

Chapter 5

Summary and Conclusions

Background

The prevalence of morbidity and mortality among women due to non-communicable diseases has been on rise in the last few decades. The major underlying causes of these diseases have been identified as physical inactivity, unhealthy diet, tobacco and alcohol consumption. Apart from these four factors menopause is a unique risk factor among women which can affect their endocrinology and body composition, making postmenopausal women predisposed to various non communicable diseases. Though menopause have the potential to increase the risk for certain diseases, early lifestyle and dietary modifications can improve the quality of life and delay or even prevent the onset of metabolic aberrations. Major upcoming metabolic risk factors for NCDs among women are vitamin B12 and folic acid deficiency, inflammation and insulin resistance. The prevalence of these metabolic factors among women and their association with other risk factors can provide a better insight for development of preventive strategies of non-communicable diseases. Incorporation of different functional foods like flaxseeds in the diet can be an effective approach towards promotion of healthy dietary habits among women. Flaxseeds are rich in α linolenic acid, lignans, different polyphenols and fiber. Regular incorporation of low doses of flaxseeds in the diet of apparently healthy overweight/obese pre-menopausal women as an early strategy to prevent development of non-communicable diseases, can lead to improvement in lipemic profile, high blood pressure, inflammation and insulin resistance in women.

Review of literature in this context lead to the following research questions:

- What are the dietary and life style habits of women residing in Urban Vadodara?
- What is the impact of menopause on lipemic, glycemic, biophysical, inflammatory and other physiological aberrations among women?



- Does age play any significant role in association between menopause and cardio vascular risk factors?
- What is the prevalence of vitamin B12 and folate deficiency among women?
- What are major causal factors of vitamin B12 and folate deficiency among women?
- What is the relationship of vitamin B12 and folate deficiency with cardio vascular risk factors?
- What are the predictor variables of inflammation and insulin resistance among women?
- What is the nutrient content of roasted flaxseeds?
- How effective would the low doses of flaxseeds be in improving the metabolic and inflammatory profile of the apparently healthy overweight/obese pre-menopausal women?

Subsequently, based on the above questions the present research was planned with the following main objectives:

1. To study the life style, behavioural and dietary differences, if any, 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 study the effect of age on association between menopause and metabolic aberrations
4. To explore the relationship of Vitamin B12 and Folic acid deficiency with risk factors of non-communicable diseases among women
5. To investigate the predictor variables of inflammation and insulin resistance among females
6. To assess the nutrient composition, fatty acid profile and antioxidant capacity of flaxseeds
7. 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

The research plan was divided into three phases as described below:

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

PHASE II: Identification of flaxseed variety for supplementation and estimation of its nutritive profile

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

PHASE I

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

A cross sectional cum factorial design study was performed to assess the nutritional status of the adult female population (30-60y) of urban Vadodara with a focus on their menopausal status. For this purpose two societies from each zone of urban Vadodara (total five zones) were selected for the study. Total 408 female subjects of 30-60 years of age were enrolled through snowball effect and data regarding general information, anthropometry and menopausal status was collected. Further 180 women were



randomly selected using 2X2 contingency table for menopause and obesity. Detailed non-invasive risk analysis for cardio metabolic diseases was performed on 131 women based on their consent and biochemical analysis was performed on 90 women. Data regarding obstetric history, family and medical history, life style pattern, dietary habits, health seeking practices, physical activity, food frequency and 24 hour dietary recall was collected using semi structured questionnaire. The anthropometric measurements, blood pressure and body fat were also measured during this appointment. Fasting blood was collected by a trained technician. The biochemical parameters analysed during this phase of study were: Hemoglobin, Complete Blood Count, Ferritin, Vitamin B12, Folic Acid, Lipid Profile, Fasting Blood Glucose, Glycated haemoglobin, Insulin, Hs-CRP, Thyroid Profile, Liver function test and Kidney function test.

RESULTS

The results of phase I were presented under following sub points:

1. Life style, health care and dietary behaviours in pre and post-menopausal women

(a) 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 hysterectomy. 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.

(b) 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 $46.4 \pm 9.97y$.

(c) Health profile of the subjects:

- Mean age of menarche and pregnancy among females was $14.44 \pm 1.8y$ and $22.9 \pm 3.1y$ respectively
- Mean age of menopause was $45.45 \pm 4.8y$.
- 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 with a significant higher proportion of post-menopausal women going for 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%).

(d) 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.
- Post-menopausal women consumed significantly higher mean amounts of oil, sugar, salt than the pre-menopausal ones.
- 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%).
- Biscuits (55.6%) were found to be consumed most frequently among the subjects followed by sev/namkeen (42.7%) and khari/nankhatai (16%).
- No significant difference between nutrient intake of pre and post-menopausal women was observed.

Conclusion

The subjects in the present study followed a fairly healthy lifestyle as indicated by low prevalence of physical inactivity, tobacco or alcohol intake. Both pre and post-menopausal women were not much attentive towards their health, though good health care practices were more pronounced in post-menopausal women probably due to escalated health issues with increase in age. Dietary practices were poor in both pre and post-menopausal women. Thus, there is a need to create awareness regarding improving health practices and dietary behaviours in post-menopausal as well as premenopausal women.



2. Physiological and metabolic aberrations in pre and post-menopausal women

- 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%) although a considerable number of women were suffering from vitamin B12 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 one fourth of the women were diagnosed with metabolic syndrome and menopause was not associated with its prevalence.
- No new case of hypothyroidism was diagnosed in the study.
- The subclinical hypothyroidism was present in 29 of women with on significant difference between pre and post-menopausal women.



- The prevalence of high levels of other liver enzymes was 12.2%, 21.1% and 10% for SGOT, SGPT and GGT respectively.
- Nearly one fourth of the women had high alkaline phosphatase levels with menopause eliciting significant risk of developing high alkaline phosphatase levels.
- 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.
- Post menopause was not found to have impact on any other liver function indicator.
- 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.
- Menopause was not found to pose any risk of developing kidney function abnormalities.
- In univariate analyses the major variables associated with menopause were high body fat percent, hypertension, diabetes, high HbA1C levels and high alkaline phosphatase levels.

Conclusion

A trend of higher prevalence of anthropometric and metabolic aberrations in post-menopausal women than pre-menopausal women was observed, though some of the differences were not considerably distinct. The major reason behind this was quite a high presence of these metabolic alterations in pre-menopausal women also. Metabolic aberrations in post-menopausal can increase the risk of NCD's and presence of obesity and deranged metabolic profile in pre-menopause can adversely affect the age of menopause, menopausal symptoms and development of non-communicable diseases vice versa. Therefore a healthy life-style should be focused from the early stages of life with extra caution during and post menopause.



3. Effect of age and obesity on the association of menopause with metabolic profile

- Mean WC, WSR, body fat, HDL-C and alkaline phosphatase levels were significantly higher in post-menopausal women after adjustment of age and obesity.
- Through logistic regression analysis menopause was found to be independently associated with body fat percent irrespective of age and obesity status of women.

Conclusion

To conclude, association of menopause with body composition alterations and tendency of central fat deposition is established through various studies and supported by the present study. Menopause can indirectly affect risk of cardio vascular disease through these fat distribution changes. However, still larger longitudinal studies are required to establish menopause as a primary risk factor for non-communicable diseases, as various other factors like age, decreased physical activity etc. can act as confounders.

4. Causal factors of vitamin B12 and folic acid deficiency and their relationship with risk factors of non-communicable diseases in women

- The prevalence of vitamin B12 deficiency was quite high (71%) in the women.
- Only 5.5% of the women studied had folic acid deficiency.
- The frequency of consumption of vitamin B12 rich food items was low in women.
- Two key causal factors identified through multivariate analysis for vitamin B12 deficiency were: Very low dietary intake of vitamin B12 ($<0.3\mu\text{g}/\text{day}$) and no regular health check-up.
- Hypertension, low HDL-C levels and high TC levels were three metabolic alterations associated with vitamin B12 deficiency as predicted by forward logistic regression.
- Mean levels of SBP, TC/HDL-C and LDL/HDL-C ratio and HsCRP were significantly higher in vitamin B12 deficient subjects as depicted by independent 't' test results. The significance was maintained for all even after adjustment of age and BMI using ANCOVA.



- Vitamin B12 deficiency posed significant higher risk for development of hypertension and low HDL-C levels through univariate analysis. After adjustment for age and BMI using logistic regression B12 deficiency emerged as independent predictor for development of hypertension among women.

Conclusion

The prevalence of vitamin B12 deficiency was found quite high in the present study in comparison to available literature. Dietary Intake and lack of health check-up have come up as two major causal factors for vitamin B12 deficiency. Regular health check-up can lead to early detection and treatment of vitamin B12 deficiency. The independent role of vitamin B12 deficiency as a risk for NCD's is still debatable, however in the present study the strongest association of vitamin B12 deficiency appeared with hypertension. The area needs to be further explored considering the very high prevalence of Vitamin B12 deficiency in the population.

5. Association of inflammation with NCD risk factors in women

- The prevalence of high Hs-CRP levels was around 64%.
- Various anthropometric indices and lipemic aberrations were significantly associated with high Hs-CRP levels in univariate analysis however on entering variables into multivariate regression model clustering risk factor i.e. Metabolic Syndrome was found to be the key variable associated with Hs-CRP.

Conclusion

Metabolic syndrome and its risk factors were well associated with inflammation measured by HsCRP in the present study. As various studies have shown higher HsCRP levels in women than men, HsCRP can be used as suggestive tool while assessing metabolic syndrome as well as diabetes and CVD risk in women.



6. Association of insulin resistance with NCD risk factors in non-diabetic women

- About 21.5% of the non-diabetic women were suffering from insulin resistance.
- Various anthropometric indices and metabolic aberrations were significantly associated with high HOMA IR values.
- The dietary factors posing high risk for development of insulin resistance were high energy and carbohydrate intake.
- Women with family history of diabetes were at higher risk of developing insulin resistance.
- The major determinants of insulin resistance predicted through logistic regression model were low HDL-C, high BMI and high energy intake.

Conclusion

As elucidated in this section, majority of the metabolic and dietary risk factors of CVDs were associated with insulin resistance in the middle aged female population of Vadodara. Therefore life style interventions and dietary changes with a focus of weight management should be promoted to reduce the risk of insulin resistance in females.

PHASE II

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

PKV-NL 260 variety of the flaxseed was selected to use for the supplementation. For analysis of flaxseed 4 packets of procured whole roasted flaxseeds (PKV NL-260) were randomly selected. 100g of flaxseeds from each packet were drawn and homogenized. It was further divided into eight parts using appropriate sampling methods. The parameters for nutritive value evaluation included carbohydrates, fats, proteins, crude fiber, sodium, potassium, calcium, iron, fatty acid profile, total phenols, flavonoids and antioxidant capacity using DPPH and FRAP methods.



RESULTS

- 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 mgGAE/100g of total polyphenols and 148.6 ± 4.0 mgRE/100g of flavonoids.
- The antioxidant capacity of the flaxseeds using DPPH RSA and FRAP methods was 1776.6 ± 80.1 mmolTE/100g and 643.7 ± 2.6 mmolTE/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 in small quantities.

Conclusion

Though the total fat content of the flaxseeds (PKV NL-260 variety) analysed was lower than the average percent reported in various studies, the linolenic acid content of the same was not compromised and provided a fair amount of ALA as required to be supplemented in the study. The presence of fair amount of polyphenols, flavonoids and antioxidant capacity along with ALA made the flaxseeds (PKV NL-260) ideal to exert beneficial impact on cardio vascular risk factors.

PHASE III

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

In the open labelled parallel randomised controlled trial 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.



Sample size was calculated as 30 subjects in each arm. This phase was divided into two parts as follows:

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

For the screening of the subjects one society from urban Vadodara was purposively selected and nearby societies in a concentric manner were purposively selected. Data regarding medical history, menopausal history, life style pattern, frequency of consumption of n-3/ALA rich foods, physical activity pattern, anthropometry, blood pressure and fasting blood glucose was collected through organizing health camp. The enrolled subjects (N=400) were further screened for identification of eligible subjects using inclusion and exclusion criteria for the study. Pre-menopausal overweight/obese female subjects (20-50y) were selected for the study and the subjects with history of chronic illnesses, allergy to flaxseeds diabetes, smoking or tobacco chewing, pregnancy, vigorous physical activity, peri and post menopause, hysterectomy, currently (for last 4 weeks) taking n-3 rich foods or supplements, rapid or dramatic weight gain or loss in last year were excluded. A detailed written consent was sought from 151 eligible subjects and out of those who consented, 90 subjects were randomly divided into three groups. Biochemical estimation and anthropometric assessment was performed for the females included in supplementation trial. Data regarding 24 hour diet pattern was also collected from the subjects. Biochemical estimation included lipid profile, fasting blood glucose, insulin, HsCRP, haematological profile, liver function tests and kidney function tests.

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

Experimental group I was asked to consume 5g of roasted flaxseeds (in form of one dose) and group II was asked to consume 10g of roasted flaxseeds (in form of two doses) for a period of 8 weeks. The control group was advised not to consume flaxseeds during the study period. 5g of flaxseeds contained 1.17g of ALA whereas 10g of flaxseed



contained 2.34g of ALA. Sachet of 5g and 10g were made for distribution and every week 7 air tight sachet in a pouch were given to the subjects. Weekly monitoring was done for the consumption of flaxseeds by the subjects through collection of empty sachet and compliance sheet. The compliance of flaxseeds consumption was 100% among the subjects who completed the study protocol. There were total 4 drop outs during the study. After 8 week of supplementation data regarding biochemical estimations and anthropometric, biophysical measurements was collected following same protocol as for baseline data collection.

RESULTS

- No significant difference in the mean values of lipid profile was observed in the two supplementation groups (5g and 10g) post supplementation.
- In the 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.
- On segregating the data based on BMI, HsCRP and HOMA IR categorization, it was revealed that elevated levels of these parameters did not alter the magnitude of impact of flaxseeds supplementation on the lipid profile of the subjects.
- Both 5g and 10g flaxseed supplementation brought better impact on the lipid profile of the subjects with initially high levels of LDL-C or AIP, though not statistically significant.
- HOMA IR and insulin values indicated a non-significant decrease after supplementations of both 5g and 10g flaxseeds.
- % insulin sensitivity fairly increased after 5g flaxseed supplementation.
- Insulin, HOAM IR and % insulin sensitivity improved greater in subjects having initially high HOMA IR levels (>1.2) post 5g flaxseed supplementation.
- Insulin ($p<0.05$) and HOMA IR ($p<0.05$) levels significantly decreased and % insulin sensitivity ($p<0.05$) significantly increased in subject with high HOMA IR levels (>1.2) post 10g flaxseed supplementation.



- Inflammatory status of the subjects remained unchanged after flaxseed supplementation as indicated by mean Hs-CRP values which showed a non-significant decrease in all three groups.
- The mean values of BMI, WC, HC, WHR and WSR remained constant throughout the study.
- Mean body fat percent ($p<0.01$) and SBP ($p<0.01$) levels significantly reduced in 5g flaxseed group post supplementation. However 10g flaxseed supplementation showed only a non-significant decrease in these parameters.
- Prevalence of pre/stage I hypertension significantly reduced in both 5g ($p<0.05$) and 10g ($p<0.05$) supplementation group.
- After supplementation, Hb values of all three groups ($p<0.01$, $p<0.001$, $p<0.001$) increased significantly than baseline.
- No difference in the values of thyroid function test was observed post supplementation.
- 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.
- Uric acid values of two supplementation groups decreased significantly ($p<0.05$ and $p<0.01$ for 5g and 10g group respectively). No such change in control group was observed.

Conclusion

Hence to conclude, flaxseed supplementation trial exhibited mixed results on the metabolic profile of the subjects. Flaxseeds exerted beneficial impact on blood pressure, insulin resistance (in subjects with HOMA IR >1.2) and body fat percent (5g flaxseed group), maintained lipid profile, anthropometric indices and failed to alter inflammation in the healthy overweight/obese subjects. Most of the studies available in literature on flaxseed supplementation have used high doses of flaxseeds and its components which is tedious to incorporate in the day to day life style of apparently healthy individuals.



Therefore hassle free strategies like inclusion of 5-10g of roasted flaxseeds in the form of “mukhwas” in the daily diet can be adapted to reduce the risk of metabolic aberrations in population.

Recommendations

- Though estrogen deficiency post menopause can bring alterations in body composition and further increase the risk of non-communicable diseases, the current study exhibited an alarming trend with high prevalence of overweight/obesity and other metabolic aberrations in pre-menopausal women also. Therefore, healthy lifestyle and dietary pattern should not just be a part of women with menopause, but it should be practised from the early stages of life.
- As the deficiency of vitamin B12 was quite high in predominantly vegetarian study population and was associated with lower health check-up visits, there is a need for at least yearly health check-up to detect deficiency of certain nutrients as well as metabolic aberrations among women.
- Flaxseed contains highest amount of ALA among all vegetarian sources along with other bioactive compounds like lignans, polyphenols and fiber. Therefore flaxseeds should be included in the regular diet of the vegetarians to meet the requirement of omega 3 fatty acids.
- Low doses of flaxseed (5-10g) supplementation showed a beneficial impact on blood pressure, insulin resistance and body fat percent of apparently healthy overweight/obese pre-menopausal women over a period of 8 weeks without any side effect and issues related to compliance. Therefore even low doses of flaxseeds have a potential to reduce metabolic aberrations if consumed on regular basis over a period of time.

