SUMMARY AND CONCLUSIONS

Background

The burden of deaths from cardiovascular and metabolic causes is rising in the Indian subcontinent as compared to worldwide and stand at 18.3 million annually (WHO 2007). It was thought to be affecting more males than females, but recent figures by the WHO observatory and country specific data from scattered population surveys, indicate that cardiovascular and metabolic deaths are equally high, if not more in females as well (WHO 2009, Gupta et al 2012, Abbasi et al 2012). The reason for this high occurrence is poorly understood, because the metabolic scenario during middle age in women is riddled with confounders related to menopausal causes. Menopausal changes occur due to depletion of estrogen production and since estrogen regulates a wide range of bodily functions in the female biology, menopause is accompanied by a host of estrogen withdrawal symptoms which are classified as vasomotor, somatic, psychological and urogenital, depending upon the target organs affected (Williams et al 2012).

In addition, these withdrawal symptoms also trigger chaotic endocrine imbalances which are closely advorcorately related to metabolic and cardiovascular health in women and result places the women at increased cardio-metabolic risk, due to development of obesity, hypertension, diabetes, bone loss, anemia and dyslipidemia, which appears to be most prevalent in menopausal women (Lovejoy et al 2008, Jilka 1998, Anderson et al 1995, He et al 2012, Clegg 2012)

To alleviate menopausal symptoms and to a certain extent for remedial measures for hypertension, diabetes and dyslipidemia, a large proportion of women worldwide are known to depend on alternate medicine therapies, for hardly any scientific evidence base is available in their support.

The ancient Indian system of healing, Ayurveda, places great importance on the herb wheatgrass, which is the grass of the common wheat plant, for the treatment of heart problems, as mentioned in ancient texts in Ayurveda. Also, wheatgrass has rudimentary evidence on its antioxidant activity, anticancer activity, hypolipidemic activity and content of heart healthy nutraceutical compounds that exert hypolipidemic activity.

Thus the review of the body of literature in this context, gave rise to following research questions:

1. What is the burden of cardio-metabolic risk conditions and menopausal symptoms in Indian menopausal women, who are in different stages of menopause?
2. What is the distribution of these risk conditions in a free-living population vis-à-vis a population that attends a clinical health check up facility?
3. Which is the most pressing problem in the menopausal women, with regard to cardio-metabolic risk factors/conditions?
4. How prompt are the health-seeking practices of Indian middle aged women when faced with a cardio-metabolic risk condition?
5. What are the longitudinal trends in the anthropometric indices and blood pressure values of Indian menopausal women?
6. What is the nutritional content of freeze dried wheatgrass?
7. Can wheatgrass powder in a freeze dried form be used as a functional food, by incorporating it in common Indian recipes?
8. What would be the acceptability of recipes that would have been developed by incorporating freeze dried wheatgrass powder?
9. How effective would freeze dried wheatgrass powder be, for the management of primary hyperlipidemia in Indian menopausal women?

Consequently, the present set of studies was planned to address the above questions, with the following main objectives:

1. To study the extent of metabolic derangements in pre, peri and post menopausal women in a free-living population and in women who attend a health check-up facility
2. To study the longitudinal outcomes of a health check-up in the after a period of 2 years, with regard to health seeking practices and anthropometric indices and blood pressure levels
3. To analyze the nutritional quality of wheatgrass powder, incorporate it in different recipes as a functional food and evaluate the acceptability of these recipes.
4. To investigate the impact of wheatgrass powder supplementation on lipoprotein status and menopausal symptoms in primary hyperlipidemic women.

The study was carried out in the following phases:

I. **Formative Research**: Clinico-Biochemical Changes across Pre, Peri and Post Menopausal Women in

   **Part A** - Women From Free-Living Population in Vadodara

   **Part B** - Women Attending a Health Check-Up Facility in Ahmedabad.

II. **Follow-up Study**: The Immediate and Longitudinal Outcomes of Health Check-Up on Women’s Health Care Practices.

III. **Translational Research**: Analysis of Nutritional Quality of Wheatgrass Powder and its Incorporation of in Different Recipes as a Functional Food and its Acceptability.
IV. **Experimental Research:** Impact of Wheatgrass Powder Supplementation on Lipoprotein Status in Primary Hyperlipidemic Women – An Open Label Randomized Controlled Trial.

**PHASE I: CLINICO-BIOCHEMICAL CHANGES ACROSS PRE, PERI AND POST MENOPAUSAL WOMEN IN WOMEN FROM FREE-LIVING POPULATION IN VADODARA VERSUS WOMEN ATTENDING A HEALTH CHECK-UP FACILITY IN AHMEDABAD.**

This study was conducted in two parts: Part A: Free-living population in Vadodara and Part B: Women attending a health check up facility in Ahmedabad. For part A, 186 women were enrolled from free-living population from each four zones of Vadodara city namely, north zone, south zone, east zone and west zone, through snowballing technique. For Part B, hospital, Jivraj Mehta Smarak Foundation in Ahmedabad, from where the women who came for a health check up were studied during the period of December 2010 to March 2011. In total, we interviewed 213 subjects aged 20-65 years.

In both the parts of the study, information pertaining to socio-economic and medical history and dietary and lifestyle habits was collected using semi structured questionnaire. Biochemical parameters studied include serum estimations of TC, TAG, LDL, VLDL, HDL, TSH, creatinine; plasma estimations of FBS & insulin; and blood estimation of Hb.

**RESULTS**

**Background Information**

- Background information revealed that majority of the subjects were Gujaratis (76%).
• Majority of them had graduate education (64%), however still, a large proportion of them (89.2%) were home makers and were not employed.
• More than three fourths of them (76%) lived in a nuclear family.

Medical History
• Majority of the women in the post menopausal category (46.6%), followed by pre menopausal stage (28.1%), hysterectomized category (13.3%) and least in the peri menopausal category (11.5%).
• The family history revealed hypertension was the most commonly reported history of illness.

Clinico-biochemical Changes in the Subjects
• The extent of menopausal symptoms in the subjects was 29% of vasomotor symptoms, 22% somatic symptoms, 20% psychological symptoms and 17% urogenital symptoms.
• The prevalence of osteoporosis was found to be 11.9% and osteopenia was 55%, indicating a prevalence of low bone mass to be as high as 67%.
• Overall obesity was found to be 67.4%, with post menopausal women having a prevalence of 75% and premenopausal women 53%.
• Abdominal obesity was the highest prevalent, with elevated WSR at 91.8% and elevated WC at 90.5%.
• The prevalence of obesity was supported by the lifestyle habits of the subjects, with an unhealthy frequency of snacking (41% more frequently than once a week), consumption of bakery/confectionery items (40% more frequently than once a week). In addition, sedentary behavior was seen in 60.7% and the mean fat intake of the subjects was 176% of the recommended daily limit.
The prevalence of pre-existing and newly detected hypertension in the study came up to 48.3%, and the major determinants included age >40, no college education, overweight/obesity, abdominal obesity, high blood sugar and high levels of atherogenic index of plasma and post-menopausal status, which was the strongest predictor of hypertension in the study (crude OR 4.6, age adjusted OR 2.0).

The prevalence of diabetes in this study was found to be 5.5%, which was higher in subjects from the free-living and the prevalence of insulin resistance was 25.7% which was higher in subjects attending the health – check up facility.

The most prevalent risk condition among cardio-metabolic parameters studied came out to be dyslipidemia, which along with abdominal obesity was the major contributor to the prevalence of metabolic syndrome in the subjects.

Univariate analysis revealed that age, low education, being post menopausal, high WC, WSR, BMI, FBS ≥100mg/dl and AIP > 0.21 had significantly high odds ratios for hypertension, indicating increased risk of developing hypertension. Multivariate analysis revealed that a model consisting of DBP, FBS, TC, LDL, TAG/H and AIP directly explained 61% of variation in SBP.

For Diabetes the significant univariate predictors identified were age, low education, being post menopausal, HOMA2 IR and AIP. Multivariate predictive model containing PG2BS, Insulin and HOMA2 IR explained maximum possible variation of 54%.

The key difference between the subjects from a clinical setting versus those from a free living population was that former had a higher prevalence of severe obesity (29% vs. 19%), insulin resistance (31% vs 21%), TC (39% vs. 27%) and LDL (73% vs. 57%), suggesting higher body fat and circulating fat.

In addition to the above, the prevalence of menopausal symptoms was also higher in subjects from a clinical setting: vasomotor sympots-30% vs 28%,
somatic symptoms – 24% vs 21%, psychological symptoms- 22% vs 19% and urogenital symptoms – 19% vs 16%, suggestive of relatively increased estrogen withdrawal.

- The above fact is supported by a higher degree of risky behavioral practices prevalent in subjects from a clinical setting: higher frequency of consumption of fried snacks (41% vs. 38%) and bakery and confectionery items (40% vs. 33%). To add to it, the proportion of subjects not engaging in regular physical activity was marginally higher (45%) in clinical setting (compared to 43% in free living population).

CONCLUSIONS

Middle aged south Asian women suffer from a high prevalence of cardio-metabolic risk factors. These factors are worse in peri and post menopausal women, suggesting the role of endocrine disturbances during menopausal transition in aggravation of cardio-metabolic health. The situation was compared between women from a free-living population and women attending a health check-up facility. It appeared that women from the clinical setting were worse off than their counterparts from free-living population, in that, the former had higher circulating and body fat and relatively higher estrogen withdrawal. This is suggestive of the fact that Indian women don’t present for a health check-up until their cardio-metabolic risk situation has aggravated to the point where there are multiple co-existing clinic-biochemical changes in their biological systems.

PHASE II: THE IMMEDIATE AND LONGITUDINAL OUTCOMES OF HEALTH CHECK-UP ON WOMEN’S HEALTH CARE PRACTICES

Of the 186 subjects enrolled from the free-living population in the formative research phase, 107 were followed up after a period of 2 years to observe what action pertaining to health was taken immediately after they obtained the results
from the health check-up, and track the anthropometric changes undergone by them over a period of 2 years. The follow-up also tracked if the women had taken any health-seeking action after the health check-up till two years. In follow-up, the subjects whose contact details were valid after 2 years were called up for an appointment at a time convenient to them and at the scheduled appointment the reported data and the physical and biophysical measurements were collected.

RESULTS

**Longitudinal Trends in Body Composition and Blood Pressure in the subjects**

- Mean weight of the subjects during the time of the initial health checkup was 64.47kg, which had mildly increased to 64.50kg after a period of 2 years
- The mean waist circumference of the subjects had also increased slightly from 95.54cm at baseline to 95.97cm at the end of 2 years
- The mean systolic blood pressure of the subjects had reduced from 130mmHg to 127mmHg SBP, which was still in the pre-hypertensive category and the DBP had reduced from 82mmHg to 79mmHg.
- A fact to be considered here is that a considerable number of people were freshly diagnosed as hypertensives in the initial health checkup and the in the subjects who were followed up had started on anti-hypertensive medication.

**Health Seeking Practices of the Subjects**

- The health seeking practices of the subjects lefts much to be desired: Very few of them actually took some action when they discovered they needed health consultation.
- Of the 107 subjects that were followed up 39.9% were not diagnosed with any risk condition
- Rest of the 60.1% had been diagnosed with risk situation(s), out of which only a mere 3.04% had seen a doctor and rest of them (57.7%) had not taken any
action after getting the results of the health check up, even after 2 years had gone by.

CONCLUSIONS

The longitudinal trends indicate a slow increase in the anthropometric indices but not blood pressure. Women tend to refrain from seeking health care even in the face of presence innumerable risk factors.

PHASE III: ANALYSIS OF NUTRITIONAL QUALITY OF WHEATGRASS POWDER AND ITS INCORPORATION IN DIFFERENT RECIPES AS A FUNCTIONAL FOOD AND ITS ACCEPTABILITY

For the nutrient component analysis, freeze-dried wheatgrass powder was procured from Aum Agri Freeze Foods, local exporting firm in Vadodara. The nutrient analysis included quantitative testing of energy, protein content, total fat, fibre, iron, moisture, ash, carbohydrate & sugar content, ascorbic acid, and β carotene.

All the recipes were standardized and wheatgrass powder was incorporated at the levels 1g, 1.5g and 2g per unit in case of Khakhra and Thepla; and per serving in case of Muthiya, Dal and Buttermilk.

The acceptability of the organoleptic attributes of the recipes was evaluated by conducting a sensory evaluation using composite rating test. The evaluation panel included 12 semi-trained members.
RESULTS

Nutrient Content Analysis

- Wheatgrass was not a significant source of macronutrients, with the energy content being 354kcal per 100g of the powder, protein being 28.7g, carbohydrates 49.9g and fats 4.4g per 100g.
- However, wheatgrass had considerable amounts of β-carotene (108100 µg) and iron (57.9mg) and fibre (25.5g).

Acceptability Testing of Wheatgrass Incorporated Recipes

- The mean overall scores of Dal incorporated with 1, 1.5 and 2g of wheatgrass was within a narrow range of 6.8 to 7.1, indicating moderate acceptability at all levels.
- The mean overall scores for the level 1.5g (7.5) was significantly higher (p<0.01) than for the level 1g (5.7) and level 2g (6.4).
- For Muthiya again, the mean overall scores did not differ significantly and the best accepted level was 1g (mean score 6.6), closely followed by the level 1.5g (6.5) and finally the 2g level (mean score 6.4).
- Khakhra was well accepted at both 1g and 2g level indicated by similar mean overall scores (7.1 and 7 respectively); while mean scores for the 1.5g level stood at 6.7.
- The best accepted recipe of the lot was Thepla, with mean overall score of 7.9 for the level 1g of incorporation of wheatgrass powder.

CONCLUSIONS

Wheatgrass is a rich source of micronutrients and phytonutrients and can be used as a health supplement. Alternatively, if incorporated into day to day recipes as a functional food, it does not significantly alter the sensory attributes and hence the acceptability of the recipes.
PHASE IV: IMPACT OF WHEATGRASS POWDER SUPPLEMENTATION ON LIPOPROTEIN STATUS, INFLAMMATION AND MENOPAUSAL SYMPTOMS IN PRIMARY HYPERLIPIDEMIC WOMEN – AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL

Design

The supplementation study was conducted using an open label randomized controlled design, involving an experimental group which was given wheatgrass powder capsules, daily for 10 weeks; and a control group that was maintained without administering any intervention. The data pertaining to background information, medical history, dietary and lifestyle habits was collected prior to and after the intervention (pre and post data).

Preparation of Treatments

Freeze dried wheatgrass powder for the supplementation study was procured from Aum Agri Freeze Foods and was encapsulated into 350mg gelatin capsules of size 0, courtesy Centurion Laboratories, Vadodara and hermetically sealed in plastic jars.

Enrollment of Subjects

Sample size using online statistical model (Length 2006-2009) adapted for a controlled trial came out to be 28 in each arm. The subjects were selected from a population of primary hyperlipidemic women. The potential participants (n=78) were subject to scrutiny by following a set of inclusion and exclusion criteria, wherein, subjects who were between 30-60 years of age, had total cholesterol levels >200mg/dl and/or a triacylglycerol level >150mg/dl, were enrolled. The subjects who suffered from diabetes, polycystic ovarian syndrome, genetic traits of dyslipidemia, hypothyroidism or pituitary disorders; or who had been initiated on statins less than 3 months before the trial or who were on hormone replacement therapy were not enrolled in the study (n=8). After enrollment of the
subjects (n=61), they were randomized into the two study groups: control and experimental groups by following the chit method for equal random allocation into two groups as delineated by Giesbrecht and Gumpertz (2004). Subjects in the experimental were supplemented with 4 capsules containing total of 1.4g wheatgrass powder each day. Only two subjects dropped out of the study, from the experimental group, leaving a total of 29 subjects in the experimental and 30 subjects in the control group.

**Outcome Parameters**

The main outcome parameters that were studied to assess the impact of the supplementation were the serum lipoprotein fractions: TC, TAG, LDL, HDL, & VLDL; apolipoproteins A & B; in order to study atherogenicity. For studying impact on inflammation, high sensitivity assay of C reactive protein (hs-CRP) was studied. Other background parameters that were studied included information on socio-economic status, medical obstetric history, dietary habits & intake, lifestyle habits and physical activity. All these data were collected using a semi structured pretested questionnaire. The clinical data included height, weight, waist circumference, hip circumference and blood pressure.

**RESULTS**

**Background Characteristics**

- The women from all the three stages of menopause viz., pre menopause, peri menopause and post menopause were more or less equally distributed in both the study groups (24-30% in controls and 24-38% in experimental group)
- Information on anthropometric variables and blood pressure revealed that the clinically the subjects in both the groups were comparable at the beginning of the study.
• Nutrient intakes of the subjects were similar across the control and experimental groups, with neither of them meeting their 100% RDA of their energy requirements.

• The iron intake was higher in the experimental group subjects (11.2mg/day) as compared to control group subjects (8.9mg/day), with the difference being statistically significant (p<0.05).

**Impact of the Supplementation on Menopausal Symptoms**

• Vasomotor symptoms saw a non-significant decline of 42% in the experimental group following the supplementation

• Somatic symptoms decreased by 33% after the supplementation, whereas psychological symptoms reduced by 50%

• Urogenital symptoms remained unchanged at 13.8% till the end of the supplementation period.

**Impact on Atherogenicity and Inflammation**

• Supplemented group experienced a significant reduction in the TC (5.3%, p<0.01), and apo B (13%, p <0.001) and near significant reduction in TAG (9.7%, p=0.07)

• The index apo B/A reduced significantly by 6.4% (p<0.05) after the intervention, while other atherogenic indices TAG/HDL and AIP reduced non-significantly by 3.6% and 5% respectively.

• FBS showed a significant decline of 5.1% after the supplementation, though the FBS levels were in the normal physiological range.

• The hs-CRP levels reduced non-significantly by 10% in the experimental group.

• The hemoglobin levels were maintained in the supplemented subjects following the supplementation, whereas they reduced in the controls.

• The prevalence of high TC (>200mg/dl, that is) went down from 83% at baseline to 66% after the supplementation. The prevalence of TAG>150mg/dl
went down from 38% initially to 31% after the intervention and the prevalence of LDL>100mg/dl went down from 96% at baseline to 90% after the supplementation.

- The prevalence of low HDL however, remained the same before and after the intervention. All the above reductions were statistically non-significant.

**Influence of Initial TC levels on Impact of the Supplementation**

- Subjects having elevated TC levels (>200mg/dl) at the beginning of the study showed a greater decline in TC (5.6%, p<0.05) and apo B (14%, p<0.001) and FBS levels (4.6%, p<0.01) compared to subjects who had normal TC levels at baseline.
- The TAG, VLDL, TAH/HDL and AIP levels had a sharper reduction in response to the supplementation in subjects who had normal TC levels (Figure 4.30) at baseline compared to those who were hypercholesterolemic at baseline (18.8%, p<0.01 for TAG, and 18.8%, p<0.05 for VLDL).

**Influence of Initial TAG levels on Impact of the Supplementation**

- Subjects with higher than normal initial TAG levels (≥150mg/dl) showed higher significant decline in the TC, TAG, LDL and VLDL levels compared to individuals who had normal TAG levels to begin with (<150mg/dl).
- The prevalence of dyslipidemia showed greater decline in elevated TAG group.
- The apo B levels, atherogenic indices, FBS and hs CRP levels were not affected by the initial TAG levels and declined significantly in all the subjects.

**Influence of Initial BMI on Impact of Supplementation**

- The effect of initial BMI was quite evident in the impact of the supplementation on the lipid profile: the TC, LDL, apo B declined significantly
in the subjects who had higher than normal BMI (> 23kg/m²) as compared to those who had a normal BMI.

- Regarding the atherogenic indices, the indicator apo B/ apo A showed a significant decline in the high BMI group compared to the normal BMI group.
- The hs-CRP levels did not show significant variations in either of the groups.

**Influence of Absence of Hypertension on Impact of Supplementation**

- Absence of hypertension was found to influence the extent of impact of supplementation on serum lipoproteins. The TC, LDL, declined significantly in normotensive group compared to hypertensives.
- Apo B was not affected by Hypertensive state and declined significantly in both the groups.
- The prevalence of dyslipidemia also showed greater decline in hypertensive subjects compared to normotensives.
- The atherogenic indicator apo B/ apo A, showed significant decline in the normotensive group, but not in the hypertensive group.
- The hs-CRP levels showed a decline in normotensive group compared to hypertensive group, where the change was non-significant.

**CONCLUSIONS**

Wheatgrass supplementation as studied at the present dose, has been found to improve the metabolic and cardiac profile of menopausal women and also assuage the menopausal symptoms in them. The effect was more pronounced in subjects with elevated TC, TAG and BMI and normal blood pressure.
RECOMMENDATIONS

- The extent of menopausal symptoms and cardio-metabolic derangements in middle aged women needs to be addressed. The women need to be sensitized to avail effective diagnostic measures and ensuring early prevention because it has been observed that they present for a health check-up well after the risk factors have build up and started clustering.

- The health seeking practices of Indian women has a long way to go and need improvement through counseling and electronic media in order to prevent clustering of risk factors and early prevention of cardio-metabolic events, also to improve the outreach of health programs.

- As wheatgrass is a source of a number of micronutrients and phytonutrients it is worth considering to be used as a health supplement for various conditions, which benefit from antioxidant supplements.

- Incorporating wheatgrass into common Indian recipes was found not to adversely affect the sensory attributes of the recipes. Thus wheatgrass can be used as a functional food at household level.

- Wheatgrass has shown to possess potent hypolidemic properties and hence can be used as a holistic adjunct therapeutic strategy in the management of primary hyperlipidemia in menopausal women.