sympathetic activity and increase in weight (Mass and Franke, 2009).The two major hindering factors to reduce the incidence of cardiac events among females are inadequate recognition and poor control of hypertension (Coylewright et al, 2008).The risk of having heart diseases among females is 10 times higher than having breast cancer (WHO-NMH, 2002). Hormonal replacement therapy (HRT) has been used to prevent chronic diseases in postmenopausal women for many years but recent studies show controversial results of the impact of HRT, as it has more harms than benefits (Mass and Franke, 2009). As the prevalence of mortality due to NCDs and its risk factors is very high, developing countries like India need to focus on early prevention care as till now treatment regimen is followed only when disease has reached to its advanced stage. There is a need to educate people about the gender specific determinants of chronic diseases (WHO-NMH, 2002). The NCD alliance (2011) had recommended carrying out more women specific clinical research in the area of chronic diseases as majority of the studies till date have a focus on men.

Vitamin B_{12} and folic acid deficiency: Emerging link with cardio vascular diseases:

Folate and vitamin B12 deficiency are the major factor responsible for increase in homocysteine levels which is a significant risk factor for cardiovascular disease (Klee, 2000). Homocystine produced during metabolism of methionine requires vitamin B12 and folate for remethylation into methionine. The Framingham study has indicated folate, vitamin B_6 and vitamin B12 as key elements of plasma homocysteine concentration in the healthy population (Lindenbaum et al, 1994).

The mechanism proposed to link Homocystine to vascular damage, stroke and cardio vascular diseases include impairment of endothelial functions, endothelial desquamation, oxidation of LDL particles, increased monocyte adhesion to the vessel wall, impaired vascular response to nitric oxide and thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction (Murthy, 2005). A retrospective study (N=1743) performed at Germany evidenced significant

association of high folate levels with favourable lipid profile irrespective of age and gender (Semmler, 2010).

The high prevalence of vitamin B12 and folate deficiency is seen worldwide without geographical differences indicating it as public health problem. As the deficiency of these vitamins have similar clinical and hematological features, at many times they cannot be differentiated easily from one another (Chandra, 2006).

Population groups at risk for developing B12 deficiency are: Strict vegetarian, lactovegetarians, pregnant and lactating women, infants and young children with low B12 intake and elderly. According to Chandra (2006) "vitamin B12 deficiency has taken over as more common micronutrient deficiency as compared to folate and the shift has still not been explained". The effects of sub-clinical deficiency, absorption, bioavailability and metabolism of vitamin B12 are yet to be established (O'Leary and Samman, 2010).

Comprising a high percentage of vegetarian population, Vadodara's women are at high risk of developing B12 deficiency. A lot of efforts have been laid in to recognize the relation or homocystine as a risk factor for CVDs but there is dearth of data to assess the direct impact of underlying causes of high homosystine levels i.e. deficiency of B12 and folate. Homocystine is a good indicator of vitamin B12 and folate deficiency but assessing homocystine levels alone does not provide insight of effect of these deficiencies individually on development of NCDs. As, the emerging effect of B12 and folate deficiency on cardiac health and the major factors lying behind these deficiencies have been understudied in India, these area need to be further explored.

Inflammation and NCD risk among females:

Inflammation plays a major factor for development of atherosclerosis and Hs-CRP had been recognized as a novel indicator to assess inflammation in humans (Ridker, 2001). Addition of hs-CRP to the definition of the metabolic syndrome can improve

the prediction of CVD. Elevated hs-CRP levels may also be predictive of development of the metabolic syndrome (Haffner, 2006). According to a study performed on 1035 subjects in Taiwan, sex difference exist between the association of metabolic syndrome and inflammation and concluded that inflammatory processes may be of particular importance in the pathogenesis of metabolic syndrome in women (Lai et al, 2010). Cook et al (2006) used Women Health study data to study the association of HsCRP and CVD risk among women. They added HsCRP into the Framingham prediction model and found that addition of HsCRP gave better prediction of actual CVD risk among women. In a study on south Indian females, HsCRP has been found to be early marker to predict the obesity related co-morbidities among obese females (Dev and Marcus, 2012). Therefore the HsCRP is a risk factor for CVDs with particular importance in females and should be studied extensively during various women centric studies.

Insulin Resistance and NCD risk among females:

According to International Diabetic Federation (2013) the prevalence of diabetes in India is around 19.1% and it has highest number of the diabetics worldwide. Around half of the people with diabetes in South East Asia are undiagnosed. The Asian Indian Phenotype characteristic (Joshi, 2003) and life style transition in the last few decades collectively poses higher risk for diabetes and other non-communicable diseases among Indian population. Cardio vascular disease is a major secondary complication of diabetes and presence of CVD risk factors can also lead to insulin resistance in early stages of life. According to Carey et al (1996) abdominal obesity can be a major factor in development of insulin resistance in obese as well as normal women. Insulin resistance has been closely interlinked with hyperlipidemia and atherogenesis as it can increase the VLDL synthesis and also contributes to the elevated plasma triglyceride levels (Ginsberg, 2000). In the recent years the concept of inflammation induced insulin resistance is coming into focus and various mechanism have been proposed for such relationship (Shoelson et al, 2006).

HOMA IR is an upcoming index to assess the insulin resistance and β cell functioning among the population. It is a reliable tool and can be used as alternate to other sophisticated techniques to detect insulin resistance (Bonora et al, 2002). It can also

serve as independent predictor of CVD among type 2 diabetes mellitus patients (Bonora et al, 2002). However there is a lack of data on HOMA IR values of different sex, age and ethnic groups and lack of standardized reference values have limited its clinical application (Qu, 2011).

Early detection of insulin resistance can help in prediction of development of diabetes, which followed by life style interventions can delay the onset of diabetes among the high risk population.

Prevention of early metabolic and inflammatory aberrations:

Various life style and dietary modification can be adopted to prevent the metabolic aberration and delay the onset of various non-communicable diseases. One such dietary modification approach is use of functional foods. There are various functional foods which have shown to exert beneficial effects in both prevention and control of metabolic derangements; like amla, wheat grass, flaxseeds, soyabean, oats, green tea, garlic etc.

Flaxseed (*LINUM USITATISSIMUM*):

Flaxseed, also known as linseed is a functional food. It is a member of the genus Linum in the family Linaceae. Flaxseed contains 37.1% fat, 4.8% crude fibre and 20.3% protein (Gopalan et al, 2007). Flaxseed has a unique fatty acid profile, being fairly low in saturated fatty acid and rich in α-linolenic acid (ALA), the essential omega-3 fatty acid. ALA gets converted to DHA in vivo which is beneficial for brain health. In animal models, dietary enhancement of DHA was shown to promote neuronal membrane excitability, increase neurotransmitter levels, and reduce neuronal damage (Lim et al, 2009).

A study conducted to see the effects of 38g of flaxseed incorporated in the breads and muffins, as part of the daily diet, on the lipid profile of postmenopausal women showed significant reductions in total cholesterol (6.9%) and LDL (14.7%). Apolipoprotein-A was also significantly lowered by the flaxseed treatment (7.4%) (Arjmandi et al, 1998).

Lucas et al (2002) examined the effects of 40g ground flaxseeds, on lipid metabolism and biomarkers of bone turnover in postmenopausal women and showed significant reductions in the serum TC by 6%, LDL by 4.7% and TG by 12.8%. Serum

apolipoprotein A-1 and apolipoprotein B concentrations were significantly reduced by 6% and 7.5%, respectively. Flaxseed had no effect on biomarkers of bone metabolism.

In a study conducted on the effects of flaxseed- derived lignan supplement on glycemic control, lipid profile and insulin sensitivity in 73 NIDDM subjects, in which subjects were given 360mg lignan capsule everyday for 12 weeks concluded that daily lignan supplementation resulted in modest, yet statistically significant improvements in glycemic control in NIDDM subjects without apparently affecting fasting blood glucose, lipid profile and insulin sensitivity (Pan et al, 2007).

A departmental study on impact of flaxseed (20g) incorporated laddoos on cognitive impairment in institutionalized elderly of urban Vadodara did not show any detrimental effect on the health of these subjects (Chauhan and Kansara, 2012).

Literature review reveals the beneficial effects of flaxseed (*Linum. usitatissimum*) on the lipid profile and glycemic response, with supplementation of 15g flaxseed or a higher dosage. 15g of flaxseed provides 3g ALA, whereas the recommendation for ALA is 1.1 g/d for women and 1.6g/day for men (US IOM, 2002). According to the American Heart Association (2002) 1.5-3g/d of ALA intake can have a beneficial effect on cardiac health. Majority of the studies have been conducted outside India. Very few studies are available on the effect of flaxseed supplementation in the Indian population. Flaxseeds are a cheap source of ALA in comparison to other supplements available in the market and small doses can be easily consumed in roasted form as “Mukhvas” (mouth freshner), commonly consumed in Gujarati society. Regular intake of moderate amount of flaxseed can exert beneficial effect even in apparently health but at risk population for developing NCDs. Most of the studies reported in literature are conducted on post-menopausal females with negligence towards pre-menopausal group. The increased prevalence of NCDs during early stages of life makes it indispensable to focus on preventive strategies for such population groups and use of functional foods like flaxseed is one of the rational approaches for the same.

Objectives

Hence the research was planned with the following main objectives:

1. To study the life style, behavioural and dietary discrepancies among pre and post-menopausal women
2. To compare the physiological and metabolic aberrations in pre and post-menopausal females in terms of:
    - Body Composition
    - Blood Pressure
    - CHO metabolism
    - Lipid metabolism
    - Inflammatory markers
    - Nutritional anemia
    - Thyroid functions
    - Liver and kidney functions
3. To explore the relationship of Vitamin B12 and Folic acid deficiency with risk factors of non-communicable diseases among women
4. To investigate the predictor variables of inflammation and insulin resistance among females
5. To assess the nutrient composition, fatty acid profile and antioxidant capacity of flaxseeds
6. To study the efficacy of two different doses of whole roasted flaxseeds on lipid profile and inflammatory markers of pre-menopausal overweight or obese female subjects

Review of Literature

The review of literature will be discussed under the following points:

1. Health and nutrition: Scenario among women

2. Nutrition transition: Impact on women's health
3. Demographic characteristics of females
4. Menopausal transition
    - Physiology, endocrinology and symptoms
    - Menopause: Role in physiological and metabolic alterations
        - Body composition
        - Blood pressure
        - Glucose metabolism
        - Lipid metabolism
        - Inflammation
        - Thyroid functions
        - Nutritional anaemia
        - Liver functions
        - Kidney functions
5. Vitamin B12 and Folic acid
    - Chemistry, functions and Sources
    - Deficiency Disorders
    - Deficiency among females
    - Association with risk factors of non-communicable diseases
6. Inflammation
    - Physiology
    - Inflammatory markers of significance
    - Inflammation, life style and non-communicable disease risks
    - Inflammation in females
7. Insulin resistance
    - Physiology
    - Markers of insulin resistance
    - Insulin resistance, life style and non-communicable disease risks
    - Insulin resistance in females
8. Approaches to prevent early metabolic and inflammatory aberrations
9. Functional Foods

- Definition
- Various functional foods and their role in prevention and management of non-communicable diseases

10. Flaxseeds
    - Taxonomy and varieties
    - Nutrient composition
    - Functional components
    - Health benefits of flaxseeds
        - Hyperlipidemia
        - Inflammation
        - Diabetes and insulin resistance
        - Hypertension
        - Cancer
        - Menopausal symptoms

Methods and Materials

The present research work comprises of 3 phases as described below:

Phase I

Nutritional status of pre and post-menopausal women (30-60y) of urban Vadodara

A formative research to assess the nutritional status of the adult female population (30-60y) of urban Vadodara was performed with a focus on their menopausal status. The study design was a cross sectional cum factorial design. The study was approved by the Institutional Ethics Committee for Human Research of Faculty of Family and Community Sciences, M.S. University, Baroda (No.: IECHR/2012/19). The details of the experimental plan are depicted in Figure 1.

Phase II

Identification of flaxseed variety for supplementation and estimation of its nutritive profile

A review of secondary data available on internet regarding various flaxseed varieties was performed. PKV-NL 260 variety of the flaxseed was selected to use for the supplementation. Details of sampling procedure for flaxseeds analysis and nutrients analysed is provided in Figure 2.

Phase III

Metabolic and inflammatory response to supplementation of whole roasted flaxseeds in pre-menopausal overweight/obese female subjects

An open labelled parallel randomised controlled trial was performed to assess the impact of whole roasted flaxseeds on the lipid profile, insulin resistance and inflammation of premenopausal overweight/obese females.

Phase III (a): Screening and collection of baseline data:

To identify the subjects for supplementation a screening of adult females of 30-50y was performed from one society and nearby societies in a concentric manner. Figure 3 illustrates the details of screening and baseline data collection.

Phase III (b): Randomised control trial to study the efficacy of whole roasted flaxseeds on lipid profile, insulin resistance and inflammation in pre-menopausal overweight/obese females

90 overweight/obese premenopausal females were selected based on the consent. They were randomly divided into 3 groups i.e. control group, experimental group I and experimental group II. The control arm was not provided with any kind of supplementation whereas group I was supplemented with 5g and group II was supplemented with 10g of roasted flaxseeds during the study period of 8 weeks. The Doses of flaxseeds were decided on the bases of Alpha Linolenic Acid (ALA) present in the flaxseeds (Table 1). Biochemical and anthropometric data was collected before and after the intervention. The study was approved by the Institutional Ethics Committee for Human Research of Faculty of Family and Community Sciences, M.S. University, Baroda (No.: IECHR/2013/7). The detailed protocol of the supplementation trial is described in Figure 4.

Table 1: ALA content of two doses of flaxseed supplementation

Control Group	Experimental Group I 5 g Flaxseeds	Experimental Group II 10 g Flaxseeds
--	1.17g ALA	2.34g ALA
No ALA supplementation	ALA provided by flaxseeds equivalent to RDA (RDA by IOM 1.1g/day)	ALA provided by flaxseeds equivalent to recommended average intake of ALA for Cardio-protective effect (AHA 2010 recommendation 1.5-3g/day)

The methods and tools used for the data collection are portrayed in Table 2.

Figure 1: EXPERIMENTAL DESIGN

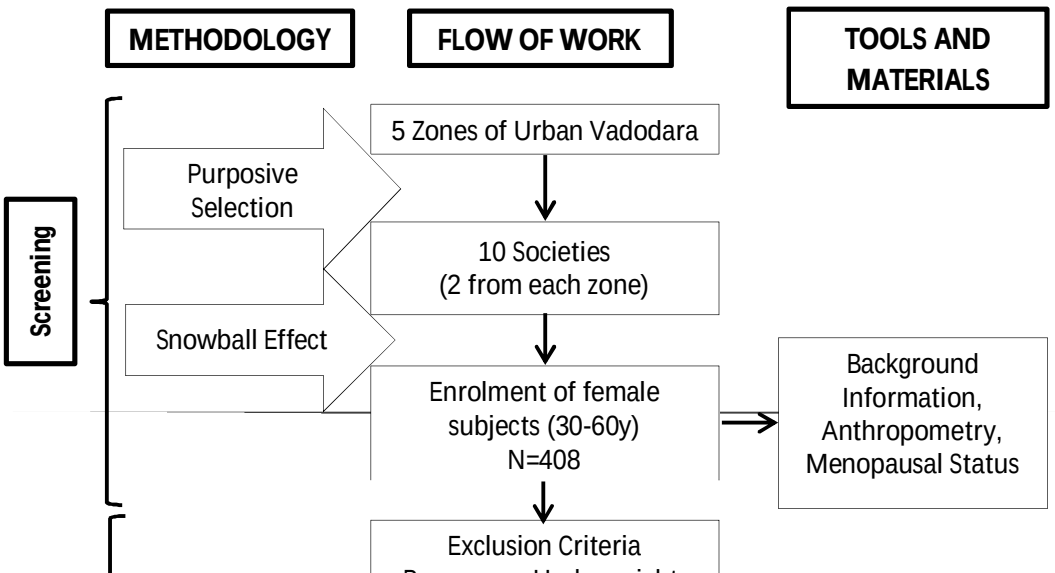

Figure 2: EXPERIMENTAL DESIGN

PHASE II

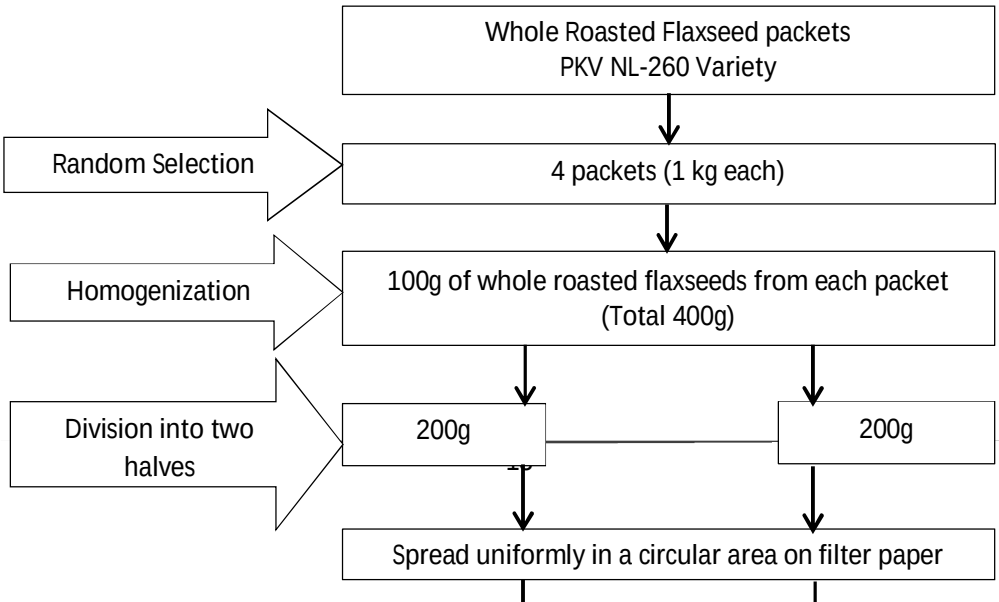

Figure 3: EXPERIMENTAL DESIGN

PHASE III (a)

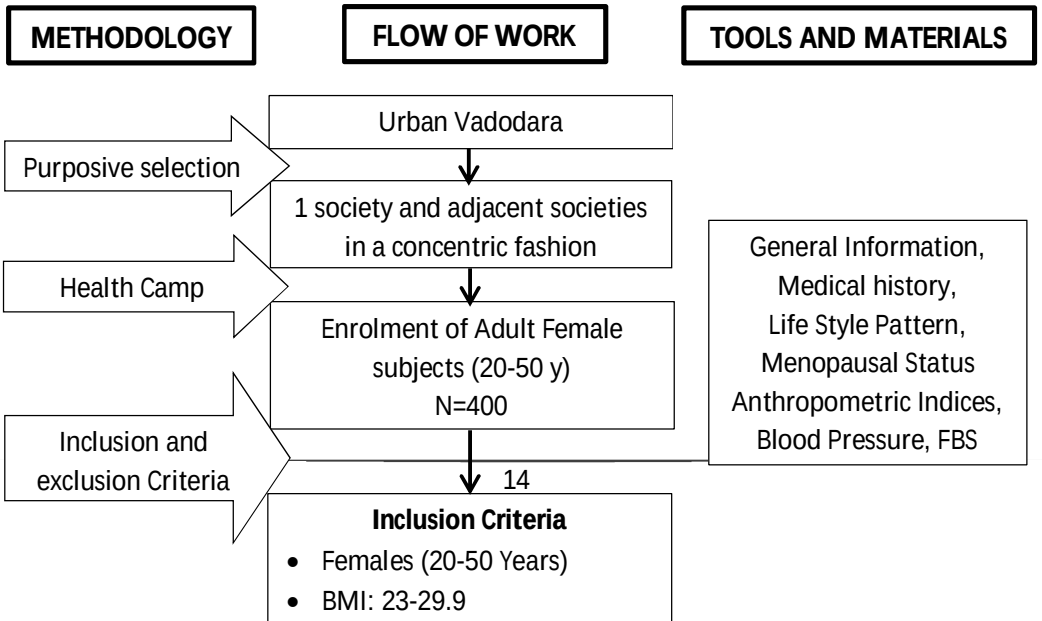

Figure 4: EXPERIMENTAL DESIGN

PHASE III (b)

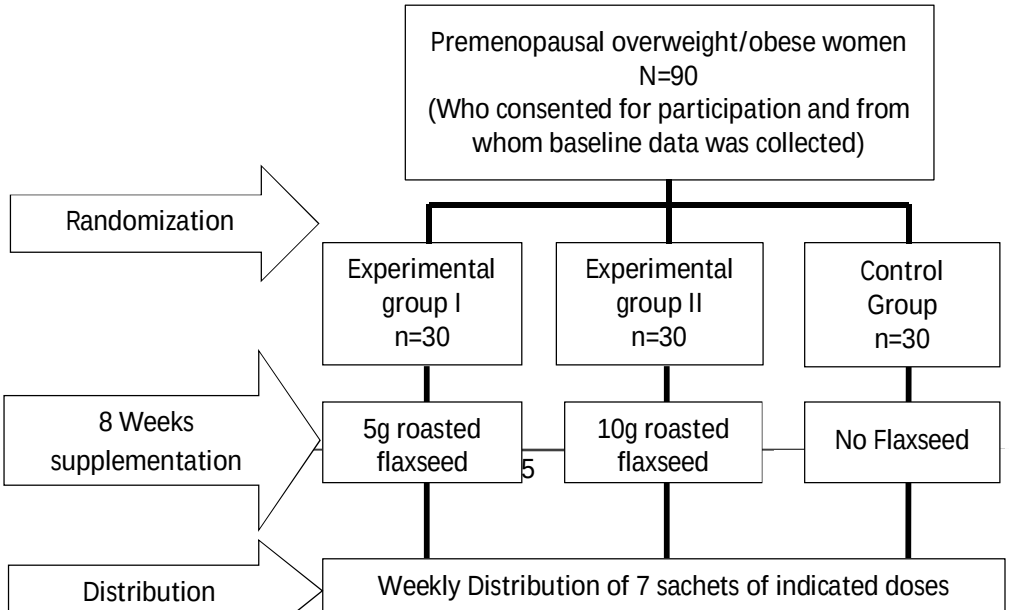

Table 2: Methods and tools for Data Collection

Parameters	Techniques
Medical and Life Style History	
General Information, Medical history, Family History, Lifestyle variables	Pre tested Structured questionnaire
24 hours dietary recall	Semi structured questionnaire
Food Frequency	Semi Quantitative Structured questionnaire
Physical Activity	International Physical activity Questionnaire (Short)
Anthropometric measurements	
Weight	Electronic Weighing Scale
Height, Waist and Hip circumference	Non stretchable Fiber Glass Tape

Biophysical Measurements	
Blood Pressure	Sphygmomanometer
Body Fat Analysis	Omron Body Fat Analyzer
Nutritional Anemia	
Haemoglobin	Cyanmethemoglobin method
Complete Blood Count	Differential Analyzer
Iron, Total Iron Binding capacity	Photometry
Folic acid, Vitamin $B_{12,}$ Ferritin	Chemiluminescence immune assay
Glucose Metabolism	
Fasting Blood Sugar	Enzymatic kit method
Fasting Insulin	Solid phase radio immune assay
HbA1C (Glycated hemoglobin)	High Pressure Liquid Chromatography
Lipid Metabolism	
Triglycerides, Total Cholesterol, High Density Lipoprotein-Cholesterol	Enzymatic kit method
Low Density Lipoprotein-Cholesterol	Derived from TC and TG
Very Low Density Lipoprotein-Cholesterol	Derived from TG
Inflammation	
High Sensitivity C-Reactive Protein	Nephelometry
Thyroid Profile	
TSH, Total T3, Total T4	Competitive chemiluminescence immune assay
Kidney Function test	
Creatinine, BUN, Uric Acid, Alkaline Phosphate	Photometry
Liver Function Test	
Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, SGOT, SGPT, GGT, Serum Albumin, Total Protein	Photometry
Parameters	**Techniques**
Flaxseed Analysis	
Fatty Acid Profile	GC-MS
Minerals (Ca, Fe, Na, K)	ICP-OES
Antioxidant capacity	DPPH, FRAP
CHO, Protein, Fats	AOAC method

Statistical Analysis

- The data was entered into Microsoft excel 2007 and verified.
- Statistical analysis has been performed using Epi Info 7 and SPSS 16.
- Data has been described using descriptive statistics (mean and standard deviations) for continuous variables.

- Data has also been depicted using proportions i.e. percentages in case of categorical variables and prevalence rates for continuous variables using well defined cut off points.
- Wherever relevant, the 95% confidence intervals in which the means and proportions lie will be calculated.
- The independent student's 't' test and Chi-square test will be performed to compare continuous and categorical variables respectively.
- Univariate odds ratio analysis will be performed to assess the risk burden of a particular risk factor and will be depicted with 95% confidence intervals.
- In multivariate analysis, stepwise forward linear regression will be applied to find out the variables that contributed to significant amount of variations in different dependent variables.
- One way analysis of variance (ANOVA) has been computed to find out the difference between baseline characteristics of three groups of intervention trial.
- Paired 't' test has been used to compare the difference between the pre and post intervention values of the outcome variables in the intervention phase.
- Stratified analysis will be done to rule out residual confounding due to covariate imbalance.
- All statistical analyses were considered significant at $p<0.05$ level.

Results

PHASE I:

NUTRITIONAL STATUS OF PRE AND POST-MENOPAUSAL WOMEN (30-60Y) OF URBAN VADODARA

Characteristics of screened subjects

- The age distribution of the subjects showed equal distribution of the subjects between three age group categories. Around 32.4% of the subjects were between 30-40y, 32.4% between 41-50y and 35.3% between 51-60y of age.

- About 46% of the subjects were pre-menopausal, 7.8% were going through peri-menopausal phase, 36% were in post-menopausal condition and 10% had faced hysteractomy. No case of pregnancy was reported by the subjects.
- Out of total 408 subjects 4.7% were underweight, 15.9% had normal weight. The prevalence of overweight and obesity was 17.4% and 62% respectively.

Background information of the subjects studied for non-invasive risk analysis

- Majority of the subjects were secondary, higher secondary pass or graduates. The per cent of higher education among subjects was higher among pre-menopausal women (50.8%) than that of post-menopausal ones (35.4%).
- Most of the subjects (92.4%) were married with a significantly higher per cent ($p<0.05$) of widows among post-menopausal females.
- Pre-menopausal women were more involved in occupational activities like unskilled labour (4.6% v/s 1.5%), service (16.9% v/s 3%) and business (6.2% v/s 4.5%) than pre-menopausal women, however majority of the subjects in both the categories were housewives (81.7%).
- The mean age of the subjects was 46.4±9.97y.

Health profile of the subjects:

- Mean age of menarche and pregnancy among females was 14.44±1.8y and 22.9±3.1y respectively
- Mean age of menopause was 45.45±4.8y.
- Around 21.1% of the post-menopausal women experienced vasomotor symptoms and 28.8% experienced somatic symptoms. Psychological symptoms were most frequently (33.3%) experienced by women with a low incidence of urogenital symptoms (3%).
- Highest prevalence of family history of hypertension (52.5%) and diabetes (39.7%) was seen among the subjects followed by CHD (27.5%), obesity (18.4%) and cancer (16.1%).
- Data on self-reported disease profile showed that highest prevalence of hypertension (21.4%) followed by diabetes (10.7%) among the subjects.

- Around 21% of the subjects suffered from acidity, 10% from gastritis and 3% from constipation.
- General nutritional deficiency symptoms showed that highest prevalence of fatigue (35%) and cramps/muscle weakness (26%) was found among the subjects.
- None of the subjects consumed alcohol and only 0.8% had habit of tobacco chewing. Physical activity of the subjects showed that around 70% of the subjects were moderately physically active and only a few (6%) were having low physical activity level.
- Around 20% of the subjects reported regular health check-up.
- Merely 32% of the subjects were aware of the breast examination practice.
- Very few subjects had undergone PAP smear (6.9%) or mammography (8.4%).

Dietary practices of the subjects:

- Most of the subjects were lacto-vegetarian (81.7%).
- The mean consumption of oil, sugar, salt was 45.2±18.6 g, 32.5±17.9 g and 9.8±4.5 g respectively showing high consumption among subjects.
- The average daily energy intake of the subjects was 1504±415 Kcal, which was below the RDA (79%) for a sedentary adult woman.
- The mean protein intake (42.8g) was also below the recommended daily intake (77.8%).
- Visible fat intake was much higher than the RDA (178%).
- No significant difference of the micro and macronutrients between pre and post-menopausal women was observed.
- Biscuits (55.6%) were found to be consumed most frequently among the subjects followed by sev/namkeen (42.7%) and khari/nankhatai (16%).

Physiological and metabolic aberrations in pre and post-menopausal females:

- The mean weight and height of the subjects were 60.02±10.7 kg and 152.5±5.99 cm respectively.
- The prevalence of overweight and obesity was high (74.8%) among the subjects.

- Abdominal obesity was assessed through three major indices (WC, WHR and WSR), and a staggeringly high prevalence was observed.
- In pre-menopausal women 9.2% of the subjects reported having history of hypertension however 15.4% were newly diagnosed as hypertensives.
- In case of post-menopausal women 33.3% of the subjects reported having hypertension and about 26% were found to be new cases of hypertension.
- About 23% of pre-menopausal and 28% of post-menopausal women had pre-hypertension.
- Leucopenia which is an indicator of low WBC levels was found to be prevalent among only 1.1% of the subjects. A minute fraction (2.2%) of the study population was suffering from thrombocytopenia (Low platelet levels).
- Around 49% of the subjects were having iron deficiency anaemia diagnosed by low haemoglobin levels.
- The prevalence of macrocytic anaemia was low among the subject (2.2%).
- The deficiency Vitamin B12 was very high (71.1%).
- Only a small per cent of the subjects (5.6%) had folic acid deficiency.
- The prevalence of high LDL-C levels was very high (72.2%).
- Around 45% had high TC levels.
- Hypertriglyceridemia was seen among 12.2% of the subjects.
- Around 12% of the subjects were found to be diabetic.
- A considerable number of the subjects had high HbA1C levels (26.7**%).**
- About 64.4% had high HSCRP levels.
- Metabolic syndrome was prevalent in one fourth of the female population
- No new case of hypothyroidism was diagnosed in the study.
- Subclinical hypothyroidism (High TSH levels) was present among around 29% of the females
- The prevalence of high levels of other liver enzymes was 12.2%, 21.1% and 10% for SGOT, SGPT and GGT respectively.
- High direct bilirubin levels were prevalent among 16.7% of the subjects.
- Occasional case (1.1% for albumin) of low serum proteins was observed among the subjects.

- None of the subjects had high high BUN levels; nevertheless 3.3% had high creatinine levels.
- Around 9% of the subjects had high uric acid level which is an indicator of Gout.

Phase II

IDENTIFICATION OF FLAXSEED VARIETY FOR SUPPLEMENTATION AND ESTIMATION OF ITS NUTRITIVE PROFILE

- PKV NL-260 variety of flaxseeds was selected for the supplementation study and whole roasted form was supplemented.
- The macronutrient analysis of the flaxseeds (PKV NL-260) showed that fat constituted around 26.6% of the flaxseeds on wet basis.
- Sodium and potassium content of the flaxseeds was 5.5mg/kg and 9.6mg/kg respectively.
- Flaxseeds contained 365.8±18.2 GAE/100g of total polyphenols and 148.6±4.0 RE/100g of flavonoids.
- The antioxidant capacity of the flaxseeds using DPPH RSA and FRAP methods was 1776.6±80.1 TE/100g and 643.7±2.6 TE/100g respectively.
- The fatty acid profile showed the highest percent (78.04%) of n-3 fatty acid in the PKV NL-260 variety of flaxseeds.
- Various forms of saturated fatty acids were present in the flaxseeds with small quantities.

Phase III

METABOLIC AND INFLAMMATORY RESPONSE TO SUPPLEMENTATION OF WHOLE ROASTED FLAXSEEDS IN PRE-MENOPAUSAL OVERWEIGHT/OBESE FEMALE SUBJECTS

Phase III (a): Screening and collection of baseline data

- Out of 400 screened subjects2.5% were overweight, 17.2% were normal, 19% were overweight, 40.8% were obese and 205% were morbid obese.

- About 68.5% of the subjects fell under pre-menopause category. Only 8.5% were undergoing through peri-menopause phase, 20% were in post-menopausal category and 3% were hysteractomized.
- About 3.8% of the subjects had history of diabetes and 10.5% were suffering from hypertension.
- None of the subjects had habit of smoking or alcohol intake.

Phase III (b): Randomised control trial to study the efficacy of whole roasted flaxseeds on lipid profile, insulin resistance and inflammation in pre-menopausal overweight/obese females

- Pre supplementation values of hemoglobin were significantly higher ($p<0.001$) in control subjects were (12.48±1.13g/dl), than 10g supplementation group (11.62±1.39g/dl) and 5g supplementation group (11.18±1.24g/dl).
- After supplementation, Hb values of all three groups ($p<0.01$, $p<0.001$, $p<0.001$) increased significantly than baseline.
- Pre supplementation mean values of kidney function tests were more or less similar in all three groups except for BUN values which were significantly higher in 10g supplementation group than other groups.
- Uric acid values of two supplementation groups decreased significantly ($p<0.05$ and $p<0.01$ for 5g and 10g group respectively. And no change in control group was observed.
- All the three groups had almost comparable mean values of pre supplementation liver function tests.
- A significant decrease in the total ($p<0.01$), indirect ($p<0.01$) and direct bilirubin ($p<0.01$) was seen among control groups. On the contrary SGPT ($p<0.01$) and GGT ($p<0.01$) values increased significantly in this group. However, all the values were within the normal range.
- Pre supplementation mean values of thyroid function tests also showed non-significant difference between three groups.
- No difference in the values of thyroid function test was observed post supplementation.

- The mean values of pre supplementation lipid profile indicated no difference in all three groups except for HDL-C values ($p<0.05$) which were significantly lower in 5g supplementation group.
- The mean AIP values ($p<0.05$) were significantly higher in 5g supplementation group.
- After supplementation no significant difference in the mean values of lipid profile of two supplementation groups were observed. However in control group a significant rise of TC ($p<0.001$), LDL-C ($p<0.001$), TC/HDL-C ratio ($p<0.001$) and LDL-C/HDL-C ratio ($p<0.001$) was found.
- The anthropometric indices and blood pressure had more or less equal mean values in pre supplementation data.
- The mean values of WC ($p<0.001$), HC ($p<0.05$), WHR ($p<0.05$), WSR (p,0.001) and SBP ($p<0.01$) showed significant decrease post supplementation.
- HOMA IR values indicated a non-significant decrease in post data.
- Inflammatory status of the subjects remained unchanged after flaxseed supplementation as indicated by mean Hs-CRP values.

Summary and Conclusion

This chapter will summarize the major findings of each individual phase of the present research and conclusions will be discussed about:

- Major focus areas for prevention of non-communicable diseases among females.
- Acceptability of flaxseed consumption in routine life
- Recommended doses of flaxseeds for maintenance of lipemic and inflammatory status for at risk female population.

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