METABOLIC DERANGEMENTS IN PRE, PERI AND POST MENOPAUSAL WOMEN AND STRATEGIES FOR THE MANAGEMENT OF PRIMARY HYPERLIPIDEMIA IN ADULT WOMEN

APRIL 2013

NITYA ELAYATH
M.F.C.Sc.
(PUBLIC HEALTH NUTRITION)

METABOLIC DERANGEMENTS IN PRE, PERI AND POST MENOPAUSAL WOMEN AND STRATEGIES FOR THE MANAGEMENT OF PRIMARY HYPERLIPIDEMIA IN ADULT WOMEN

Thesis Submitted In Partial Fulfillment Of The Requirements
For The Degree Of
Doctor Of Philosophy (Foods And Nutrition)

By

NITYA ELAYATH

M.Sc. (F.C.Sc.)
Registration No. 313

Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Vadodara.

Certificate

This is to certify that the research work presented in the results of this thesis has been carried out independently by Ms. Nitya Elayath in pursuit of a Doctoral Degree in Foods and Nutrition and this represents her original work.

Prof. (Dr.) Uma Iyer
Head and Guide,
Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja Sayajirao University of Baroda
Vadodara.

Date: 25th April 2013

ACKNOWLEDGEMENTS

I thank whatever gods may be, for my unconquerable soul...
...I am the master of my fate; I am the captain of my soul.

-William Ernest Henley
(*Invictus, 1888*)

Accomplishments in nature are possible when the circumstances favor them to happen. Conducting this research and writing this doctoral thesis, has been a huge accomplishment for me indeed, and as for all accomplishments, I had several enabling elements facilitating the execution of this research. I therefore would like to express my due gratitude to all who have helped me fulfill this research, before I present its findings.

I gained realization of the ancient Hindu verse "Acharya devo bhava" by firsthand experience during this research; because my guide, mentor and guru, Prof Uma Iyer, has been just that to me. Her foresight, brilliance, objectivity, steadfastness and rock-solid confidence encouraged me constantly to strive for perfection and to never give up in the face of hindrances. I would like to take this opportunity to convey my utmost sincere thanks and gratefulness to her.

I acknowledge the University Grants Commission, New Delhi for providing the financial input for conducting this research, The M. S. University of Baroda and Reliance Industries for providing the infrastructural support and The Jivraj Mehta Smarak Health Foundation, Ahmedabad for both. I am grateful to Dr. Kiran Shinglot for training me in clinical estimation of blood pressure and Dr. Narendran for helping with the nutrient component analysis.

Another beam of support and source of valuable inputs for this research has been Dr. Swati Dhruv, our all beloved Swati mam.

Dr. Shonima Venugopal (Shonima di), senior and friend, has provided inputs that have helped me in personal and professional spheres of life.

Friends who made this journey a memorable experience include Trushna, Smriti di, Kuhu, Shruti di, Rachana di, Vandana, Aditika, Pooja and Arti di.

My sister, Nisha (who has always been by my side in all my endeavors) has always been a great support throughout. Vinodettan has been a godfather always and has helped shape all my significant decisions; I hold him in the highest of respects. Cheering up my life all the way has been Shivangi, my adorable niece.

Family I believe is the substratum that keeps you grounded and averts any misfortunes in this world from uprooting you. My parents have been my bedrock and nurturers of my abilities and confidence, and plainly put, there wouldn't have been any of this without them. However much I try, I cannot thank them enough and such is their input. Mummy and Papa & Praveen in Bahrain helped me a lot during the entire time I was there and I am ever grateful to them for inspiring me to aim still higher.

This research experience brought me face to face with some really tough times, where if it were not for the brainwave and inspiration provided by my partner, Deep; I have no idea what the shape of events would have been. He has been a pillar of support, friend, husband, guru and even a therapist in times when I thought I would give up, but he never gave up in me and reminded me 'Its only during the tough times that we know what we are made of...'

Thanks a lot, all of you...!

Nitya

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ADA	American Dietetic Association
AHA	American Heart Association
AIP	Atherogenic Index of Plasma
ALA	Alpha Linoleic Acid
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
ATP	Adult Treatment Panel
BIS	Bureau of Indian Standards
BMD	Bone Mineral Density
BMI	Body Mass Index
BUA	Broadband Ultrasound Attenuation
CETP	Cholesterol Ester Transfer Protein
CHD	Coronary Heart Disease
CHO	Carbohydrates
CLIA	Chemiluminescent Immuno Assay
CNS	Central Nervous System
CO	Carbon Monoxide
CUPS	Chennai Urban Population Study
CURES	Chennai Urban Rural Epidemiological Study
CVD	Cardio Vascular Disease
DBP	Diastolic Blood Pressure
DEXA	Dual Energy X-ray Absorptiometry
DM	Diabetes Mellitus
E2	Estradiol
EGCG	Epi Gallo Catechin Gallate
ELISA	Enzyme Linked Immuno Sorbent Assay
ERα	Estrogen Receptor α
FBS	Fasting Blood Sugar
FFA	Free Fatty Acids
FRAP	Ferric Reducing Power Assay
FSH	Follicle Stimulating Hormone
FT4	Free Thyroxine
GDP	Gross Domestic Product
GLUT	Glucose Transporter
GLV	Green Leafy Vegetables
GnRH	Gonadotropin Releasing Hormone
GSH	Glutathione
Hb	Hemoglobin
HbA1C	Glycated Hemoglobin

HC	Hip Circumference
HDL	High Density Lipoprotein
HL	Hepatic Lipase
HMG CoA	
HOMA	Homeostasis Model Assessment
HRT	Hormone Replacement Therapy
hs-CRP	high sensitivity C Reactive Protein
IDA	Iron Deficiency Anemia
IFG	Impaired Fasting Glucose
IGF	Insulin like Growth Factor
IGT	Impaired Glucose Tolerance
IL	Interleukin
IR	Insulin Resistance
JHW	Jaipur Heart Watch
JNC	Joint National Committee
LCAT	Lecithin Cholesterol Acyl Transferase
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
Lp(a)	Lipoprotein (a)
MDA	Malondialdehyde
MS	Metabolic Syndrome
MUFA	Mono Unsaturated Fatty Acids
NHANES	National Health And Nutrition Examination Survey
NNMB	National Nutrition Monitoring Bureau
NOS	Nitric Oxide Synthase
NPY	Neuropeptide Y
Ob	Obese
OR	Odds Ratio
OW	Overweight
PA	Physical Activity
POD	Peroxidase
PP2BS	Post Prandial Blood Sugar
PPAR	Peroxizome Proliferator Activated Receptor
PUFA	Poly Unsaturated Fatty Acid
qUS	Quantitative Ultra Sound
RDA	Recommended Dietary Allowance
RIA	Radio-Immuno Assay
RLU	Relative Light Units
RNA	Ribo Nucleic Acid
SBP	Systolic Blood Pressure
SCH	Sub Clinical Hypothyroidism
SERM	Selective Estrogen Receptor Modulator
SFA	Saturated Fatty Acid
SHBG	Sex Hormone Binding Globulin

SNP	Single Nucleotide Polymorphism
SOD	Super Oxide Dismutase
TAG	Tri Acyl Glycerol
TC	Total Cholesterol
TCA	Tri Carboxylic acid Cycle
TDF	Total Dietary Fiber
TNF	Tumor Necrosis Factor
TSH	Thyroid Stimulating Hormone
UN	United Nations
USFDA	United States Food and Drug Administration
VCAM	Vascular Cell Adhesion Molecule
VDR	Vitamin D Receptor
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHI	Women's Health Initiative
WHO	World Health Organization
WHR	Waist Hip Ratio
WSR	Waist Stature Ratio

ABSTRACT

Menopause has been known to disrupt the harmony in the cardio-metabolic and related systems in females. The different level of saturation of risk factors in women, together with their interaction with female hormones, plays an important role in the development of cardiovascular disease. This coupled with the distressingly high statistics of adverse cardio-metabolic events occurring in women worldwide raises a concern and given that middle aged women form a sizeable element of the Indian demography, the health expenses incurred towards chronic disease alleviation by this huge segment of the population would be a cause of grave concern for the stake holders. However, to sketch conclusive decisions on the interventions and the extent of coverage, comprehensive studies spanning the complete picture of the metabolic and cardio-vascular risk factors across a significant part of the Indian population is a pre-requisite. But in this regard, most of the studies are on the western population and data on ethnic women is lacking.

Research in the area of menopausal health has speculated that nutraceutical compounds and functional foods are promising and so is the case with herbal supplements with regard to hyperlipidemia. However discreetly designed trials on Indians are scarce and fail to provide any conclusive evidence. One breakthrough that the field of nutraceuticals has witnessed is Wheatgrass, which is proposed to have remarkable antioxidant capacity, which can prove to be a functional food for the management of chronic diseases.

Thus the following set of studies was conducted with the following objectives:

- To study the clinic-biochemical changes in pre, peri and post menopausal women in a free-living population versus in women from a clinical setting
- To study the longitudinal outcomes of a health checkup in middle aged women

- To study the nutrient content of freeze-dried wheatgrass powder and acceptability of wheatgrass incorporated common Indian recipes
- To study the impact of wheatgrass powder supplementation on atherogenicity, inflammation and menopausal symptoms in primary hyperlipidemic women

For studying the clinico-biochemical changes in pre, peri and post menopausal women in a free living population, 186 women (30-65years) were enrolled from four zones of the city. For comparison with women from a clinical setting, 213 pre peri and post menopausal women attending a health check-up facility in Ahmedabad were enrolled. The cardio-metabolic risk factors were studied using standard physical, bio-physical and bio-chemical methods in both the settings.

Overall obesity was found to be 67.4%, with post menopausal women having a prevalence of 75% and premenopausal women 53%. Prevalence of high waist circumference was the highest (90.5%). Extent of menopausal ranged from 17% - 22%. Almost 49.3% had Hypertension and 25.8% were pre-hypertensive. 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.

Around 6.1% of the subjects were diagnosed with diabetes while as high as 25.7% had insulin resistance (HOMA 2). Almost 33.8% of the subjects had hypercholesterolemia, 17% had elevates triglycerides. An astounding 65.2% had high LDL cholesterol and 47.3% had low HDL and 35.8% had metabolic syndrome. An unexpected 46.4% of the subjects were found to be anemic. Osteoporosis was diagnosed in 11.9% of the women, in a subsample of 67 women. As high as 86.5% of the subjects had >30% calories from fat. Post menopausal status was found to be a significant predictor of hypertension and diabetes (OR 4.6 and 5.4 respectively; 95% CI: 2.4 – 8.8 and 1.7-19.1 respectively; $p<0.001$). A model consisting of BMI,

WC and SBP was able to explain 58% of variance in SBP. 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 and higher prevalence of menopausal symptoms, suggesting increased estrogen withdrawal.

Thus, the burden of cardio-metabolic derangements in middle aged women is alarmingly high and is aggravated by the menopausal transition. Anthropometric parameters are able to predict much of the cardio metabolic risk scenario in these women. The situation is worse off in women from a clinical setting, indicating the fact that they present for a check-up after the risk situations have aggravated quite a bit. A holistic intervention to target endocrinological changes and resulting metabolic changes is the need of the hour.

For studying the longitudinal trends in the body composition and blood pressure of menopausal women, the women studied in the first phase from a free-living population, were followed up after 2 years. During the exploratory research, the women who had elevated levels of risk factors studied in that phase, were informed of their high risk situation and were asked to see a doctor for further diagnosis and treatment, if any. After a period of two years, of the 186 subjects studied in the exploratory research phase, 107 could be followed up, because 27 had permanently moved, 42 were temporarily unavailable because of either being out of station or having changed their contact details, 7 were not willing to share any details and 3 unfortunately, had expired. The results indicated that the 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 2years. 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 blood pressure of the subjects had reduced from 130mmHg to 127mmHg SBP, and DBP had reduced from 82mmHg to 79mmHg. Regarding the health seeking practices of the subjects, it was observed that of the 107 subjects

that were followed up, 39.9% were not diagnosed with any risk situation, of the remaining 61.1%, 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. This takes attention to the fact that the health seeking practices of women in India is abysmally low, awareness needs to be created among them so that they realize that health consultation if sought early, will revert most of the adverse health conditions they are predisposed to.

The nutrient component analysis included quantitative testing of energy, protein content, total fat, fibre, iron, moisture, ash, carbohydrate & sugar content, ascorbic acid, and β carotene. It was found that wheatgrass has excellent nutrient content as reflected by its high iron content (57.9mg) and β-carotene content (360µg). For testing the acceptability, five recipes: *Khakhra, Thepla, Muthiya, Dal* and Buttermilk, which involve different method of cooking were selected and wheatgrass was incorporated at levels 1g, 1.5g and 2g per serving in case of Muthiya, dal and Buttermilk and per unit in case of *Khakhra* and *Thepla*. The acceptability of the organoleptic attributes was evaluated using sensory evaluation employing the Composite scoring test, by a semi trained panel of 12 subjects. The mean scores for *Dal*, buttermilk, *Khakhra*, *Muthiya* and *Thepla* were 7.0, 6.5, 6.9, 6.5, 7.3 respectively, which was indicative of good acceptability. It was observed that the acceptability was higher in recipes with low water content like *Khakhra* and *Thepla*, than recipes with high water content viz., *Dal*, Buttermilk and *Muthiya*. Therefore, wheatgrass will have excellent acceptability if used as a functional food component in day to day recipes and its nutritional quality can serve to ameliorate various chronic health conditions.

For evaluating the effect of wheatgrass (*Triticum aestivum* L.) on menopausal health, hyperlipidemia and inflammation, a randomized controlled study design was employed wherein, 59 mildly hyperlipidemic menopausal women served as participants. Here 3.5g of freeze-dried wheatgrass powder in encapsulated form was administered to the participants in the intervened group (n=29) daily for a period of 10 weeks, while the control group (n=30) received no intervention. At the end of the

intervention period, the prevalence of menopausal symptoms saw non-significant, but noteworthy reductions: vasomotor symptoms came down by 42%, somatic symptoms 33%, psychological symptoms 50%, while urogenital symptoms remained unaltered. Experimental group showed significant 5.3% reduction in total cholesterol ($p<0.01$) and 13% reduction in apo B ($p<0.001$); near-significant reduction of 9.7% in triacylglycerols ($p=0.07$); and significant decrease of 6% in high density lipoproteins ($p=0.05$); while C – reactive protein levels remained unaltered. Thus the abundance of nutraceutical compounds in wheatgrass exerts beneficial effects on both the atherogenic indices and menopausal symptoms in hyperlipidemic women.

Thus the present study found that women have a high degree of cardio-metabolic risk factors coupled with equally high degree of endocrine imbalances attributable to menopausal transition. However, their poor, untimely health seeking practices do not make up for this elevated risk situation. Freeze-fried wheatgrass powder showed promising hypolipidemic effects and menopausal symptoms alleviating properties in primary hyperlipidemic women and hence can be a useful holistic adjunct therapeutic strategy in the management of primary hyperlipidemia in menopausal women.

INTRODUCTION

Despite extensive strides being made in health science research and health policy research and related areas, the burden of non-communicable diseases reported continues to be alarmingly high around the world, which adds to the public health spending for seeking treatment of these conditions. The latest World Health Organization fact sheet (WHO 2012) reports more that 36 million deaths accountable to non-communicable diseases across the world, out of which 80% occur in low and middle income countries. These countries include India and it is a concern given the fact that this spending exerts a drag on the already fraught Indian economy.

A major part of above reported deaths is directly accountable to cardiovascular and metabolic causes, which are responsible for 18.3 million deaths annually (WHO 2007). The main behavioral risk factors identified by WHO in this regard are all instrumental in precipitating a metabolic and physiological situation which is highly conducive to occurrence of adverse cardiovascular events. Cardiovascular deaths have been projected to rise to 25 million by 2030 (WHO 2012).

Another disturbing trend that has been observed in the face of high prevalence of non-communicable diseases, especially cardiovascular diseases and risk factors, is the characteristic dominance of these diseases in middle aged women, particularly in Indian populations (Ghosh et al 2010, Abbasi et al 2012, Gupta et al 2012). WHO (2009) observes that cardiovascular disease, often thought to be a problem more for males, is the leading cause of death in women, responsible for one third of all deaths in women (Global Health Observatory 2010). The major causative factor for such high prevalence in middle aged women is more often than not, the complex endocrino-metabolic changes that the female body undergoes around menopausal transition (Hart, Charkoudian and Miller 2011).

MENOPAUSAL TRANSITION

Menopause is an important physiologic phase and denotes the end of reproductive lifespan in women. Its defining feature is termination of periodic menstrual cycles and it is clinically defined as absence of menstrual periods for atleast 12 consecutive months or more (Soules et al 2001). On an average, menopause occurs at the age of 51-52 years (Copeland 1993). The phenomenon of menopause is not a one point event, rather is a gradual endocrinological progression that culminates with stoppage of menstruation. Depending on the clinical stage of menstrual cycles, reproductive stage classification of women categorizes them into either

- Premenopausal (reproductive/ fertile phase),
- Peri menopausal (phase of transition from pre-menopause to cessation of menstrual cycles) or
- Post menopausal (after the menopausal transition is complete and menstrual cycles have completely stopped)

The instrumental factor for occurrence of menopause is the decline in ovarian follicles that are responsible for ovulation and production of sex steroids, including estrogens in women. By middle age, the reserve of ovarian follicles is exhausted and the endogenous estrogen production also declines. This is main hormonal feature that precipitates a range of physiological, biochemical and clinical changes in the female body (Williams 2012). The reason attributable to this occurrence is that estrogen is a key regulator of a wide variety of bodily functions and estrogen receptors are found in a host of organ systems in females. When the estrogen production falls, all these functions suffer estrogen withdrawal effects (Burger et al 2008). These effects range from physiological symptoms to altered metabolic milieu. The symptoms experienced hence are termed as the menopausal syndrome and depending on the system affected are classified into the following

a. Vasomotor symptoms

b. Psychological symptoms

c. Somatic symptoms and

d. Urogenital symptoms

MENOPAUSAL SYMPTOMS

Vasomotor Symptoms: These include hot flashes and night sweats, where the person experiences sudden rise in temperature and feels unusually hot even in cool climates, which results in unusual perspiration as well. Estrogens are involved in major pathways in the temperature regulation center in the hypothalamus; hence estrogen withdrawal leads to vasomotor symptoms during menopause (Freedman 2001). Vasomotor symptoms in Indian populations have been reported to be as high as 56% in North Indian women, while it is 38% in Gujarat (Nair and Chauhan 2006).

Psychological Symptoms include irritability, depressed mood, anxiety, sudden mood swings, and crying spells among others. Estrogens are closely associated with neuroeffector mechanisms and the production and functioning of neurotransmitters dopamine and serotonin, which are key mood regulators. Thus loss of estrogens affects dopamine synthesis and reuptake, leading to changes in moods and the psychological equilibrium in general (Glazer et al 2002). Psychological symptoms in Indian women are reported to be around 36.4% (Kapur et al 2009).

Somatic Symptoms, interchangeably called as physical symptoms, include most commonly, headaches, joint pain/ aches, dizziness and fatigue. Estrogens exert vasodilatory effects on blood vessels; hence during low levels of estrogen, like in menopause, there is concomitant vasoconstriction which often results in headaches (Lucchesi et al 2012). Fatigue is related to lack of sleep due to night

sweats and anxiety spells, and tends to be physical and mental in nature (Moller et al 2013). Singh (2012) estimated the prevalence of menopause related somatic complaints in Central Indian women from Hyderabad to be 32%. In south India, this figure was reported to be 43% (Bairy et al 2009).

Urogenital Symptoms affect both the urinary system and the genitals, and include urine incontinence, drying and itching in the vagina and decline in sexual drive. The genito-urinary system has a number of estrogen receptors for regulation of various functions, and decline in estrogen leads to atrophy of vaginal muscles and urethral muscles, leading to urine incontinence and loss of secretions in the vagina (Warren, Shu and Dominiguez 2004). Singh (2012) estimated the prevalence of urogenital complaints in India populations to be 15.5%.

In addition to the menopausal syndrome, menopausal transition affects the cardio-metabolic processes, leading to increased prevalence of risk conditions in women and putting them at higher risk of developing adverse cardio-metabolic events. Following are the clinic-biochemical changes brought about by menopausal endocrinological shifts in female biology.

MENOPAUSAL TRANSITION AND CARDIO-METABOLIC CHANGES

BODY COMPOSITION, OBESITY & OSTEOPOROSIS

Sex steroid hormones are known to regulate energy metabolism pathways and hence affect body composition in females (Chen, Brown and Russo 2009). Misso et al (2005) emphasized the role of androgens and endogenous estrogens in the homeostasis control over energy balance and adipogenesis, in a way comparable to leptin. Bhatia and Wade (1993) demonstrated that estrogens regulate either the activity and/ or the expression of the enzymes/proteins involved in the glucose transport pathway: Glycolysis / glucoegenesis → TCA

cycle → MRC-mediated electron transport / oxidative phosphorylation → ATP translocation. Toda et al (2001) demonstrated in mouse model that the absence of estrogen suppresses the mRNA expression of enzymes concerned with fatty acid metabolism: very long fatty acyl-CoA synthase, medium chain acyl-CoA dehydrogenase and peroxisomal acy-CoA oxidase. Consequently, depletion of estrogen results in an imbalance in the energy balance and frequently leads to obesity after menopause.

With regard to abdominal fat as well, it has been reported that peri menopause has links to increase in the fat depot and its redistribution to the abdomen, which leads to a shift from gynoid to android form of distribution of adipose tissue (Davis et al 2012). Obesity is reported to be prevalent to the order of 31% to 67% (Ebrahim et al 2010; Prasad et al 2011; Midha et al 2011; Gupta et al 2012; Singh et al 2012). The prevalence of abdominal obesity is even higher: 82.3% - 93% (Jyothi and Nayak 2010; Ghosh and Bhagat 2010; Khokhar, Kaur and Sidhu 2010; Singh et al 2012)

Estrogen withdrawal after menopause also affects the skeletal tissues and leads to loss of bone mass. Estrogens are involved in the biological phenomena of bone formation and inactivation of estrogen receptor ERRα, resulting in increased bone loss that is not compensated with bone formation (Gallet et al 2013). Epidemiological studies have reported that the menopause, coupled with obesity, puts women at increased risk of fractures, which is an indicator of fragile bones resulting due to bone loss (van der Voort et al 2001). A recent large scale longitudinal study, Global Longitudinal study of Osteoporosis in Women (Compston et al 2011) looked into changes in bone at menopause and by considering endpoint as fractures, reported that post menopausal women had significantly higher number of ankle and leg fractures, particularly obese women, compared to pre menopausal normal weight women. The prevalence of osteoporosis in postmenopausal women in Southern Indian has been reported to be as high as 50% at any site (Paul et al 2008), while in North India, it has been found to be 53% (Aggarwal et al 2011).

VASOMOTOR INSTABILITY AND HYPERTENSION

Estrogen receptors are closely linked with vasomotor activities (baroreflex functioning) and central sympathetic activity, imbalance in both of which causes hypertension (Sadeghi et al 2011). Endogenous estrogens and androgens co-ordinate to enable a balance between vasodilatation and vasoconstriction; loss of this balance, leads to persistent vasoconstriction and eventually in the long run, results in hypertensive states. A number of studies have observed an epidemiological link between menopause and prevalence of hypertension, that suggest causative links between menopausal transition and occurrence of hypertension in women (Freedman and Woodward 1995; James et al 2004; Zanchetti et al 2005; Sadeghi et al 2011). Hypertension in middle aged women in several cities across all zones in India has been found to be ranging between 30%-54.4% (Bharti et al 2011; Meshram et al 2012; Gupta et al 2012; Gupta Deedwania and Achari 2013).

GLUCOSE HOMEOSTASIS AND DIABETES

Menopausal transition brings about distributional body composition changes resulting in substantial increase in visceral fat depot. Abdominal fat depot is regarded as an endocrine organ in itself owing to its ability to secrete adipokines among other substances that are directly linked with metabolic diseases such as insulin resistance, diabetes and the metabolic syndrome. Additionally, decrease in levels of sex steroids leads to increased levels of the transport protein for sex steroid hormones in humans, serum sex hormone binding globulin/ SHBG, as positive feedback mechanism. High circulating SHBG has been found to be a potential independent indicator of risk of developing insulin resistance (Goodman-Gruen and Barret-Connor 1997; Thadani et al 2003; Jayagopal et al 2004) and diabetes (Perry et al 2009). SHBG has been increasingly associated with the pathogenesis of diabetes and cardiovascular diseases (Jorde et al 2006, Ding et al 2009, and Peter et al 2010). In postmenopausal women, SHBG levels

are negatively correlated with an adverse adipokine profile and visceral fat (Wildman et al 2012).

Diabetes is single handedly responsible for 1.8 million deaths worldwide and the number of people with diabetes is projected to increase from 171 million right now to 366 million in 2030 (Wild et al 2004). The prevalence of diabetes runs high in Indian populations, with Indian having the second most number of people with diabetes in the world, second only to China (International Diabetes Federation 2012). Bharati et al (2011) estimated the prevalence in 1370 South Indian women to be 8.5%. Gupta et al (2012) reported the age adjusted prevalence in 288 North Indian women to be 10.8%. Prasad et al (2012) reported the crude prevalence in 1178 adults from Eastern India to be 15.7%. Insulin resistance too is found to be high in South Asians, especially Indians. Deepa et al (2002) reported the prevalence of insulin resistance to be 18.7% in the middle income group in Southern India, while Kumar et al (2005) found the the prevalence to be 11.8% in 350 adults from North India. Khoo et al (2011) reported insulin resistance to be higher in Asian Indians compared to Chinese and Malays. Petersen et al (2006) also reported insulin resistance to be 2-3 fold higher in Asian Indians compared to Eastern Asians, Blacks and Caucasians.

Lipid Homeostasis And Dyslipidemia

Endogenous estrogens are known to be linked to lipid homeostasis in addition to lipid and energy metabolism. The pathways through which estrogen modifies lipids and atherogenic mechanisms are believed to be non-genomic and genomic as well (Herman et al 2010). The genomic pathways include transcriptional regulation of genes encoding for athero-protective genes, for example vascular endothelial growth factor and insulin-like growth factor-1 and concurrently downregulating the genes that code for pro-atherogenic states, including interleukin 6 and other inflammatory cytokines. The non-genomic pathways are

speculated to be activation of eNOS [endothelial nitric oxide synthase] (Nilsson et al 2001, Liu et al 2005).

Pandey et al (2011) estimated the prevalence of elevated total cholesterol (TC) levels in urban women to be 28%. Gupta et al (2012) reported the prevalence in urban women to be 27%. The most recent documentation of lipid aberrations in Indian women is from a large scale study, Jaipur Heart Watch (JHW), spanning 739 subjects in North India (Gupta et al 2012), which reported the age adjusted prevalence of hypercholesterolemia to be 33% in women while that of low HDL levels was as high as 55.3%.

INFLAMMATION AND METABOLIC SYNDROME

Deleterious increases in inflammatory chemokines and adipokines have been observed to have a strong relationship with visceral adiposity during menopause. In a recent animal model study by Kireev et al (2010) it was reported that generation of proinflammatory cytokines IL-1b, IL-6 and TNFa, was higher in liver homogenates of old female rats coupled with a decreased IL-10 concentration, which is anti-inflammatory. The authors found that treatment with 17 β estradiol tended to inhibit the production of proinflammatory cytokines, resulting in reduced levels of marker of oxidative stress.

Estrogens have also been demonstrated to upregulate antioxidant genes in mitochondria in female Wistar rats, resulting in a decreased mitochondrial oxygen free radical production, eventually helping in prolonged longevity in females compared to males (Vina and Borras 2010).

The concurrent metabolic, inflammatory and energy metabolism changes taking place in the female biological system on account of fluctuations in ovarian steroids, puts the woman at a situation where she is at an increased of a cluster of cardio-metabolic risk factors, in other words, the metabolic syndrome. Because of menopausal transition, the above mentioned changes occur concomitantly and precipitate a condition where increased abdominal fat,

increased blood pressure, glucose dysregulation and imbalances in serum lipids co-exist. This has also been confirmed by epidemiological trend of increased prevalence of metabolic syndrome in middle aged women, especially in India. Sinha et al (2012) reported the prevalence to be almost 30% in a study of 300 women in South Delhi. Sawant et al (2010) found the prevalence in Western India to be 19.5% in a study on 548 adults. The highest prevalence reported so far is by Das et al (2011), where the urban women from Eastern India were found to have 57.8% metabolic syndrome prevalence. This implicates that one in every women has multiple risk factors and is at a high risk situation to develop cardiovascular complications.

THYROID HORMONE EQUILIBRIUM AND SUBCLINICAL HYPOTHYROIDISM

Thyroid hormone regulation may change with changes in reproductive hormone changes. The prevalence of anti-thyroid antibodies and hypothyroidism has been found to increase with menopause (Sawin et al 1985). Thyrotropin (TSH) and prolactin levels were not found to be different between younger and older postmenopausal women, however there was a significant decline in the triiodothyronine (T3) concentration, indicating the stronger role of menopause than aging. The finding that the TSH and prolactin levels were strongly positively correlated over time despite a dramatic decrease in T3 levels led the researchers to deduce a slow intermittent pulsatility of TSH and prolactin and an impaired negative feedback on the hypothalamic-pituitary unit in the elderly menopausal women (Rossmanith et al 1992). Thyroid hormone functioning is also known to be affected by insulin resistance. Topsakal et al (2012) reported an exploratory study on 141 obese post menopausal Turkish women, where it was demonstrated that higher insulin resistance values were associated with significantly higher TSH vales ($p<0.001$), reduced FT3 and FT4 ($p<0.05$). In Indian women, the prevalence of hypothyroidism is found to be prevalent to the order of 21.4%-37% in adult women (Ray et al 2009, Marwaha et al 2012).

The concurrent increase in the clinico-biochemical changes in middle aged women as seen above corroborates the complex endocrinological links of menopausal transition to cardio-metabolic changes and precipitation of risk conditions in middle aged women. Among these risk conditions, ***hyperlipidemia has been found to be the most pressing problem and is highly prevalent in the middle aged female population across India***. This necessitates a holistic remedy which would attenuate the risk situation of Indian middle aged women. In this regard, dietary interventions, focusing on various active bio-molecules have shown positive impact in the management of primary hyperlipidemia, and prove to be the way forward.

NATURAL PLANT BASED INTERVENTIONS FOR PRIMARY HYPERLIPIDEMIA

Plant based products have been used in the traditional alternative healing systems since ancient times, before the development of modern medicine. Much of scientific research has also gone into many of these plants and plant products in the past few years and molecular research has helped pave way for the identification of active compounds in these herbs/fruits/plant products that are responsible for the desired effect. These compounds called phytochemicals or phytonutrients or nutraceuticals, are the cornerstone of recommending natural food and plant products in alternative therapy for disease condition despite availability of synthetic pharmaceutical alternatives. Some of such interventions that have been documented in literature are reviewed below.

The bark of Terminalia arjuna tree (a deciduous tree native to the Indian subcontinent), has been found to contain a huge assortment of phytochemical compounds, namely treiterpenoids, saponins, gallic acid, phytosterols, proanthocyanidins and tannins, among others (Karthikeyan, Sarala Bai and Gauthaman 2003). Two recent systematic reviews on Terminalia arjun and cardiovascular interventions (Dwivedi 2010; Maulik and Talwar 2012) concluded that there are ample studies which provide for clinical evidence of hypolipidemic

effect of the 1-5g/day of bark of Terminalia arjun tree in individuals with and without cardio-vascular complications.

The seed coat of black soya beans (*Glycine max L*.) has been found to contain abundance of anthocyanins that have been purported to exert hypolipidemic actions. Kwon et al (2007) investigated the effect of black soya bean anthocyanins on rats fed on high fat diet to study the changes in weight and lipid profile in them. The results indicated a favorable effect of the 10% black soya bean diet (0.037% anthocyanins) on reduction in weight gain ($p<0.05$) as well as significant reduction in serum TAG and TC ($p<0.01$) and increases in HDL ($p<0.05$).

Spices have an important place in the Indian diet, nutritionally too, the role holds relevance. Many Indian spices have been studied to confer health benefits, one of the most popular one being turmeric (*Curcuma longa*). Several *in vivo* intervention studies in animal model have demonstrated hypolipidemic activity of turmeric with significant reductions in TC, TAG and LDL (Dixit, Jain and Joshi 1988; Babu and Srinivasan 1997; Khouri 2006; Jin et al 2011) and the active compound identified that bestows these benefits is Curcumin (Srivastav 1989, Khouri 2006). A recent review (Zingg, Hasan and Meydani 2013) studied the molecular mechanisms of the hypolipidemic action of curcumin. The authors reported free radical scavenging, induction of signal transduction of the Akt and AMPK pathways and regulation of expression of genes involved in lipid homeostasis (HMG-CoA reductase, carnitine palmitoyltransferase-I) to be the likely mechanisms implicated in molecular and genetic studies reviewed. Additionally, Jin et al (2011) also reported 23-40% inhibition of cholesteryl ester transfer protein / CETP activity by 10μg/ml of turmeric extracts to be one of the hypolipidemic mechanisms.

Artichoke leaf extract has also been speculated to exert hypolipidemic effects. A recent Cochrane review on randomized controlled trials on artichoke leaf extracts (Pittler, Thompson and Ernst 2002) on 167 participants reported significant

reductions in TC, TAG and LDL following supplementation with 1800mg artichoke leaf extract ($p<0.0001$). A yet another Cochrane review of three randomized controlled trials on artichoke leaf extracts (Wider et al 2009) also concluded that the intervention with 1280 mg of artichoke leaf extract has good potential of significantly reducing the serum lipids ($p<0.05$) in mildly hyperlipidemic subjects.

Various interventions in the Department have also shown positive results with fruits and herbal and botanical products. Iyer et al (2010) investigated the effect of fresh Panchratna juice (made from 50g gooseberry, 10g basil, 5g turmeric, 20g mint and 10g ginger) on 35 diabetic subjects for a period of 45 days, and found a non-significant but noteworthy 3.9% reduction in TC and 13.4% in TAG.

Iyer et al (2009) also found a significant reduction of 5.7% in TC, 9.4% in LDL and 8.3% in Non-HDL following gooseberry (*Emblica officinalis*) supplementation (35g/day) for a period of 60days in 45 diabetic subjects.

Venugopal et al (2012) studied the impact of subatmospheric dehydrated barley grass powder (1.2g/day) on 59 stable diabetic subjects for a period of 60 days. The results indicated a 5.1% drop in TC, 8.2% decline in LDL and 7.7% fall in non-HDL levels and a rise of 5% in HDL levels, all values being statistically significant. The TAG levels also declined but non-significantly.

Mehta et al in 2007 (unpublished M.Sc. dissertation) evaluated the effect of 3 months of soy supplementation on 20 geriatric individuals and found and significant reduction in TC and significant 10% rise in HDL.

Mani et al (2011) conducted an open label supplementation study on flax seed (*Linum usitatissimum*) powder (10g/day) on 29 diabetic subjects for a period of one month. The authors reported a non significant reduction of 14.3% in TC, 17.5% in TAG, 21.8% in LDL and apolipoproteins B and an 11.9% increase in HDL at the end of the supplementation.

Nambiar et al (2010) reported hypolipidemic effects of dark chocolate supplementation (50g/day) for a period of 1 month in 40 healthy individuals. The supplementation saw a 12% reduction in TC, 17.7% in LDL and a 20% fall in non-HDL ($p<0.05$).

Thus among the many foods that have shown a hypolipidemic action, the ones that have been extensively investigated and proved to be beneficial include gooseberry, basil, flax seeds, soya bean and cocoa, among many others.

Thus, food and plant based natural interventions show promising results in treating hyperlipidemia, on account of their excellent phytochemical content. One such similar natural product popularly used for various ailments in the alternative healing systems is wheatgrass, which also hold promising possibilities to alleviate hyperlipidemia, on account of its nutraceutical content.

WHEATGRASS (*Triticum aestivum*) – THE WONDER HERB OF AYURVEDA

Wheatgrass is prepared from the cotyledons of the plant common wheat. Botanically, the grass of the wheat belongs to the family *Poaceae* (grass family) and the genus is *Triticum* (wheat), the species being *Triticum aestivum* (common wheat), thereby having the botanical name *Triticum aestivum*. It is an annual plant that grows to 4 feet in height and resembles any other grass.

The grains of wheat have been traditionally used in the ancient Indian system of healing, Ayurveda and the documentation of its uses is found in ancient Hindu treatise on medicine and healing, the Charaka Samhita, compiled by the Indian physician, Charaka.

In modern history, the use of wheatgrass was popularized by Anne Wigmore, in the 1950s, when advocated and started healing cancer patients with wheatgrass

juice. Later on she went ahead and started the Hippocrates Health Institute in 1961 to aid her wheatgrass therapy.

Qualitatively wheatgrass has been found to contain a huge variety of phytochemical compounds: Saponins, gums & mucilages, fructo oligosaccharides, phenolic compounds, sitosterols, triterpenes, hydroxycinnamic acids were found in the aqueous extracts of wheatgrass (Tulloch and Hoffman 1973; Carpita 1989; Estiarte et al 1999; Shirude 2011; Kothari 2011). Even more phenolic compounds are found in the alcohol extracts of wheatgrass: 834 to 1206mg per kg of hexane extracts of wheatgrass by weight is phytosterols, of which, 74% is β-sitosterol, and remaining constituents include campesterol and stigmasterol, all of which have been associated with hypolipidemic action (Dunford, Irmak and Jonnala 2009).

The mineral composition of wheatgrass indicates presence of silicon, phosphorus, calcium, potassium, chlorine and sulphur (Hodson and Sangster 1988). Enzymes carboxypeptidases have also been found in wheatgrass (Mikola 1986). A diverse variety of antioxidant compounds that have been found in wheatgrass include α tocopherol, β carotene, glutathione, in addition to the phenolic compounds stated above (Bartoli et al 1999); and antioxidant enzymes catalase, ascorbate peroxidase, glutathione reductase, superoxide dismutase and glutathione peroxidase, in addition to the enzyme that produces endothelium protective nitric oxide: nitrate reductase, have been detected in young leaves (Rosales et al 2011, Devi, Kaur and Gupta 2012; Duke 2013). The nutrition content of wheatgrass is depicted in Table 1.1

Therapeutic value of wheatgrass has been investigated by several authors in relation to conditions like ulcerative colitis, anemia, thalassemia, iron overload, myelodysplastic syndrome, chemotherapy induced myelotoxicity, hyperglycemia, insulin resistance, oxidative stress, among others, but less so in case of hyperlipidemia.

TABLE 1.1 NUTRITIONAL CONTENT IN WHEATGRASS

Nutrient	Amount per 100g
Calories	500 kcal
Fat	0 g
Carbohydrate	66.7 g
Dietary Fiber	33.4 g
Protein	33.4 g
Amino acid score	76
Vitamin A	50,000 IU
Vitamin C	233 mg
Vitamin D	~
Vitamin E (Alpha Tocopherol)	10.7 g
Vitamin K	1.17 mg
Thiamin	366 mg
Riboflavin	8.7 g
Niacin	8.4 g
Vitamin B6	1.3 g
Folate	~
Vitamin B12	3.3µg
Pantothenic Acid	1.2 g
Choline	166 mg
Calcium	500 mg
Iron	266 mg
Magnesium	130 mg
Phosphorus	466 mg
Potassium	3.4 g
Sodium	~
Zinc	2.1 g
Copper	56.7 mg
Manganese	4.7 g
Selenium	116 mg

Source: Self Nutrition Data 2012

Kothari and co-workers (2008) investigated the hypolipidemic effects of wheatgrass in rat model by administering 5ml and 10ml/kg of wheatgrass juice for a period of 21 days to normocholesterolemic rats. The supplementation resulted in dose dependant significant decline in the levels of total cholesterol (TC), triacylglycerols (TAG). Low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL). It also resulted in non-significant increase in the high density lipoprotein cholesterol (HDL).

The same authors replicated the experiment in hyperlipidemic rats (Kothari et al 2011). The duration of the supplementation was reduced to 14 days, but still, the rats fed with 10ml/kg of fresh wheatgrass juice demonstrated significant reduction in TC, LDL, TAG and VLDL. The authors also reported an increased fecal fat excretion, indicating that the mechanism through which wheatgrass renders hypolipidemic effect is inhibition of cholesterol absorption in the gut.

Shirude (2011) conducted a rat model study to investigate the hypoglycemic properties of wheatgrass. Wheatgrass was administered at100mg/kg for a period of 14 days to hyperglycemic rats; parallel gliclazide and control group were also maintained. The results revealed that wheatgrass showed significant ($p<0.05$) reduction in the blood glucose levels of the supplemented rats, comparable to gliclazide.

With regard to wheatgrass's anti-inflammatory role in ulcerative colitis, Ben-Arye et al (2002) conducted a randomized placebo controlled trial on twenty three patients with distal ulcerative colitis, where oatients in the experimental group were supplemented with 100ml of wheatgrass juice for a month. The intervention resulted in significant reduction in the disease activity index ($p<0.05$) and the rectal bleeding ($p<0.05$), exhibiting antioxidant healing properties in inflammation states.

The sole human trial conducted on wheatgrass targeting hyperlipidemia, is by Shyam et al (2007), which investigated the efficacy of 500mg wheatgrass for 30 days in attenuation of oxidative stress in adult subjects. The findings reflected

that wheatgrass supplementation resulted in significant decline in the malondialdehyde levels ($p<0.05$), which is a marker for oxidative stress; and a parallel increase in the antioxidant ascorbic acid levels and superoxide dismutase levels.

Studies conducted in the department on wheatgrass have focused on its effects on alleviating anemia. Iyer et al (2010) evaluated the acceptability and lipemic responses of wheatgrass incorporated common Indian recipes. It was found that the level of incorporation at which the recipes were most acceptable was 15g of wheatgrass per serving of the recipe. At this level, incorporation of wheatgrass also reduced the lipemic responses of the recipes with the rise in serum TAG after ingestion of the recipe to be around 1.5 to 32%.

Sharma et al in 2001 (unpublished M.Sc. dissertation) investigated the impact of 100ml wheatgrass juice supplementation on 80 adult women for a period of 30 days and found a significant increase 0.85 g/dl ($p<0.05$) in the mean hemoglobin levels of the supplemented group.

It can be inferred from these studies that the extent of clinic biochemical changes in Indian middle aged from different parts of India is alarming and hence the need of the hour is a comprehensive remedy that targets the complex endocrinological changes that occur in middle aged women. For this different food and plant based interventions have been evaluated, but their efficacy has not been established by a judiciously designed trial. Therefore more research is needed to investigate and explore the benefits and toxic effects of natural plant products and their utility to manage cardio-metabolic risk conditions.

Evidently, the above review leaves behind certain research questions that need to be addressed. Following are some of them:

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 from 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 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.

REVIEW OF LITERATURE

DEMOGRAPHIC PICTURE OF MIDDLE-AGED WOMEN

Worldwide, ageing is a phenomenon that grips every nation as the productive population will gradually age into geriatric population, a greater proportion of which will be women given that 55% of elderly in the world are women (UN 1988, WHO 2002). In India, middle-age population is increasing rapidly and forms the productive population. The latest 2011 census (Chandramouli 2011) estimates roughly half of the women (47.6%) in India are in the age group of 15-65 years. Demographic studies estimate that, in 1990, there were 467 million postmenopausal women in the world. Population projections based on these demographic studies predict that, by the year 2030, the number of postmenopausal women will increase to 1.2 billion. At this time, approx 47 million women will be entering menopause each year (Hill 1996). Furthermore, it is estimated that women in developed countries will spend about 30 years of their life in the postmenopausal state. This translates into increased public health spending on chronic diseases and eventually burdens the economy.

The health spending is highest, owing mainly to chronic diseases in middle-age and elderly population. This is evidently reflected in India's increasing spending on the health, the major part of which is in the private sector (4.5% of GDP as compared to 1.2% by the state in India's total public health spending of 5.2%). Half of this contributed by women, who unlike men, don't enjoy a smooth transition into the middle-age health risks, mainly on account of menopause. Moreover, there is evidence that situation of Indian women may be relatively worse than that of men concerning many of the risk factors for CHD, particularly post-menopausal women (Vlassof 2007, Silander et al 2008, Njelekela 2009, Ghosh et al 2010, Abbasi et al 2012).

MENOPAUSAL TRANSITION

Menopause signifies the cessation of ovarian function in women which marks the end of the reproductive life span in them. Clinically menopause is defined as absence of menstrual periods for atleast 12 consecutive months or more (Soules et al 2001). Although menopause is a discrete event in the reproductive lifespan, the stoppage of menstrual periods is not sudden; the event itself represents the culmination of an altered endocrinological situation, the origins of which usually precede the menopause by more than a decade. Based on this biological phenomenon of menopause, the reproductive classification of women is done into

Pre-Menopausal: Before the menopausal transition starts

Peri-Menopausal: the phase of transition from pre-menopause to menopause

Post-Menopausal: the period after menopause has occurred

Contrary to the popular belief, where the entire phase of transition into menopause is referred to as menopause; clinically menopause is considered as a single event that marks end of menstrual periods.

The mean age of occurrence of menopause is 51-52 years (Copeland 1993)**.** But recent trends indicate a shift of this mean age a little earlier in life, and the factors disputed to be causative here are unhealthy dietary patterns and sedentary lifestyle and exposure to higher levels of environmental pollutants. But not only does any documentation exist on this shift in mean menopausal age in the Indian context, but also the role of diet and lifestyle has not been studied. Hence mapping needs to be done in Indian women and influence of diet and lifestyle needs to be reviewed.

It is worthwhile to note that while menopause is a natural phenomenon, it is listed as a ‘disease’ in International Classification of Diseases – 9 & 10, Disease Data

Base, e-Medicine, and Medical Subject Headings. Menopause evidently alters the function of human body resulting in menopausal symptoms collectively called as ‘menopausal syndrome’ (Govil 2010).

Women are categorized clinically into either of the reproductive aging categories (namely reproductive, menopausal transition or post menopausal) by ascertaining their menstrual status (Table 2.1). Since the time that a girl begins to menstruate to the point of time that she has regular menstrual cycles, is referred to as the Reproductive age. When the length of the cycles starts to vary highly, which would start to happen around the time when the woman is in her 40s, till the time that the menstrual cycles completely cease, is called the menopausal transition. Following the menopausal transition, the woman is said to be in her post menopausal stage (American Society for Reproductive Medicine 2007).

MENOPAUSAL SYNDROME

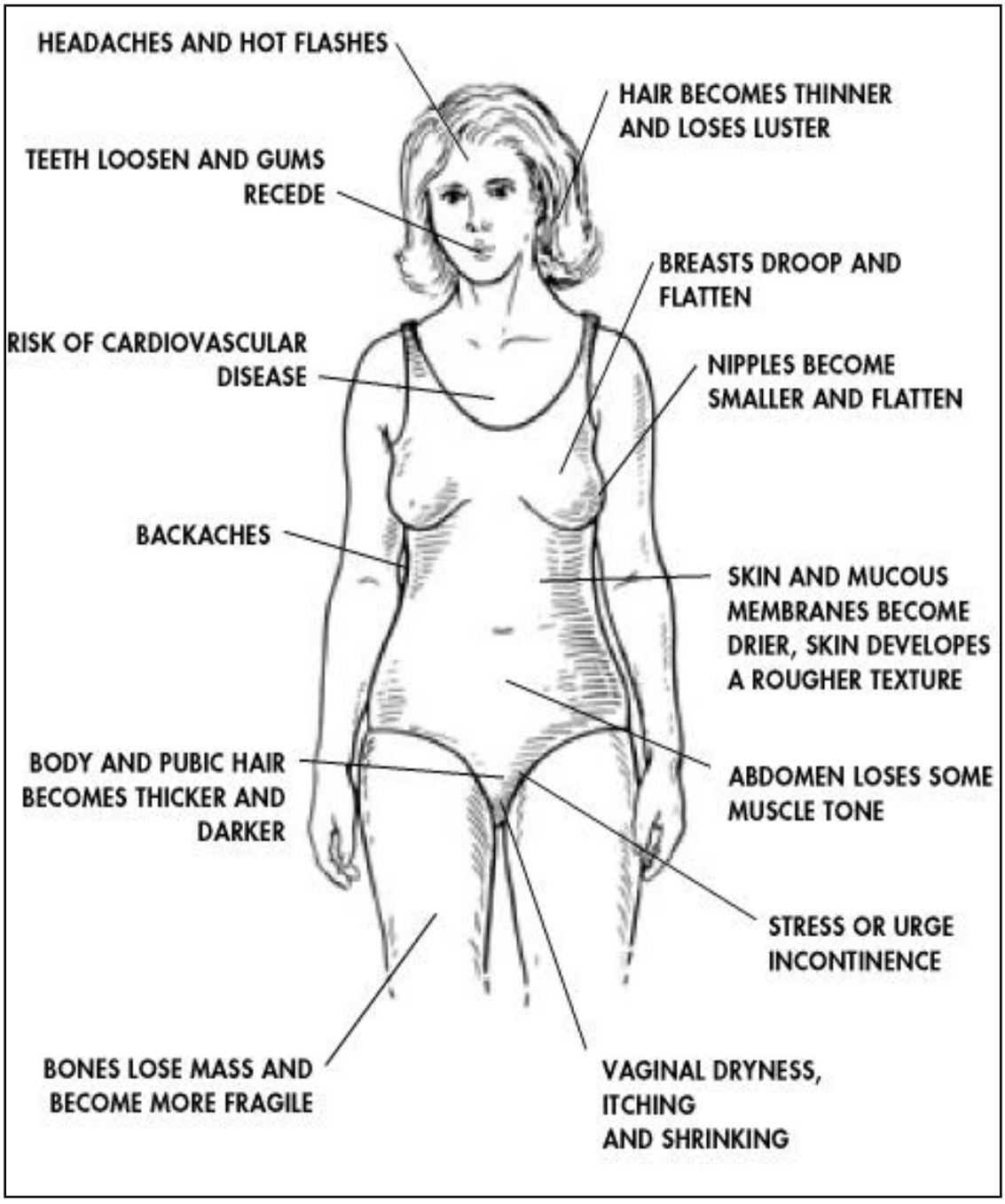
In females the biological effects of estrogen deficiency that occurs when the ovarian follicular stores decline, results in a variety of symptoms with varying degrees of severity and discomfort. This syndrome is called menopausal syndrome. Various organs and organ systems in the human body have estrogen receptors, which belongs to the nuclear hormone receptor superfamily and has two isoforms: ERα and ERβ. Presence of either of these receptors is indicative of the fact that estrogens are either instrumental or have a regulatory role in the functionality of that specific organ. During menopausal transition, with decline in estrogen levels, these organs experience estrogen withdrawal and these phenomena manifest themselves as the menopausal syndrome (Williams 2012). The specific areas where the lack of estrogen effect is felt due to menopausal transition are outlined in Figure 2.1. These areas are the temperature regulating center of the hypothalamus which causes hot flashes and night sweats, vascular endothelium which increases risk of CVD, bone which undergoes more resorption, vaginal muscles which atrophy and become thin, urinary bladder

TABLE 2.1 CLINICAL REPRODUCTIVE AGING IN WOMEN

	Average Age	Menstrual Cycles	Signs & Symptoms
Reproductive Years	First Period: 9-15years	Variable	-
	16-30 years	Regular	-
	31-42 years	Regular	Fertility progressively declines
Menopausal Transition	Early Transition: 40s	Lengths of cycles vary increasingly	-
	Late Transition: late 40s, early 50s	2 or more skipped periods	• Hot flashes • Irritability • Sleep disturbances • Bone loss begins
	Final Period: 51 years	No periods	• Hot flashes • Irritability • Sleep disturbances • Bone loss begins
Post-menopause	50s and beyond	No periods	• Vaginal dryness • Bone loss • Hot flashes can persist • For a few women, hot flashes continue into their 60s and 70s

Source: American Society for Reproductive Medicine 2007

FIGURE 2.1 MANIFESTATIONS OF ESTROGEN WITHDRAWAL DURING MENOPAUSE



Source: Williams 2012

muscles, which lose a little bit of tone resulting in incontinence. And finally there is adipose tissue beneath the skin which atrophies resulting in rougher and looser skin.

Depending upon the organ system being affected, menopausal symptoms are classified into following four classes:

1) Vasomotor Symptoms
2) Somatic Symptoms
3) Psychological Symptoms
4) Urogenital Symptoms

A. Vasomotor Symptoms

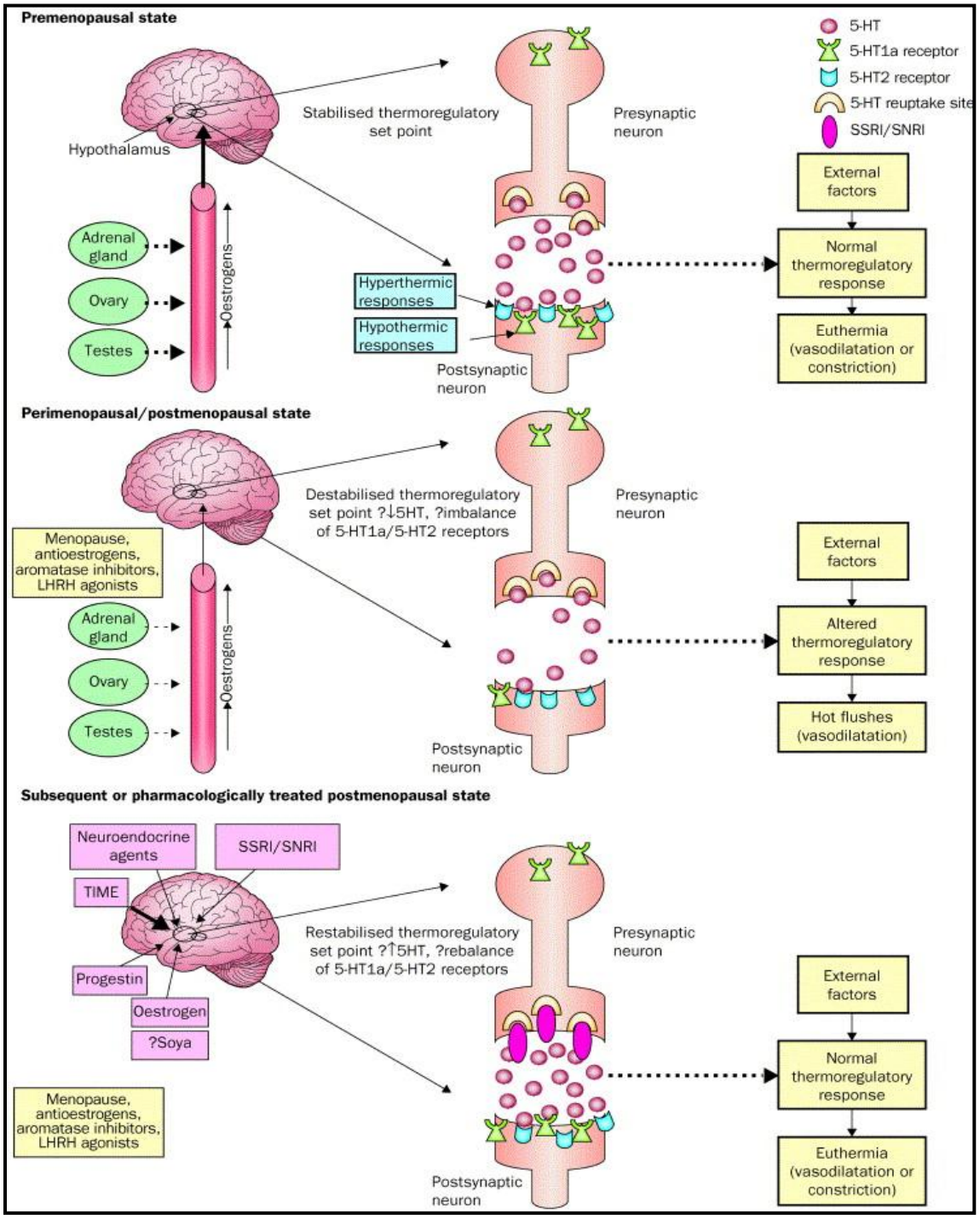
Vasomotor symptoms in menopause include hot flashes and night sweats. Often, the expressions 'hot flash' and 'hot flush' are used interchangeably because they are almost synonymous which represent a condition characterized by a sudden sensation of heat and sweating, most notably on the upper body. Hot flashes are more frequent and intense in peri- and postmenopausal women. They typically occur when levels of sex steroids drop abruptly and rapidly. Examples of sudden estrogen decline in women include removal of the ovaries in premenopausal women, and administration of selective estrogen receptor modifiers (SERMs) as part of chemoptherapy in breast cancer patients (for example, raloxifen and tamoxifen). Men also tend to experience hot flashes, when there is a sudden fall in testosterone levels (for example medical or surgical treatment for prostate cancer). In both women and men, whenever there arises a situation where there is a rapid drop in sex steroid hormones it results in hot flashes.

The physiological mechanism underlying manifestation of vasomotor symptoms includes effects of estrogen withdrawal on autonomic nervous system, which is essential for regulating heart rate and arterial pressure through the arterial baroreflex. Because multiple central nervous system neurons express estrogen receptors, estrogen directly influences the central transduction of messages from neurons to the autonomic ganglia (Burger et al 2008).

The activity of these autonomic neurons is centrally controlled through nuclei which transmit excitatory or inhibitory information to the pre-ganglionic autonomic fibers in the sympathetic and/or parasympathetic pathways. Sex steroids affect the activity of these pathways directly, thereby altering peripheral sympathetic and parasympathetic neuronal activity, and consequently, the cardiovascular function. Thus, peripheral vasodilatation associated with vasomotor symptoms of menopause is most likely mediated centrally by estrogens through modulation of hypothalamic regions associated with temperature regulation (Thurston et al 2010).

Diagrammatic representation of the neuromodulatory mechanisms through which sex steroids affect the thermoregulatory actions in the temperature control centre in the hypothalamus; is given in Figure 2.2. It can be seen that in premenopausal women, estrogens seem to offer stability to the CNS thermoregulatory set-point, by ensuring a balance in the serotonin (5-HT) uptake by the post-synaptic neuron and adequate re-uptake by the pre-synaptic neuron. This results in a normal thermoregulatory response to external thermal stimuli which can be either peripheral vasodilatation or constriction. On the other hand, in women undergoing menopause, estrogen concentrations are abruptly decreased, resulting in instability of the CNS thermoregulatory set-point, resulting in an altered vasodilatory thermoregulatory response to external thermal stimuli (Freedman 2001). The mechanisms under play here can either be reduced serotonin production and/or reduced expression of serotonin uptake and re-uptake receptors. With time, once the menopausal transition in over, the CNS thermoregulatory set point readjusts itself and become restabilised, if no pharmacological intervention is administered. Such interventions also help in restabilizing the thermo-regulatory set point and include exogenous hormones, serotonin re-uptake inhibitors and phytoestrogen therapy, among others (Stearns et al 2002).

FIGURE 2.2 PHYSIOLOGY OF VASOMOTOR SYMPTOMS: EFFECT OF SEX STEROIDS ON THERMOREGULATORY MECHANISMS



Source: Stearns et al 2002

Prevalence of vasomotor symptoms runs high in most populations including Indian population (Figure 2.3). Gold et al 2002, reported the comparative prevalence across various ethnic populations and concluded that women of African origin had the highest prevalence (45.6%), followed by Hispanic women (35.4%), Caucasians (31.2%), Chinese (20.5%) and finally Japanese women (17.8%). Mahajan et al 2012 reported the prevalence of hot flashes in Himachali menopausal women in North India to be as high as 56% and night sweats to be 52%. Sharma, Tandon and Mahajan (2007) reported the prevalence of hot flashes in middle aged women in Jammu, to be 53.8%. Nair et al (2006) reported the prevalence of vasomotor symptoms in menopausal women from Vadodara to be intermediate, that is, 38%.

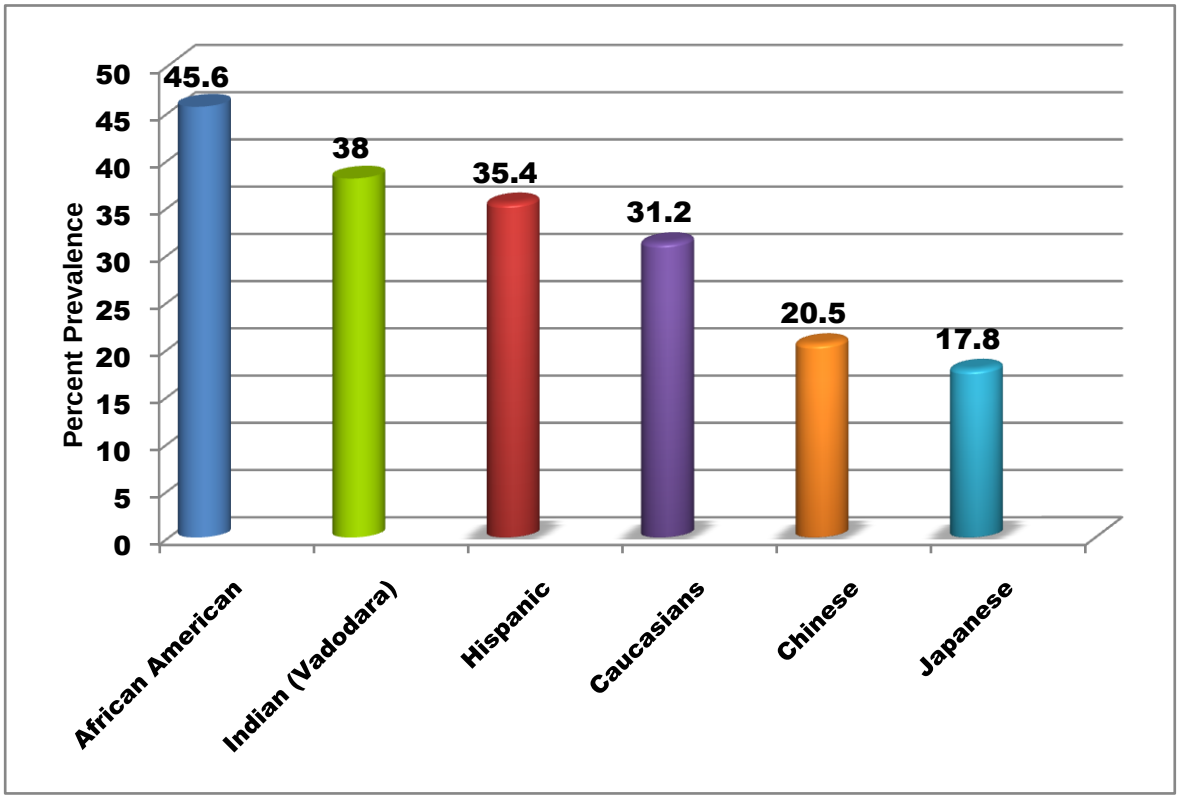
B. Somatic Symptoms

The most common somatic symptoms reported by menopausal women include: headaches & dizzines, joint pain, general aches and fatigue.

Headaches and Dizzines

Headaches precipitated by hormonal fluctuations can either be due to decline in sex steroids or by elevated levels of sex hormones. Severe fluctuations in estrogen levels immediately preceding menopause can cause both types of headaches. Estrogen has vasodilatory effects through autonomous nervous mechanisms, whereas progesterone exerts vasoconstriction. As the levels of both these hormones fluctuate, the blood vessels are forced to dilate and constrict, resulting in intense pain in the head (Oh et al 202, Lucchesi et al 2012). Typically, the headaches begin suddenly, without warning and there is a throbbing pain. The location of the headache usually varies, with the pain appearing on either or both sides of the head. In addition to this, nausea, vomiting and sensitivity to light and noise, abdominal pain, which attenuates after vomiting, have also been observed. Apart from these, other symptoms of migraine headaches also include a sudden change in eyesight, seeing bright

FIGURE 2.3 PREVALENCE OF VASOMOTOR SYMPTOMS ACROSS VARIOUS POPULATIONS



Source: Gold et al 2000 and Nair et al 2006

spots or zigzag lines, double vision, numbness and tingling of the lips, face, hands (on one or both sides), dizziness, weakness of an arm or leg, unsteadiness in walking, mild confusion while thinking, drowsiness, and slurred speech (Karli et al 2012).

A person may have only one or a few of these symptoms, though they do tend to occur in the same combination in each attack. The symptoms may last from five minutes to 15 or more (Terauchi et al 2013).

Dizziness can be a direct consequence, as well as an indirect manifestation of other consequences associated with menopause. Therefore, the causes of dizziness might be linked to the changes in the physiology or result from medication being taken for other symptoms of menopause. Dizziness is more frustrating than it is fatal, but can easily be a cause for a fatal accident. In some cases presence of constant dizzyness may indicate a serious underlying health concern (Terauchi et al 2013). For this reason, it is important not to overlook presence of this menopause symptom and appropriate solutions should be sought.

Fatigue

Another common somatic symptom that is frequent during menopausal transition is fatigue. The distinguishing feature of fatigue from general exhaustion is that there is a constant feeling of exhaustion in fatigue. This symptom differs from normal sleepiness in that it includes a constant lack of energy, mood changes, and inability to perform one's normal day-to-day tasks as usual (Greenblum et al 2013).

Fatigue often affects both an individuals' physical and mental state. The way it would affect mental state is while the body may experience drowsiness and muscular fatigue, an individual may begin to feel apathetic towards important matters, struggle to recall information, or have difficulty concentrating at work and at home. The mechanisms under play include the adrenal and reproductive systems in the female body. Estrogen synthesized in the adrenal glands & the ovaries and progesterone released by the ovaries control the quantum of energy

generated in the cells in the body. When sex steroid production declines during perimenopause, fatigue sets in. In addition, estrogen and progesterone regulate the sleep cycle, which may lead to restless nights (Moller et al 2013, Greenblum et al 2013).

Fatigue and menopause act as self-perpetuating cycle, with one causing the other. Common causes of fatigue are often common menopausal symptoms themselves, such as sleep apnea, night sweats, anxiety and depression (Lucchesi et al 2012).

Distribution of somatic symptoms across populations has been found to vary. Liu et al (2013) reported a prevalence of 82.7% in 1686 menopausal women in Beijing. Sweed et al (2012) reported the prevalence in 400 Egyptian middle aged women to be 80%. Chunni and Sreeramareddy (2011) reported physical and mental exhaustion in a sample of 729 Nepalese women to be 73.5%. Women from urban Nigeria were reported to have prevalence of 43% of physical and mental exhaustion, as studied in a descriptive community based study of 1189 middle aged women (Olaolorun and Lawoyin 2009).

This variability is also found across Indian populations. Sharma, Tandon and Mahajan (2007) studied the extent of menopausal complaints in 117 women from Jammu in North India. They found that prevalence of fatigue to be highest (72.9%), followed by headaches (55.9%) and rheumatic pains (48%). Kapur, Sinha and Pereira (2009) reported that the most prevalent problem in menopausal women in Uttarkhand in India was muscle and joint pains (55.8%), followed by fatigue (51.2%) and then headaches (43.4%). More recently, Mahajan, Aggarwal and Bagga (2012) reported the prevalence of fatigue in Himachali middle aged women to as high as 62% and backaches to be 51%. Singh (2012) reported the prevalence of physical ailments in a cross sectional study of 1765 menopausal women from Hyderabad in Central India, to be 32%. Bairy, Adiga, Bhat et al (2009), studied a comparative prevalence of menopausal symptoms in a cross-section of 352 South Indian women, and found that most

prevalent were fatigue, aching muscles and joints and lower backache, while sexual and urologic problems were less frequently reported.

C. Psychological Symptoms

Menopausal transition also brings about an imbalance in the psychological functioning in females, apart from the physical symptoms. Changes in estrogen levels exert a direct effect on the neurotransmitters serotonin, norepinephrine, dopamine, and melatonin and all of these chemicals play an integral role in emotion and mood regulation (Bruce and Ewen 1999, Bruce and Ewen 2001, Glazer et al 2002, Heikkinen et al 2002). Hence, disruptions caused by fluctuations in estrogens can lead to anxiety, depression and mood disorders during menopause.

Depression

Depression is a common yet potentially serious symptom of menopause. It entails more than the occasional bout of sadness and, if not treated, can lead to more severe mental disorders and a lessened quality of life. Women are especially susceptible to depression and when approaching menopause are even more: Women ages 45 to 55 are four times more likely to have depression than women who have not yet reached that stage in life. The general use of the term depression refers to a mental state characterized by a pessimistic sense of inadequacy, feeling of sadness, and a despondent lack of activity. Fall in estrogen levels are highly likely to cause depression- like states in women because of its effects on a number of neurotransmitter systems in the brain (Bruce and Ewen 1999).

Irritability

In addition to myriad physical effects, emotional symptoms are a common feature of the menopausal transition. In fact, up to 50 percent of all perimenopausal women experience disturbances in mood, including irritability (Cuadros et al

2012). While several factors can contribute to irritability in our daily lives, hormonal fluctuations characteristic of menopause are often the prime cause of irritability and other negative emotional states during this major life transition. Irritability is defined as an excessive response to stimuli. Other menopausal symptoms, such as hot flashes, sleep disorders, loss of libido, vaginal dryness**,** and more, can cause or contribute to irritability (Cohen et al 2006).

Anxiety

While anxiety is the result of a complex interplay of social, biological, and psychological factors, hormonal changes are often the root cause of anxiety during menopause. Because of changing levels of estrogen, a menopausal woman may experience marked differences in the way she feels. She may find that she is easily irritated, worries more than she used to, feels down, or suffers from a general sense of anxious tension (Bauld and Brown 2009). Anxiety and stress manifest more at the time of menopausal transition because sex hormones and the hypothalamo-pituitary-adrenal axis are also closely correlated, with changes in one, affecting the other. Therefore, vacillations in estrogen and progesterone are bound to affect the adrenal function and give rise to stress and anxiety in menopausal women (Fernandez-Guasti et al 2012)

Because the exact causes of anxiety are complex, it is important for a woman experiencing this symptom to understand all of the possible causes. This can greatly help her to determine the best way to control and manage her anxiety. It is often comforting for a woman to understand that her anxiety is likely the result of normal hormonal changes.

A cross sectional study on 1025 Greek women (Grigoriou et al 2013) reported the prevalence of psychological symptoms in post menopausal women to be 21.3%. Jonusiene et al (2013) studied the determinants of sexual function in Lithuanian postmenopausal women and reported that anxiety and depression associated with menopause were main risk factors for the probable development of sexual dysfunction. A cross-sectional survey of 1686 menopausal nurses in

Beijing (Liu et al 2013), revealed that the prevalence of irritability was as high as 70.2% in them. An exploratory study in Florida (Greenblum et al 2013) studied the effect of clustering of menopausal symptoms rather than individual symptoms on the quality of life in middle aged women and found that anxiety, mental fatigue ad sleep disturbances were together best able to explain the variance (16.7%) in the quality of life in the women studied.

With regard to Indian populations, Kapur, Sinha and Pereira (2009) reported the prevalence of depressed mood to be 36.4% in middle aged women of Uttarkhand in Northern India.

D. Urogenital Symptoms

There is abundance of estrogen receptors in the genitor-urinary system in females especially are found in the urethra, trigone of the bladder and in vagina and the vulva. Consequently, an estrogen deficiency results in the atrophy of these tissues and affects functionality, which manifest as urine incontinence, dryness of the vagina, dyspareunia (loss/decline of sexual desire), itching/irritation in the vagina and painful intercourse among others.

The changes occurring in the vagina following menopause include atrophy of muscles in the vaginal wall and diminished elasticity in them. The vaginal secretions also decrease and the functional lubrication during coitus is also reduced in face of sexual stimuli. As estrogen levels decrease, there is a loss of lactobacilli, causing the vagina to become more alkaline, which promotes colonization by fecal flora and other pathogens in the vagina (Brincat and Calleja-Agius 2009). Warren, Shu and Dominiguez (2004) also reported that after menopause, “the vulva becomes flattened and thin as a result of the loss of collagen, adipose tissue and the ability to retain water. The urethra also becomes thinner and less efficient, with detrusor pressure at the urethral opening decreasing, both during and after voiding. Estrogen deficiency also leads to an increase in fibrosis of the bladder neck, reduced collagen in surrounding tissues,

and a decrease in the number and diameter of the muscle fibers in the pelvic floor. Estrogen stimulates the maturation of the vaginal epithelium and its production of glycogen. These changes increase a woman's risk of vaginal and urinary tract infection. Atrophic genitourinary tissues are also at increased risk of injury by trauma. Estrogen replacement therapy may significantly lessen these problems.

The changes occurring in the genitourinary tract may lead to dyspareunia, which is characterized by a diminished interest in sexual intercourse. Worsening of this decreased interest is caused by fatigue and depression that accompany vasomotor symptoms and also sleep disturbances of menopause. Decreased levels of endogenous testosterone, both in women who have undergone surgical menopause, as well as in those who experience natural menopause, may cause decreased libido. Women who complain of lack of sex drive may be candidates for androgen replacement, as well as estrogen. In general, androgen levels do not decrease abruptly at menopause but decrease gradually as women age so that decreased libido may be a problem of older postmenopausal women" (Warren, Shu and Dominiguez 2004).

Legendre et al (2012) reviewed the extent of urinary incontinence in French women across 482 articles; and found that prevalence ranged from 15-30%, with an annual incidence of 5-10%. Pastore et al (2004) reported the prevalence of vaginal dryness in 98,705 American women in a large scale study (Women's Health Initiative), to be as high as 27% and vaginal itching to be 18.6%. Bozkurt et al (2007) studied prevalence of all urogenital symptoms in 510 Turkish post menopausal women, to find that an overactive bladder was the most frequent complaint, reported by 16.5% followed by incontinence which was reported by 10.4% of the participants. The authors also found that prevalence of dyspareunia to be 10% and vaginal dryness to be 9.6%.

In Indian populations, the prevalence of urogenital symptoms tends to be less frequently reported, probably because most of them find it embarrassing to report

such problems (Bairy, Adiga, Bhat et al 2009). Ismael (1994) reported prevalence of menopausal symptoms and health seeking practices in a mixed population of Indians, Malays and Chinese in Malaysia. The author found that despite a considerable number of women experiencing urinary incontinence and dyspareunia, around 80% of them did not seek medical advice, because they were regarded as embarrassing complaints. Singh (2012) reported the prevalence of genitourinary symptoms in a multi-centric study across India, to be only 15.5%, while the vasomotor symptoms were as high as 75.3%. Huang et al (2010) studied the prevalence of sexual symptoms related to menopause in a mixed population of Caucasians, South Asians, Pacific Islanders and East Asians in the US. The authors found that non-white race was significantly associated with vaginal dryness (OR: 1.53 95% CI: 1.04–2.27).

ENDOCRINOLOGY OF MENOPAUSE

The onset of menopause is marked by irregularity in menstrual cycles and finally culminating in complete termination of menstruation. The endocrinologic changes of menopause result from interplay between declining ovarian function and reciprocal changes in circulating gonadotropins. This occurs due to the feedback control mechanism that is in place for regulation of sex steroid production in the body, which is accomplished by the hypothalamo-pituitary-ovarian axis in females. The normal sex-steroid feedback control loop (depicted in Figure 2.4) starts when there is stimulus from the hypothalamus to the pituitary, in the form of gonadotropin releasing hormone (GnRH), which causes pituitary to release gonadotropins, namely luteinizing hormone (LH) and follicle stimulating hormone (FSH). The gonadotropins further stimulate the gonads, in this case, the ovaries, to release the sex steroids (estrogen, pregesterone). The circulating levels of these steroids act as a feedback regulatory mechanism for hypothalamus to stop releasing GnRH. Along with this feedback control, another inhibitory mechanism is also in place: the release of hormone Inhibin by the ovaries, which also signals the hypothalamus to regulate GnRH production (Williams and Kriegsfeld 2012).

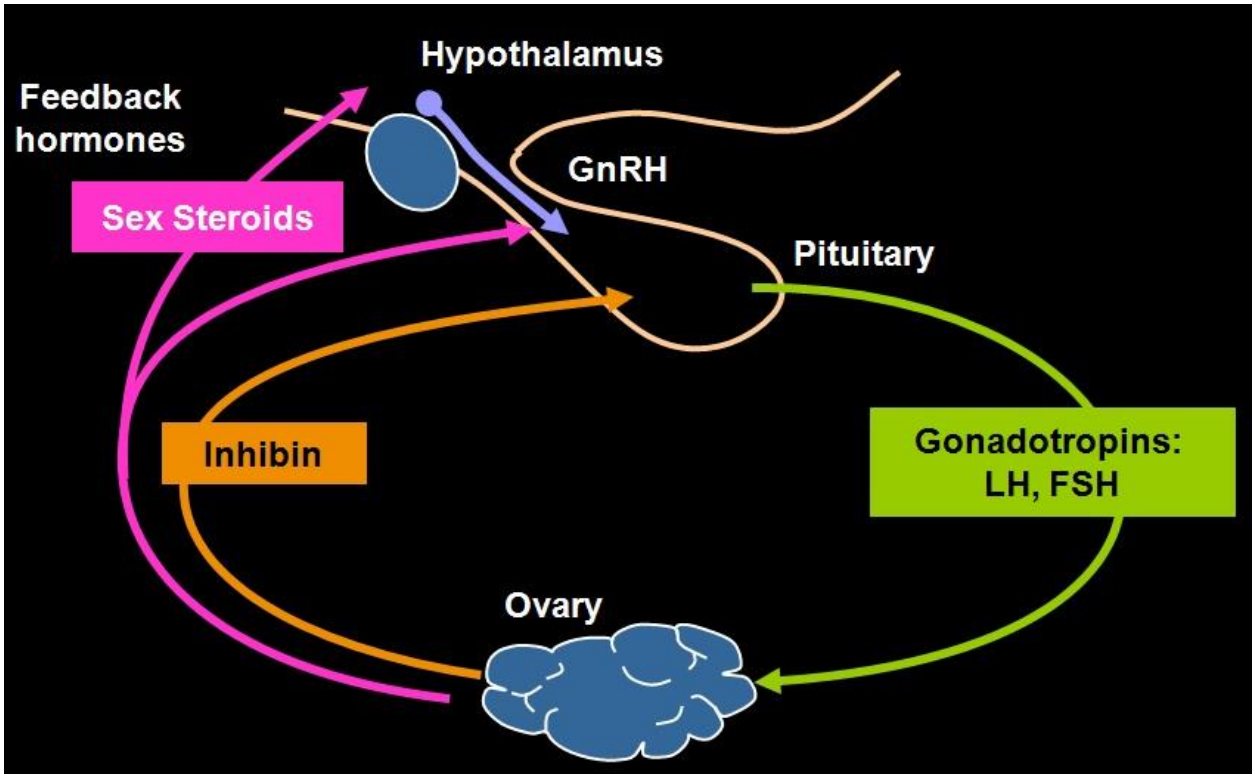
The decrease in ovarian function begins as early as five to seven years before the onset of the last menstrual period. Thus one of the main ovarian hormones, Estrogen begins to diminish, which disrupts the feedback control loop of estrogen release and throws the whole hypothalamo-pituitary-ovarian axis into chaos. This manifests as increased levels of Follicle Stimulating Hormone (FSH), secreted by the anterior pituitary for stimulating the ovaries to increase the production of estrogen.

Both steroids and protein hormones from the ovary control pituitary production and secretion of LH and FSH. The principal ovarian steroid hormones are estradiol (predominant in the follicular phase) and progesterone (predominant in the luteal phase). These steroids regulate gona dotropin production and release via feedback loops of the hypothalamic-pituitary-ovarian axis. In addition, several peptide hormones (inhibin, activin, and follistatin) produced by granulosa cells influence FSH synthesis and secretion.

Concurrently, the production Inhibin B, which is a hormone secreted by the small antral follicles and is a major regulator of FSH, declines due to age-related decline in the number of follicles in the ovary, which in turn, leads to further increase in FSH (Warren and Dominguez, 2004). Women older than the age of 45 exhibited menstrual irregularity when the average number of primordial follicles per ovary decreased to approximately 100 (Burger et al 2008).

The role of inhibin in the regulation of FSH secretion has received considerable attention given the dynamic changes in serum concentrations that occur over the menstrual cycle. There are two types of inhibin, each consisting of the same aa-subunit combined with a either bA- or bB-subunit to form inhibin-A and inhibin-B, respectively. These dimeric inhibins show different patterns of secretion during the menstrual cycle. Levels of inhibin-A are low during the follicular phase, rise with ovulation, and peak during the luteal phase (Groome et al 1994). In contrast, inhibin-B levels are highest during the midfollicular phase, decline at midcycle, and display a transient rise shortly after the LH surge (Groome 1996)

FIG 2.4 SEX STEROID SECRETORY FEEDBACK CONTROL LOOP



Source: Thomas Addison Unit Endocrinology Modules 2008

One of the most consistent endocrinologic changes associated with onset of the perimenopause is the monotropic rise in FSH (Sherman and Korenman 1975, Lenton et al 1988). It has been hypothesized that this change in FSH may result from diminished function of the granulosa cell compartment of the ovary, manifested by decreased production of estradiol, inhibin, and/or insulin-like growth factors (IGFs)

Early studies showed that elevations in FSH are often accompanied by decreases in circulating levels of estradiol (Sherman, West and Korenman 1976, Hee et al 1993, Burger et al 1995*)* and inhibin (Hee et al 1993, Batiste et al 1995*).* Other studies of the perimenopausal transition have shown no significant change in estradiol levels (Reyes, Winter and Faiman 1977; Lee et al 1988*)* or elevated estrogen levels (Kliein et al 1996; Blake, Adel and Santoro 1997*).* These apparent conflicts in the literature may reflect differences in the timing of the sample collections over the perimenopausal transition. Perhaps, initially, the increase in FSH compensates for decreasing ovarian function and results in increased estradiol levels. Then, as the ovary continues to age in the latter part of the perimenopausal transition, a decline in estradiol occurs. Declining inhibin rather than estradiol production by the granulosa cells during the early phase of the perimenopause may be important in initiating the monotropic rise in FSH (Pellicer, Simon and Remohi 1995*;* Seifer et al 1996*).* On the other hand, some studies using a polyclonal antibody to the a-subunit have failed to show a change in serum inhibin concentrations associated with the monotropic rise in FSH (Klein, Battagalia, Miller et al 1996; Lenton et al 1991; Klein, Battagalia, Fujimoto et al 1996*),* & decreased secretion of inhibin-B has been shown to be associated with elevation in FSH (Klein, Illingworth, Groome et al 1996*).* Thus, decreased inhibin-B may reflect diminished function of the granulosa cells of older women and play a role in the regulation of FSH during the perimenopause (Seifer et al 1997*).* These changes disrupt the feedback control loop of estrogen release and throw the whole hypothalamo-pituitary-ovarian axis into chaos. As a result a typical pattern of plasma hormonal levels

(shown in Figure 2.5) results in the phases surrounding menopause. In this pattern, there are two estrogen level peaks following FSH peaks and a progesterone peak following an LH peak in premenopausal phase. In the peri menopausal phase, the sex hormone production starts declining resulting in highly erratic increases in releasing hormones. Finally after the menopausal transition is complete, the ovarian sex hormone production drops to the minimum with releasing hormone levels remaining elevated throughout. Figure 2.6 summarizes the pattern of estrogen levels during the lifetime of a woman

In summary, the earliest endocrinologic evidence of diminished ovarian reserve may be diminished inhibin-B secretion and the monotropic rise in FSH. This may occur in the presence of elevated circulating levels of estradiol.

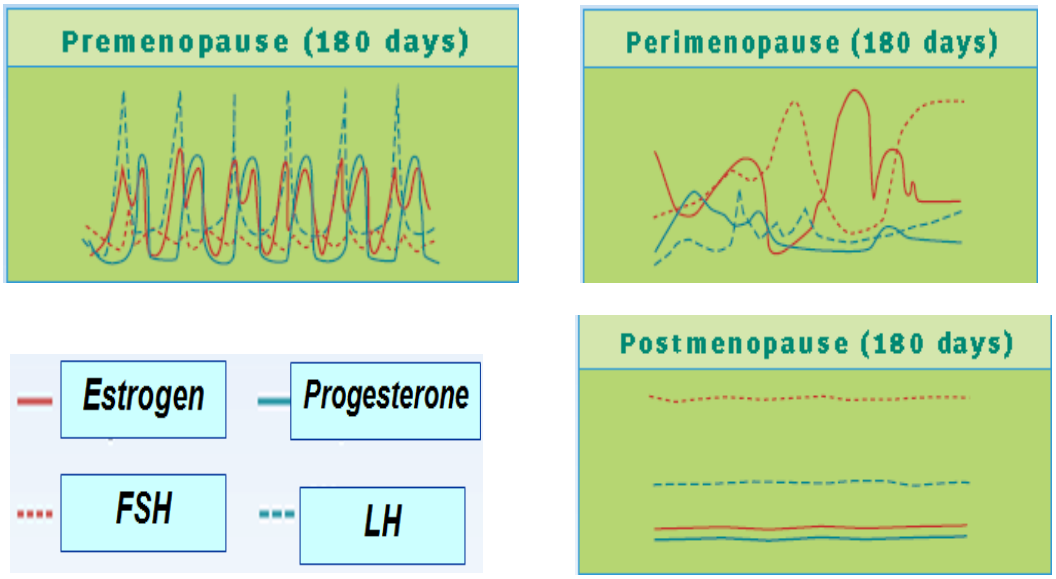
DIMINISHED OVARIAN RESERVE

Peri Menopause

The locus of reproductive aging is the ovary. It is here that the seeds of menopause are sown, because the ovary contains a finite number of irreplaceable primordial follicles. The perimenopausal years are marked by their accelerated attrition. As the number of ollicles dwindles, elaboration of ovarian hormones appears to change somewhat unpredictably. The menstrual regularity a woman experiences during the perimenopausal years appears to be more related to her remaining primordial follicle number than to her age (Richardson, Senikas and Nelson 1987). As the number diminishes, irregular bleeding can occur after an estradiol peak without subsequent ovulation or corpus luteum formation. Both normal (Sherman, West and Korenman 1976; Lenton et al1988) and inadequate (Santoro et al 1996; Reyes, Winters and Faiman 1977) corpus luteum secretion of progesterone have been described in perimenopausal women.

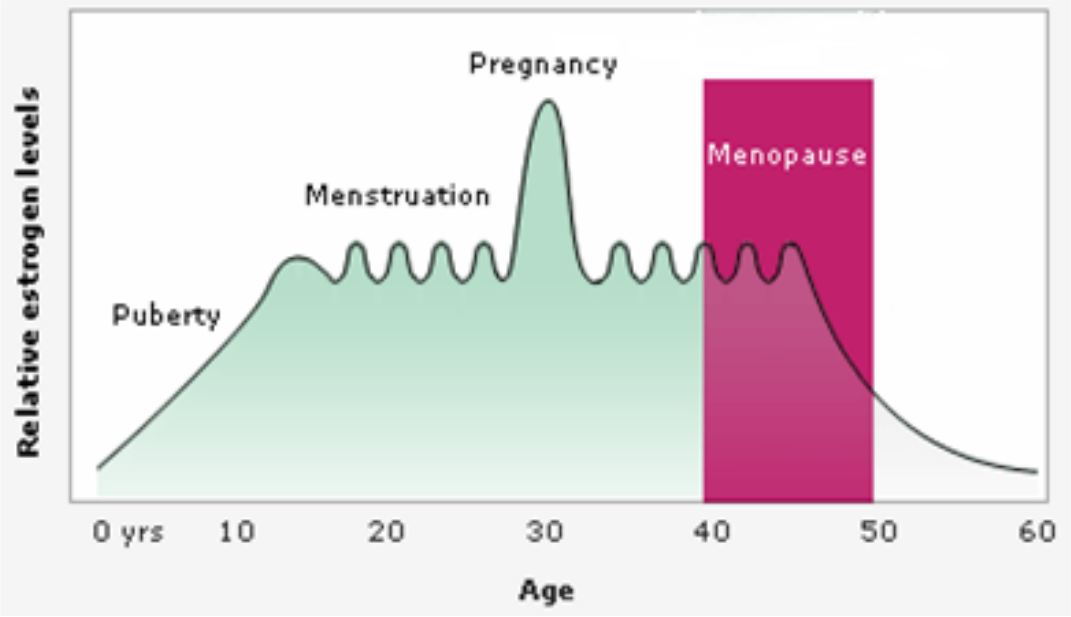
Coordinately, early follicular phase estradiol concentrations are elevated in peri menopausal women compared with mid reproductive-aged women (Unger and

FIG 2.5 CHANGES IN HORMONE LEVEL PATTERNS OVER SIX MONTHS BEFORE, DURING AND AFTER MENOPAUSE



Source: Harvard Women's Health Watch 2006

FIG 2.6 ESTROGEN LEVELS FROM PUBERTY TO MENOPAUSE



Source: Promensil USA 2009

Meeks 1996; Klein, Illingworth, Groome et al 1996; Santoro et al 1996; Klein, Battaglia, Miller et al 1996; Shedeler et al 1989). In two small studies of women aged 43 and older who were still cycling, ovulatory cycles with high estrogen production were observed (Santoro et al 1996; Shideler et al 1989), suggesting that this accelerated folliculogenesis could be exuberant throughout. In other words, the ovary, less responsive to FSH, requires greater circulating quantities of FSH to initiate folliculogenesis. Once started, FSH induces an overshoot of estradiol and consequently, hyperestrogenemia occurs. These elevations in estrogen may be a feature of the early peri menopause, with reduced estrogen accounting for the menstrual cycles immediately preceding menopause. It is clinically important to understand how commonly diminished progesterone secretion might be coupled with hyperestrogenic cycles, since this combination predisposes women to menorrhagia, endometrial hyperplasia, dysfunctional uterine bleeding, and even endometrial cancer.

Glycoprotein hormones elaborated by the granulosa cell include inhibin, a disulfide linked heterodimer, which has been shown to decrease over the peri menopausal transition (Buckler et al 1991; Burger 1994). A decrease in inhibin secretion by the granulosa cells begins at approximately age 35, but accelerates dramatically after age 40. The decline in inhibin, which probably reflects both lesser follicular competence and a smaller ovarian follicular pool, is believed to facilitate the early follicular phase rise in FSH. Activin, a homodimer of the inhibin b-subunit, may be increased locally in the perimenopausal ovary, since inhibin a-subunit is declining at this time of life (Buckler et al 1991). This increase in activin may further increase circulating FSH, and, in an animal model it has been shown to lead to hyperestrogenic superovulation (Erickson et al 1995).

Thus, the early peri menopause is heralded by the appearance of elevated FSH, possible elevations in estrogen, and decreased progesterone secretion. The hormonal milieu is one of relatively unopposed estrogen, and this may promote the growth of uterine pleiomyomata and a variety of disconcerting bleeding problems. The perimenopausal reproductive hormonal environment should not

be regarded as a simple waning of ovarian function over time. It is a waxing and waning process, at times more like a "roller coaster" in its hormonal dynamics.

Menopause

At the time of menopause, the ovary is nearly devoid of primordial follicles (Gosden 1987). Granulosa cell estrogen production is essentially nonexistent. The circulating level of estrogen in women shows a very steep decline over the first 12 months after the menopause, with only a very slight further decline in the years thereafter (Longcope et al 1986; Meldrum et al 1981). The daily production rate of estrogen falls nearly eightfold to a level of approx 48 mg per 24 hours. Essentially, all estrogen in the postmenopausal woman is derived from the peripheral conversion of androstenedione. Indeed, postmenopausal women who have undergone bilateral oophorectomies for endometrial cancer show no significant reduction in their circulating levels or urinary excretion rates of estrogen (Procope 1968; Bulbrook and Grenwood 1957).

Glucocorticoid suppression dramatically reduces the circulating level of estrogen whereas adrenalectomy effectively eliminates measurable estrogens from the urine (Barlow et al 1969). The circulating level of estrone in postmenopausal women is approx 30.70 pg/mL. The circulating level of estradiol is even lower, approx 10.20 pg/mL, as most is derived from the peripheral conversion of estrone (Judd et al 1982). Estrone sulfate is an inactive metabolite of both estradiol and estrone, which diminishes in a similar manner postmenopausally. However, it still is present in higher concentrations than its precursors in both plasma and breast tumor tissue. It may have significant biological effects as culture studies with rat mammary tumor cell lines show nearly complete desulfation of the hormone and tumor colony proliferation (Santen et al1986). Sporadic and transient increases in estradiol concentrations, neither accompanied nor followed by elevations in progesterone, have been noted in some postmenopausal women (Metcalf et al1982). Such instances may represent residual follicular activity without subsequent ovulation, or perhaps are

associated with stromal hyperplasia. Ovarian stromas possess a limited capacity to aromatize androgens and therefore directly contribute more to the circulating pool of estrogen. Immunohistochemical examination of ovarian stromal cells has also recently demonstrated the presence of aromatase cytochrome P-450 in both pre and postmenopausal ovaries (Inkster and Brodie 1991). Whatever ability to aromatize androgens the postmenopausal ovary may possess in vivo, it is generally agreed to be at most quite limited. This may be because of a disproportionately lower concentration of FSH as compared to LH receptors in the ovarian stromal cells.

Androgen Production

As women traverse the menopause, ovarian androgen secretion declines. Midcycle testosterone and androstenedione have been reported to be decreased at midcycle in women in their mid-40s who are still having regular menstrual periods, when compared to younger, midreproductive-aged women (Mushayandebvu et al 1996). This aside, a solid foundation of evidence exists that demonstrates the postmenopausal ovary to remain a highly functional androgen-secreting organ. Histologic examination reveals the stromal cell of the ovarian cortex and the hilar cell of the ovarian medulla to be responsible for this production.

.

EFFECTS OF MENOPAUSAL ENDOCRINOLOGICAL CHANGES ON BODY COMPOSITION, CHRONIC DISEASE PHYSIOLOGY AND METABOLISM

(EXPERIMENTAL EVIDENCE)

Estrogen receptors are present in various organ systems in the body, hence depletion in estrogen, exerts an effect of deficiency on these organ systems, one among these being the cardiovascular system. The effects estrogen has on cardiovascular system can be viewed as rapid effect which follows the non-genetic route and includes estrogen mediated vasodilatation. Then there are slow effects which are genetic in nature and include production of vasodilative substances, beneficial effects in lipid profile and resistance to atherosclerosis.

Thus low estrogen levels during menopause, has detrimental effects on the vasculature, lipid profile, coagulation and fibrinolytic systems (Wood and Cox 2000).

Effects on Body Composition

A number of studies have reported the association of menopausal transition and changes in body composition, especially increase in the visceral fat depot in women (Ley et al 1992, Hunter et al 1996, Tremollieres et al 1996, Reubinoff et al 1995, Lovejoy et al 2008). Franklin et al (2009) followed up 8 pre menopausal women for 8 years till they were one complete year into their menopause and studied the changes in total body fat and distribution. The results indicated that total abdominal fat, visceral fat and subcutaneous fat were found to be significantly higher after menopause ($p<0.05$).

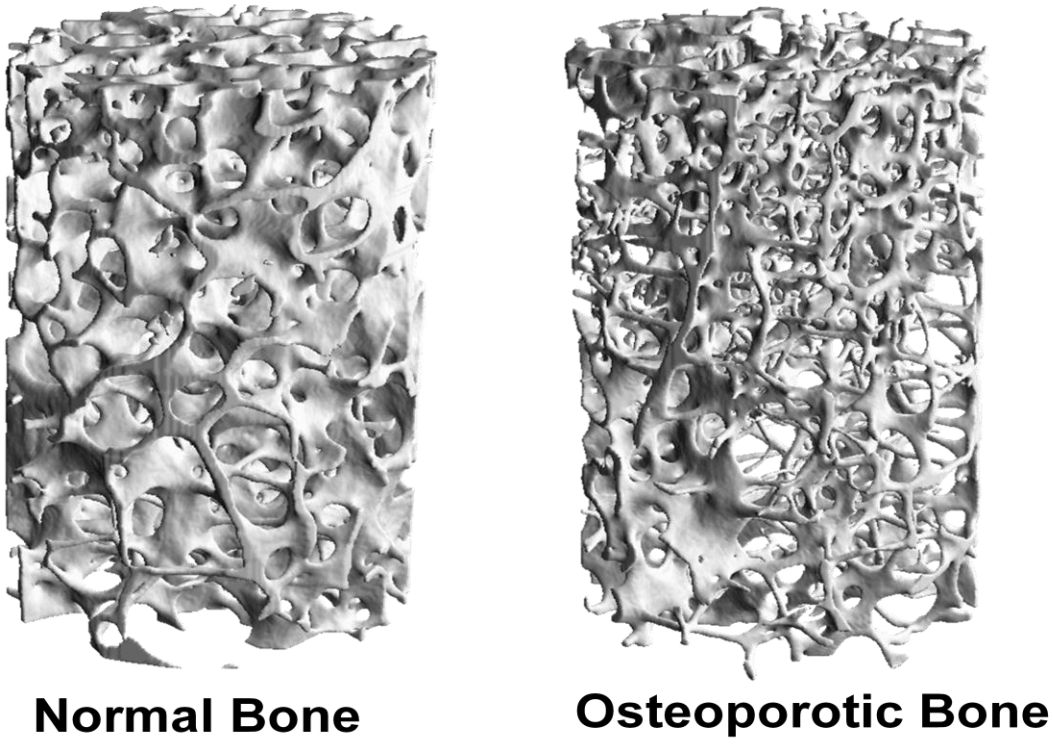
Panotopoulos and co-workers (1996) compared the regional fat distribution and lean mass distribution across European pre, peri and post menopausal obese women using DEXA and found that post menopausal women had a higher

percentage of fat mass in the trunk area while lower percentage of fat as well as lean mass in the thigh and leg regions as compared to premenopausal obese women, after adjusting for total fat and age. Similarly Douchi et al (2001) looked into the relative contribution of menopause and aging to changes in the fat content and lean mass content in 566 adult pre and post menopausal Japanese women using DEXA. The results revealed that after menopause, the lean tissue content and bone mineral density decreased while the total body fat, truncal fat and trunk-leg fat ratio increased with age after menopause ($p<0.001$). However, the lean tissue was inversely correlated with menopausal status ($p<0.001$) but not with age. Svendsen, Hassager and Christiansen (1995) also observed similar results in 407 healthy Danish women, alongwith the independent effect of age and menopause it was found that the total fat content, % fat mass and % abdominal fat mass had significant associations with menopause and years since menopause, independent of age.

Changes in Bone Structure

The skeletal mass also changes as the menopausal transition progresses. In the adult skeleton, approximately 5–10% of the existing bone is replaced every year through remodeling (Baron 1996). Remodeling begins with bone resorption by osteoclasts, which is followed by bone formation by osteoblasts. This is how a balance of the bone mass is maintained. The maintenance of a normal, healthy, mechanically competent skeletal mass depends on keeping the process of bone resorption and formation in balance. Failure to match bone formation with bone resorption results in net bone loss and osteroporosis (Manolagas and Jilka 1995). Figure 2.7 shows the structural changes in an osteoporotic bone where the lacunae in the bone are larger, making the bone brittle and prone to fractures. The remodeling process is controlled by systemic and locally produced cytokines mainly TNFs, interleukin-1 and interleukin-6, and is closely regulated by estrogens (Horowitz 1993). Apart from this, the bone forming cells, osteoblasts

Figure 2.7 Structural Changes in the Skeletal Tissues With Osteoporosis



have estrogen receptors which promote bone formation. Therefore, menopausal transition brings about an imbalance in bone remodeling and results in loss of bone mass in post menopausal women (Jilka 1998, Martin and Udagawa 1998).

A recent study by Mitra and co workers (Mitra, Desai and Ikram 2006) also revealed that Vitamin D Receptor (VDR) gene polymorphisms were associated with BMD in postmenopausal Indian women and may influence determinants of bone metabolism. Another study done by Vupputuri et al (2006) reported that variation in BMD at spine and forearm was related to parathyroid hormone levels and VDR gene polymorphisms and at hip to vitamin D deficiency in vitamin D deficient/ insufficient urban Asian Indians. In addition, estrogen receptor α (ERα) gene polymorphisms may also be associated with BMD in Indian women and may influence some determinants of bone metabolism resulting in accelerated age related bone loss (Mitra, Desai and Ikram 2006).

EFFECTS OF ESTROGEN WITHDRAWAL ON THE PHYSIOLOGY OF THE HEART AND VASCULATURE

The addition of estrogen has been shown to increase cardiac output, arterial compliance, and myocardial perfusion, and to decrease vascular resistance and systolic and diastolic blood pressure both in animals and humans. The effect of the physiologic removal of estrogen with menopause on cardiovascular function is less clear.

Changes in Blood Flow

The endothelium plays a critical role in the control of blood flow in the interaction between the blood and the vessel wall. Endothelial function has been assessed in patients by measuring coronary hemodynamic response to intracoronary administration of an endothelium dependent vasodilator, acetylcholine.

Coronaries with normally functioning endothelium exhibit acetylcholine-induced dilation, manifested by an increased epicardial cross-sectional area and coronary flow augmentation. In patients with atherosclerosis or dysfunctional endothelium, paradoxical acetylcholine-induced constriction is manifested by decreases both in area and blood flow (Klapholz and Buttrick 1989). Of note, acetylcholine-induced changes in coronary tone mimic those to common vasomotor stimuli, such as exercise and mental stress and, thus, are useful in experimental settings. Endothelial dysfunction is increasingly recognized as an important factor in the progression of cardiovascular disease. Numerous studies suggest that estrogen has a beneficial effect on endothelial dysfunction and, thus, declining estrogen levels with menopause and the subsequent negative effect on vascular tone, could be an important mechanism by which atherosclerosis occurs in postmenopausal women (Anderson et al 1995).

Effects on Vasculature

Most of the recent literature has focused on the effects (acute and chronic) of estrogen administration to postmenopausal women with atherosclerosis and impaired vascular tone. A few cross-sectional studies have looked at the direct effect of menopause (and, thus, estrogen withdrawal) on vascular tone. A group of investigators used high resolution ultrasound to evaluate endothelial responsiveness in the brachial artery which has been shown to be an effective proxy for coronary endothelial function (Anderson et al 1995). Flow-mediated dilation was preserved in young male subjects and then declined after 40 years of age. In women, however, flow mediated dilation was maintained until the early 50s, and then declined significantly more than it did in men. Another recent study looked at both normotensive and hypertensive males and females and found that age-related endothelial dysfunction is attenuated in premenopausal women both with and without hypertension as compared to males. This gender difference was not seen postmenopausally (Taddei et al 1996). The same authors who studied changes in forearm blood flow used brachial artery strain gauge

plethysmography to measure the effect of surgical menopause on vascular tone in a small series of women who were scheduled to have TAH/BSO for uterine leiomyoma. In association with dramatic drops in estrogen levels these women had a significant reduction in acetylcholine-induced vasodilation compared to their presurgical baseline. These changes were significantly attenuated in a small subset of the women who received estrogen replacement over the next three months (Pinto et al 1997).

There are data that both short and longterm estrogen administration improves endothelial cell-mediated vasodilation in ovariectomized monkeys fed an atherogenic diet (Pinto et al 1997; Wiliams et al 1990). Among recent studies looking at the effects of estrogen administration on vascular tone in postmenopausal women most have looked at the acute effect of estrogen on vascular reactivity. Earlier studies used cardiac catheterization to measure coronary flow resistance in cross-sectional areas before and after intravenous estrogen. Later studies used brachial strain gauge plethysmography and brachial artery high-resolution ultrasound. There is limited data on the effects of longterm estrogen administration and coronary endothelial cell function. One study, in ovariectomized monkeys treated with hormonal replacement for 26 months, has shown a beneficial effect (Wiliams et al 1990). A study by Lieberman (Lieberman et al 1994), treated 13 postmenopausal women with hormone replacement therapy in a double-blind placebo controlled crossover trial. Measurements of flow-mediated vasodilation of the brachial artery taken at the end of each 9 week treatment suggested statistically significant changes in flow-mediated vasodilation in postmenopausal women on shortterm hormone replacement therapy. In contrast, Gilligan (Gilligan et al 1995) found no improvement after 3 week of hormone replacement therapy in contrast to the effect of acute estrogen administration on flow mediated dilation using the same method. The recent study by McCrohon, which was a cross-sectional study comparing postmenopausal women who had taken HRT with age-matched controls (who had never taken HRT), demonstrated statistically significantly greater flow

mediated dilation in women taking HRT, as measured by brachial artery high-resolution ultrasound (McCrohon et al 1996).

In summary, clinical studies suggest a role for acute estrogen in the improvement of endothelial-dependent flow-mediated vasodilation. The data for shortterm or chronic hormone replacement therapy is less clear. There are a number of possible reasons for these differences. First, the plasma level of estradiol achieved by acute infusion, when measured in studies, was 34 times higher than what would be achieved by usual doses of hormone replacement therapy. It is also possible that chronic estrogen administration acts through different cellular mechanisms in regulating vascular tone. Finally, studies to date have been limited to small sample sizes, suggesting the possibility of a beta error (i.e., inability to detect a small benefit in vasomotor responsiveness in patients on chronic hormone replacement therapy).

Endothelium Dependent Vasodilation

The endothelium consists of a monolayer of cells that lines the intimal surface of the entire cardiovascular system. It plays a major role in regulating vascular tone through the release of dilator and constrictor substances that act upon vascular smooth muscle. There is accumulating evidence that impairment of endothelium-mediated vasodilation is an important early feature in the development of vascular disease not only in patients with known atherosclerosis but also with patients with hypertension, hypercholesterolemia, smoking, and diabetes (Zeiher et al 1991; Creager et al 1992).

Nitric Oxide

Endothelium dependent vasodilators, such as acetylcholine stimulate the endothelium to produce endothelial-derived relaxing factor (EDRF), which is nitric oxide (NO). Nitric oxide is released by normal vascular endothelium in response

to many types of clinical and physical stimuli, including neurotransmitters (acetylcholine), catecholamines, platelet products (serotonin), shear stress and changes in oxygen tension. NO causes vasodilation in endothelium intact coronary arteries and is a product of the conversion of L-arginine by nitric oxide synthetase (NOS) to NO and citrulline. NO is released in response to many factors, including acetylcholine, causing a subsequent relaxation of the blood vessel. In arteries damaged by atherosclerosis, however, acetylcholine causes constriction suggesting that atheroma impairs endothelium mediated dilation of the coronary arteries. Patients with central hypertension also have impaired endothelium dependent vasodilation. At least one study has demonstrated that abnormal endothelial function of patients with central hypertension is related to a defect in the endothelium-derived nitric oxide system, because of reduced synthesis, release, or diffusion of nitric oxide to vascular smooth muscle (Panza et al 1993).

NO has several actions that are cardioprotective including vasodilation, inhibition of platelet adhesion and aggregation, and inhibition of smooth muscle cell proliferation and the amount of available NOS in a cell. NO has also been observed to slow the development of atheroma by inhibiting smooth cell proliferation or stimulating proliferation of endothelial cells. Estrogen is also a potent antioxidant of lipids and oxidized lipids inhibit NO. Estrogen may, therefore, protect the vascular tone by enhancing and/or prolonging the half-life of released NO. The time course for this effect is unknown and effects may only be seen with long-term estrogen therapy. In one study of HRT in postmenopausal women, researchers measured NO2 and NO3 levels as markers for NOS synthase activity and found an increase in women who were on estrogen alone (Roselli et al 1995). One study in guinea pigs has suggested that long-term administration of estrogen up-regulates the transcription of nitric oxide synthase. A recent study in humans has demonstrated variations in expired NO production with cyclical hormone changes in premenopausal women. NO levels

peak at the middle of the menstrual cycle suggesting an influence of hormones on the synthesis and release of NO in humans (Kharitov et al 1994).

Calcium Antagonism

Vascular smooth muscle (VSM) contraction is enhanced by intravascular calcium. Substances that block the flow of calcium into cells cause VSM relaxation and decreased vascular tone. It has been hypothesized, based on animal models, that some of the cardiovascular benefit of estrogen replacement therapy may be because of a calcium antagonistic effect of estrogen (Collins et al 1993). These properties have been demonstrated in several animal models. 17 β Estradiol was shown to have a negative inotropic effect on single-isolated guinea pig ventricular myocytes by inhibiting inward calcium currents and so reducing intracellular free calcium (Jiang et al 1992).

Prostaglandins

Prostacyclin is a prostaglandin produced by endothelial cells. Its synthesis is thought to be coupled to NO release. It has been shown to induce vasodilation and inhibition of platelet activation in animal models. Evidence in humans is scant, but there is an indication that estrogen may effect coagulation and vasodilatation by its effects on prostacyclin (Beale and Collins 1996).

Inhibition of Constrictor Factors

Animal studies suggest that estrogen inhibits the release of or response to vascular constrictor factors. Vasoconstrictors include endothelin and fibronectin. There is a correlation between high endothelin levels and the development of atherosclerosis in humans (Lerman et al 1991). One study demonstrated that plasma endothelin levels tend to be higher in men than women and lower still in pregnant women (Polderman et al 1993). As a corollary, the same authors demonstrated in transsexuals that sex hormones may modulate endothelin

levels, with male hormones increasing and female hormones decreasing the level. The effect of declining levels of estrogen with menopause on vascular constrictor factors is still unclear.

Estrogen also inhibits angiotensin II-induced constrictor effects in animal studies suggesting an inhibitory effect on the renin-angiotensin system (Beale and Collins 1996). In males elevated activity of serum angiotensin-converting-enzyme (ACE) may be associated with an increased risk of developing CAD. To date, there are no studies looking at ACE levels in women pre- and postmenopausally and correlating them with increased risk of developing CAD. In one of postmenopausal women treated with 6 months of hormone replacement therapy, ACE-activity was reduced by 20% in 28 treated women as compared with 16 untreated controls (Proudher et al 1995).

Effects on Vasoactive Neurotransmitters

Epinephrine and norepinephrine are released from sympathetic and parasympathetic nerve endings in the arterial wall and, thus, can cause vasoconstriction and vasodilation, playing an important role in the maintenance of vascular tone. Estrogens and progestins are thought to influence the release of these neurotransmitters by several mechanisms (Sarrel 1994). Of note, vasomotor instability (VMI) the hallmark of estrogen deficiency occurs with rapid fluctuations in serum epinephrine and norepinephrine concentrations. Medications that decrease central noradrenergic activity, such as clonidine, have been shown to successfully treat hot flashes. The decline of estrogen levels that is seen with menopause is also associated with a relative increase in catecholamine release associated with physical and mental stress (Matthews et al 1994).

Effects on Vascular Wall Composition: Animal studies have shown that vascular smooth muscle hyperplasia and collagen biosynthesis are reduced by estrogen administration (Samaan and Crawford 1995). In one clinical study,

postmenopausal estrogen use was associated with significant borderline reductions in measured common carotid artery wall intimal medial thickness even after controlling for other risk factors such age, smoking, lipids, etc. (Manolio et al 1993).

In a subanalysis of the Asymptomatic Carotid Atherosclerosis Progression Study (ACAPS), women who used ERT (preparation and dose not specified) were assessed for carotid artery wall intimal-medial thickness (IMT) by carotid ultrasonography. IMT, which is a marker for atherosclerosis, appeared to be retarded and to possibly reverse in women who took estrogen without receiving lipid-lowering therapy (Espeland et al 1995).

Changes in Vascular Compliance and Blood Pressure

A newly recognized marker for hypertension and atherosclerosis is reduced vascular compliance. The latter describes the condition of the arterial wall that influences the relation between volume and pressure. In stiffer vessels, a smaller volume change will cause a greater pressure rise as compared to a normally compliant system. Vascular compliance is known to decrease with menopause.

One direct measure of vascular stiffness is the pulsatility index (PI). This represents the impedance to blood flow downstream from the point of measurement. An increase in PI is closely correlated with the time elapsed after the menopause. Decreases in arterial waveform pulsatility index in the uterine and carotid arteries have been demonstrated in postmenopausal women after chronic estrogen replacement suggesting an improvement in arterial compliance (Gangar et al 1991). In another recent study, patients were treated with estrogen and progesterone for 1 year and a significant decrease in PI was observed at 48 weeks. Arterial compliance is increased with pregnancy but returns to normal within 8 week postpartum suggesting that these changes were not secondary to a change in vascular structure, but to a reduction in smooth muscle tone (London et al 1995).

Premenopausal women have lower systolic blood pressure than men of a similar age. After menopause, however, systolic blood pressure tends to be higher than in age-matched males. One study has also shown that an increase in pulsatile components of blood pressure is associated with higher cardiovascular risk in postmenopausal women (Darne et al 1989). The changes in blood pressure with menopause were explored in a study of both premenopausal and postmenopausal women who were compared with age-matched men (London et al 1995). Using ultrasound/Doppler to measure vascular flow, the authors found that premenopausal women had lower systolic blood pressure in their peripheral arteries, but not in their central (i.e., carotid) artery. Males had greater peripheral blood pressure that was attributed to amplification of blood pressure from central to peripheral arteries, which increased with body height and decreased with arterial distensibility. In contrast, in postmenopausal women, arterial distensibility was similar to that of age-matched men and no longer compensated for smaller body size, resulting in a persistent increased defect of wave reflections in central arteries, and greater peripheral blood pressure (London et al 1995).

In a related study, 18 women with essential hypertension were followed for 3 years, during which time they went through menopause, to investigate whether a natural decrease in sex hormones in hypertensive women caused an increase in the stiffness of the aortic root (Karpanou et al 1996). The authors found that aortic root distensibility decreased significantly in women who had gone through menopause as compared with age-matched controls, suggesting an important role for declining estrogen levels in this process.

Changes in Cardiac Function

Estrogens affect hemodynamic parameters through several different mechanisms. There is less evidence about the effects of declining estrogen levels with menopause on hemodynamic function. In one study, which followed women through the menopause transition, no significant changes in echocardiographic measurements of end-diastolic and end-systolic dimensions

were found after menopause. However, significant decreases in rest Doppler measurements of left ventricular contractility appeared progressively over the years after menopause in women not treated with hormone replacement therapy (Pines et al 1992). These factors appeared to be modified with hormone replacement therapy suggesting a positive inotropic effect of estrogen (Pines et al 1991).

METABOLIC CHANGES WITH MENOPAUSE

Changes in Lipid Metabolism

Several epidemiologic studies have suggested increases in levels of total cholesterol, low-density lipoproteins and triglyceride rich lipoproteins associated with menopause. He et al (2012) have reported significantly higher prevalence of elevated total cholesterol, triacylglycerols and LDL levels ($p<0.05$) in post menopausal Chinese women compared to premenopausal counterparts. Eshtiaghi et al (2009) reported significantly higher adjusted odds ratio (OR: 1.01, 95% CI: 1.00 – 1.02, $p<0.001$) for post menopausal Iranian women of having elevated non-HDL levels compared to premenopausal women in a cross-sectional analysis on 940 adult women. Cagnacci et al (2012) in a retrospective cross-sectional study on 951 post menopausal women, found that presence of menopausal symptoms was significantly associated with TC/HDL ratio ($r=0.35$, $p<0.0001$), TAG ($r=0.35$, $p<0.0001$), TAG/H ($r=0.42$, $p<0.0001$) and glucose ($r=0.39$, $p<0.0001$).

In general, HDL levels are stable in the years after menopause, although there may be a small reduction in HDL2 subfraction. Presumably, these changes with menopause are secondary to reduction in endogenous hormones. This is certainly supported by the beneficial effect of postmenopausal hormone therapy on lipoprotein metabolism in postmenopausal women. Studies suggest that estrogen use is associated with elevations in high-density lipoprotein (HDL) cholesterol, especially HDL2 by as much as 20% and reduction in low-density lipoprotein (LDL) cholesterol by as much as 19%

An elevated Lp(a) level is independently associated with the development of CAD in women (Boston et al 1994) as well as men. Lp(a) is a modified form of LDL to which an apolipoprotein is attached. Its genetic structure is similar to plasminogen and, thus it interferes with the binding of plasminogen to sites of cells and molecules. Levels of Lp(a) are primarily determined by genetic and, as such, there are no abrupt changes in Lp(a) with menopause. However, estrogen therapy appears to reduce Lp(a) levels. An elevated plasma homocysteine level is an independent risk factor for CAD especially premature atherosclerosis. Levels are known to increase in both genders with age. After menopause, fasting homocysteine levels may increase or stay the same (Mayer et al 1996). Thus, the impact of declining estrogen levels on homocysteine levels is unclear.

In animal studies, estrogen appears to interfere with cholesterol deposition in the arterial wall (Adams et al 1994) and in laboratory studies to reduce arterial smooth muscle cells proliferation. Oxidative modification of LDL cholesterol may be an important step in atherogenesis. In animal studies, the oxidized form of LDL appears to be more effective than inactive LDL in impairing endothelium-dependent vasodilation. One recent study suggests that endothelium mediated vasodilation is improved with lipid lowering drugs in patients with elevated cholesterol particularly if the lipid lowering therapy lowers rates of LDL oxidation (Anderson et al 1996). In vitro studies suggest that 17-β estradiol appears to inhibit LDL oxidation and reduce cholesterol ester formation (Rifici et al 1992). In one study, 17-β estradiol administration significantly reduced the oxidation of LDL cholesterol from postmenopausal women (Sack et al 1994).

Changes in Clotting

Certain hemostatic variables change with menopause with a potential impact on both thrombosis and fibrinolysis. After menopause, fibrinogen levels increase as do levels of factor VII and antithrombin III. Higher levels of PAI-1 an antagonist of fibrinolysis in humans have been noted in postmenopausal women in the

Framingham Offspring Study (Gebara et al 1995). Studies of HRT in postmenopausal women suggest a decrease in fibrinogen (Writing Group for the PEPI trial 1995), and a decrease in PAI-1 (Koh et al 1997). Animal studies also suggest that estrogen inhibits platelet aggregation.

Symptoms of Vasomotor Instability

Symptoms of vasomotor instability include palpitations and, in a small percentage of women, symptoms of chest pressure. Although they occur most often in conjunction with hot flashes, an increase in palpitations can be seen in the absence of other symptoms. The severity of these cardiac symptoms appears to be related to the severity of the hot flashes (WHO 1990). Vasomotor symptoms and associated cardiac symptoms are more severe in patients who experience a sudden drop in their estrogen level (e.g., surgical menopause). In one longitudinal study of 200 perimenopausal women from Scandinavia, palpitations figured prominently in the symptomatology in association with other vasomotor complaints (Holte 1992). In another survey of 501 women, 12.20% of those who were postmenopausal noted pressure in chest and 36.47% noted a change in heart rate in association with their hot flashes (Kronenberg 1992).

Effects on Glucose Metabolism

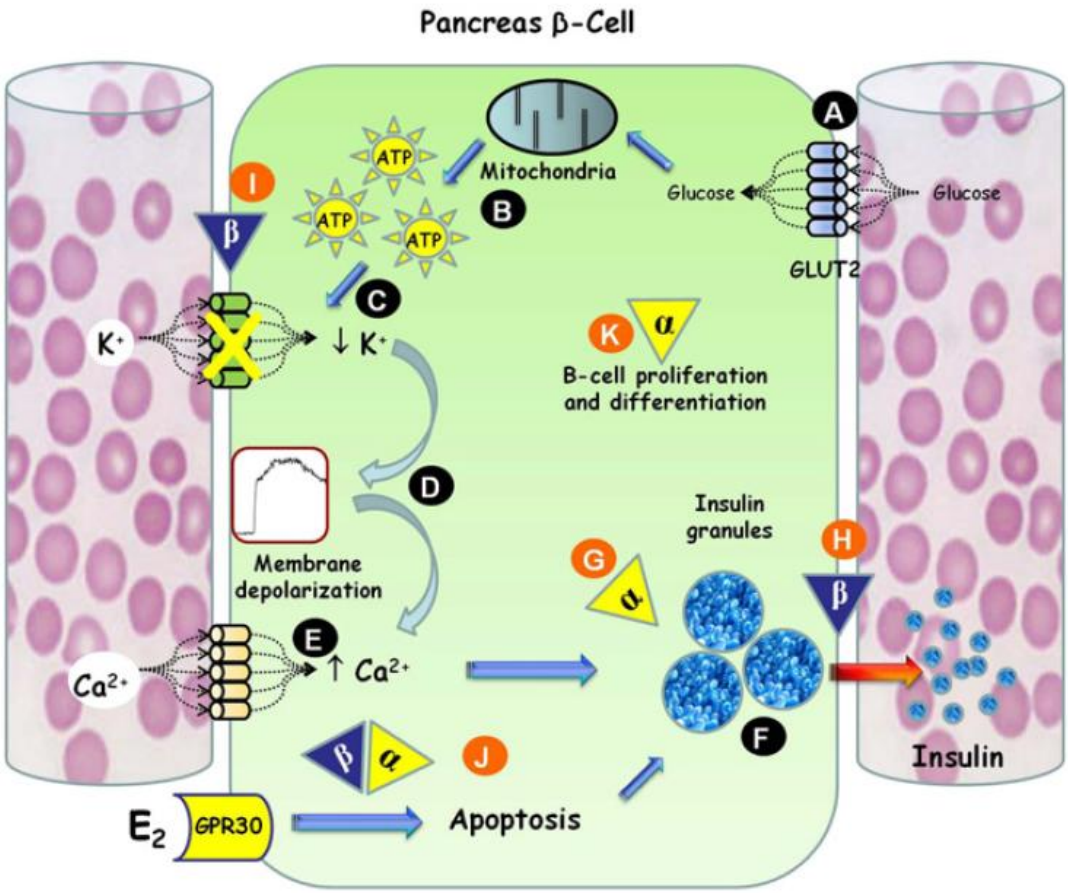
Estrogens have been known to affect energy metabolism, lipid metabolism, both of which are closely related to glucose homeostasis and glucose impairment and entails disruption in both (Barros, Machado and Gustafsson 2006). The link is also evident by presence of estrogen receptors in pancreatic islets, which are key regulators of glucose utilization (Clegg 2012). Several experimental studies involving estrogen and estrogen receptor agonists have demonstrated beneficial effects of estrogens on glucose metabolism. Lin et al (2008) developed a selective estrogen β receptor ligand, butyl 4-(butyryloxy) benzoate, which was found to induce increased GLUT 4 expression in the body, suggesting the fact that estrogenic action improves glucose transport across the cell (Liu et al 2008)

Tiano and coworkers (2011) studied the role of estrogen in pancreatic β cells of male diabetic obese rats. The authors supplemented the rats with estrogen in a series of experiments, while hypothesizing based on their earlier findings that ovarian steroids proved to exert a protective role with regard to glucose homeostasis and pancreatic β cell functioning. The study findings indicated that supplementation with 17β estradiol diminished the synthesis and accumulation of free fatty acids in Zucker diabetic obese male rats and shielded them from β cell failure. The authors triangulated the findings by pharmacological activation of the estrogen β receptor in pancreatic islet cells, which resulted in antilipogenic effects. The impairment in glucose metabolism was reverted to euglycemia, upon supplementation. Further, removal of β receptors resulted in enhanced lipid accumulation and dysregulated glucose levels, in face of a high fat intake (Tiano et al 2011).

Ahmed and Hassanein (2012) also supplemented 17 β estradiol in streptozocin-induced diabetic male rats for a period of 15 days. The supplementation resulted in reduced plasma glucose levels and better plasma insulin levels, increased expression of insulin receptors and improved histological structure of pancreatic β cells (Ahmed and Hassanein 2012).

The supposed mechanism of action of estrogen receptors was non-genomic pathways and has been represented in Figure 2.8(Barros and Gustafsson 2011). Briefly, Figure 2.8 depicts the events involved in glucose uptake by pancreatic islet cells and subsequent release of insulin granules by the β cells, and also the role of estrogen receptors in insulin release. Influx of glucose into the islet cell causes closure of KATP channels, resulting in depolarizing of the membrane. This leads to opening of calcium channels and resulting increase in intracellular calcium ion concentration, which promotes the release of insulin into the blood stream. Estrogens promote the cellular actions which are essential for release of insulin from pancreatic islet cells. These events include closure of KATP channels, protective effects on apoptosis, which avert decline in insulin release. Estrogen α receptor also augments β cell proliferation in the pancreas.

FIGURE 2.8 NON-GENOMIC PATHWAYS OF ESTROGEN REGULATION OF GLUCOSE HOMEOSTASIS



Source: Barros and Gustafsson 2011.

E2: estrogen; A-F: Glucose uptake by pancreatic islet cell; G-K: Sites of estrogen action for release of insulin granules into the blood stream; α and β: estrogen receptors

Effect on Thyroid Metabolism

Thyroid dysfunction appears to be more prevalent in women over the age of 50 years, which is roughly the mean age of menopause in most populations (Pearce 2007). When the endocrinological changes associated with the reproductive system and thyroid function go hand in hand during menopause, the already increased risk for cardio vascular, skeletal and metabolic diseases is amplified even more.

Not only overt hypothyroidism, but even sub clinical forms of hypothyroidism are associated with atherosclerotic disease, even when dyslipidemia is not present (Brenta et al 2007). The effect of TSH elevations in sub clinically hypothyroid menopausal women was studied by Brenta et al (2007) and the response to levothyroxine treatment. The authors found that TSH levels were associated with increased hepatic lipase activity and in turn TAG rich LDL particle. This characteristic pattern of lipoproteins was indicative of a pro atherogenic trend in women who were sub clinically hypothyroid.

Badawy, State and Sherief (2007) hypothesized that much of the menopausal symptoms appeared to be consequences of thyroid dysfunction in menopausal women. The authors investigated the effect of hypothyroidism treatment on attenuation of menopausal symptoms by supplementing levothyroxine in the hypothyroid group and estrogen replacement therapy. The findings indicated that many of the menoapuse linked symptoms were alleviated when treated for hypothyroidism, indicating the role of thyroid dysfunction in precipitation of menopausal symptoms. In a similar study, by Hernandez et al (2008), the investigators studied the effect of levothyroxine treatment in 45 menopausal women with subclinical hypothyroidism. They reported the frequency and severity of menopausal symptoms decreased significantly ($p<0.05$) with levothyroxine treatment. Lambrinoudaki et al (2009) studied the apo E and paraoxonase 1 polymorphisms and thyroid hormone levels in 84 healthy postmenopausal women. The observations reflected that in ase of apo E gene polymorphisms, carriers of E2 or E4 allele were found to have lower levels of FT4 ($p<0.001$)

compared to women carrying E1 or E3 alleles. Similarly, in case of paraoxonase gene polymorphisms, the carriers of B allele were found to have lower FT4 levels ($p<0.05$) compared to women having wild-type gene composition.

Thus, to summarize, sex steroids exert multiple beneficial effects on cardiometabolic systems by influencing endothelium dependant vasodilation, production of nitric oxide, inhibition of constricting factors, vasoactive neurotransmitters, blood pressure, lipid metabolism, clotting function and glucose metabolism. As a consequence, during menopause the resulting estrogen deficiency sets off a cascade of metabolic events starting with obesity, insulin resistance, and vascular effects leading to hypertension and dyslipidemia and metabolic syndrome. Figure 2.9 summarizes this chain of events that pervade through multiple organ systems in the body and precipitate a pro-inflammatory pro-thrombotic state, leading to adverse coronary outcomes and stroke.

This partly explains why the epidemiological trends which indicate that the prevalence of cardio-metabolic derangements runs high in middle women population in India. A gist of plight of Indian women can be had by looking at the burden of clinic metabolic aberrations in them, as reviewed in the epidemiological studies in the following section.

MIDDLE AGE HEALTH RISKS IN WOMEN

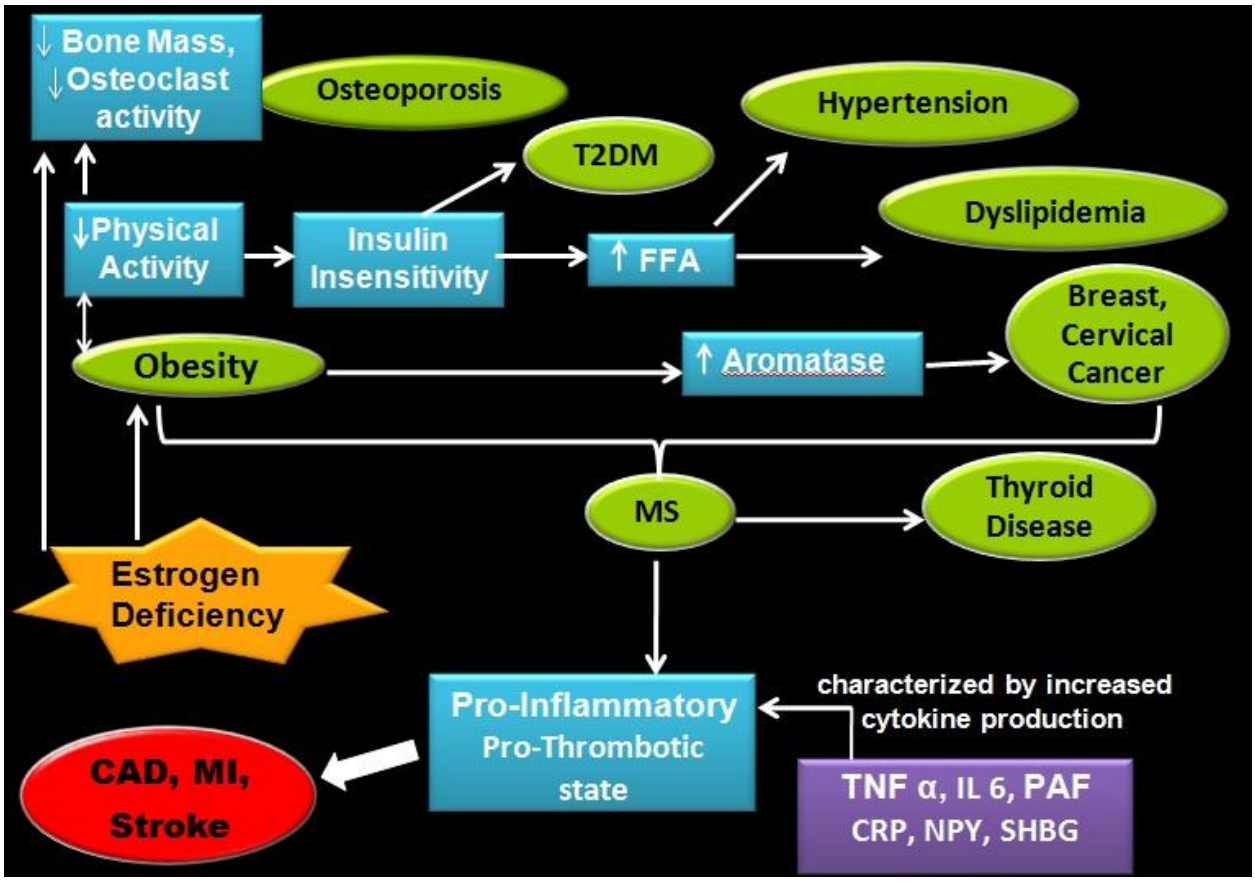
EFFECTS OF MENOPAUSAL ENDOCRINOLOGICAL CHANGES ON BODY COMPOSITION, CHRONIC DISEASE PHYSIOLOGY AND METABOLISM

(EPIDEMIOLOGICAL EVIDENCE)

Iron Deficiency

Anemia has been emerging as a potent risk multiplier of mortality risk in middle-age population. Earlier considered to be merely a disease marker, it is now envisaged as having profound implications as a cormorbid factor for other illnesses while posing a serious health risk on its own. Anemic patients have a

FIG 2.9 CASCADE OF METABOLIC EVENTS DUE TO ESTROGEN DEFICIENCY



Source: Horowitz 1993, Tremollieres et al 1996, Pearce 2007, Clegg 2012

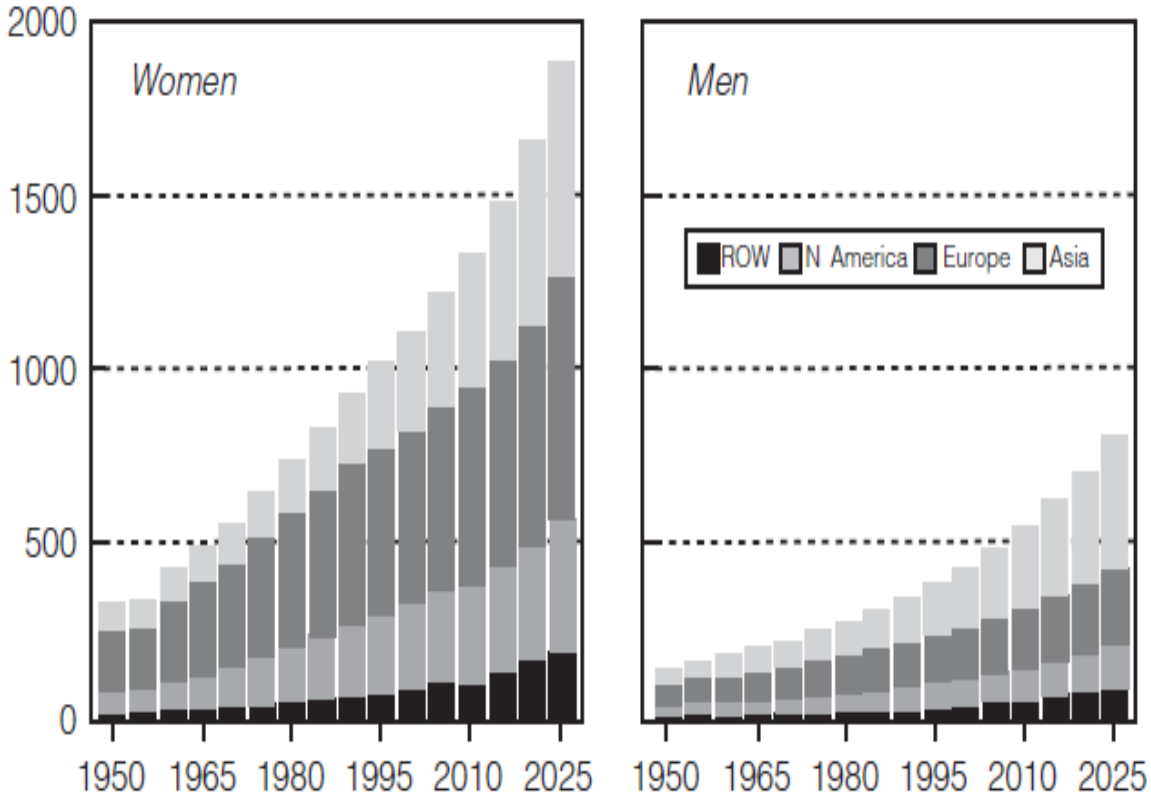
shorter survival than their nonanemic, age-matched counterparts (Ania et al 1997) and anemia is also an independent risk factor for mortality in heart disease (Caro et al 2001), cancer (Penninx et al 2004), renal disease (Mozaffarian, Nye and Levy 2003) and HIV infection (Semba et al 2002).

The prevalence of anemia according to NHANES III (1988-1994), in US women of age-group 50-64 years, was reported to be 6.8% as compared to 4.4% in men and 8.5% in 65-74 year old women and further 10.3% in 75-84 year old females. However, the prevalence of anemia continues to be higher in pre-menopausal women than in those experiencing menopause: 11% in pre-menopausal versus 19% in perimenopausal women in US, 2002 (National Center for Health Statistics 2008). Extent of anemia in women of reproductive age group has been studied extensively. The National Family Health Survey 3 (International Institute for Population Sciences 2007) reports the prevalence of iron deficiency anemia in women of reproductive age group to be 51% in urban sector, 57% in rural sector. Departmental studies have estimated the prevalence in reproductive women to be ranging from 40-45% in Vadodara [Mehta et al 2010, Nambiar et al 2010 (unpublished M.Sc. dissertations)]. Dhruv et al (2012) estimated the prevalence of anemia in young adult women to be as high as 87% in a cross-sectional study on 1258 women aged 18 to 26 years. Prevalence in Indian middle-aged women needs to be studied, due to lack of data in this regard and especially in view of emergence of anemia as an independent risk factor for heart disease, which affects women in middle-age.

Osteoporosis

In 1990 more than 1.25 million hip fractures were reported worldwide in women, and 500,000 in men. In the United States the estimated numbers of hip and vertebral fractures in women annually were more than 250,000 and 500,000, respectively (WHO 2003). Figure 2.10, shows the prevalence of hip fractures across the world, it can be seen that the prevalence is highest in Asians and is

FIGURE 2.10 GLOBAL PREVALENCE OF HIP FRACTURES (THOUSANDS)



Source: WHO 2003

twice as high in Asian women than their male counterparts.

The consequences of osteoporosis include diminished quality of life, decreased independence, and increased morbidity and mortality. The pain and kyphosis (curvature of spinal column and loss of height), height loss, and other changes in body habits that occur as a result of vertebral compression fractures erode quality of life for both women and men. In addition, the functional status of patients who have had vertebral crush fractures may also decrease. These patients may be unable to bathe, dress, or walk independently. Increased mortality is related primarily to hip fractures and 20% excess mortality occurs in older persons in the year following hip fracture. In addition, approximately 50% of women with hip fracture do not fully recover prior function (Manolagas et al 1995). Thus, in older adults, it is important to prevent as many fractures as possible.

Overweight and Obesity

World Health Organization (WHO) defines overweight as having a Body Mass Index (BMI) >25kg/m2 and obesity as BMI > 30kg/m2. There are approximately 350 million obese people and over 1 billion overweight people in the world (Kelly, Yang, Chen et al 2008). The 2012 World Health Statistics report states that one in six adults is obese in the world, which in itself is a cause of alarm because, over all about 2.5 millions deaths are attributed to overweight/obesity worldwide (WHO 2012). Prevalence of obesity in India, as estimated by the National Family Health Survey 3 (NFHS 2006) in rural and urban women, was reported to be highest in age-group of 40-49 years: 6.4% as compared to only 2.3% in males in the same age group; followed by 3.9% in 30-39 year age group (men 1.8%) and 1.2% in 20-29 year age group (men 0.7%). Similar trends were reported in case of overweight prevalence: highest prevalence was in women in 40-49year age

group (23.7%) compared to 15.2% in men of same age group; followed by 17.4% in 30-39 year age group compared to 13% in men and 8.2% in 20-29 years age group compared to 6.5% in men. The World Health Statistics report also confirms the fact that all around the world; women are more likely to be overweight and obese than men (WHO 2012). Thus, overweight and obesity is prevalent highest in women at and around menopausal age.

Scattered studies from different parts of India have consistently reported a high prevalence of overweight and obesity in India. Sharma et al (2012) conducted a cross-sectional study where they studied 453 administrative employees working at a hospital. The authors reported a prevalence of high BMI of 77.3% in the sample and 80.7% central obesity in women.

The effect of rural to urban migration on obesity was studied by Ebrahim et al (2010). Findings indicated that the prevalence of obesity was highest in urban women (53.5%) and lowest in rural men (18%) and indicated that the rural urban migration increases the risk of obesity in Indian populations.

Gupta and co-workers studied the association of educational status and cardio-vascular risk factors in 2772 women and 3426 men (Gupta et al 2012); and found that the prevalence of overweight or obesity in women to be 45.2% as compared to 41.1% in men. Extent of abdominal obesity was reported to be 57.5% in women compared to the 35.7% in men.

Prevalence of obesity in working postmenopausal women of Punjab was reported in a cross sectional study by Khokhar, Kaur and Sidhu (2010), to be 75%, as compared to 70% in premenopausal women. Central obesity was prevalent to the order of 89% in post menopausal women, as opposed to 74.5% in pre menopausal women.

Ghosh and Bhagat (2010) investigated the body composition characteristics in menopausal women from Eastern India. Results reflected that central obesity was significantly higher in post menopausal women (chi square 9.73, $p<0.05$), indicating a vital role of menopause in altering the body composition resulting in higher visceral fat.

The latest results in the series of Jaipur Heart Watch (JHW) series (Gupta et al 2012), revealed age adjusted prevalence in women to be 50.7%, while men had a prevalence of 46.2%. Elevated waist circumference was found in 26.6% women and in 12.9% of men.

Midha and co-workers studied prevalence and determinants of obesity in population from Kanpur. The findings revealed astonishingly low prevalence of obesity: 4.7%, however the authors reported higher prevalence in women and the odds ratio (OR=0.36) for female gender for development of obesity was significantly high.

The prevalence of obesity and related risk factors was studied by Singh et al (2012) in 670 pre and post menopausal women in Chhattisgarh in Eastern India. The study identified 32.7% overweight and 30.6% obesity in post menopausal women compared to 4.8% overweight and 12.6% obesity in pre menopausal women. The disparity in the prevalence of central obesity was very high, with 86.3% being abdominally obese compared to 31.2% of premenopausal women.

The Chennai Urban Rural Epidemiology Study (CURES) determined prevalence of obesity across the district of Chennai by sampling 2350 subjects. The results showed an age standardized prevalence of 47.4% in women compared to 43.2% in men. The prevalence of abdominal obesity was found to be way higher in women (56.2%) compare to that in men (35.1%).

Thus, the above review strongly suggests that epidemiological trends support experimental evidence on the role of menopause in pre-disposing women to adverse changes in body composition including decreased bone mass and increased visceral fat depot and android obesity.

Hypertension

Hypertension, defined as Systolic Blood Pressure (BP) higher than 120mmHg and/or Diastolic BP higher than 80mmHg, is the highly prevalent threat to cardio-vascular health. Globally, 26.1% of women have hypertension (Kearney et al 2005). The Jaipur heart Watch 4 - JHW 4 (Gupta et al 2005) reported the

prevalence of hypertension in urban women to be 29% in 30-39 year age-group, which rose to 67.3% in 40-49 years, 72.7% in 50-59 years and reaching peak at 91.2% in 60 years and above age group. The JHW 5 (Gupta et al 2012) reported the age adjusted prevalence in the same cohort to be 24.6%.

The most recent survey on hypertension (Gupta et al 2012) which was done across 7 major cities in India (Delhi, Kochi, Jaipur, Kolkatta, Haryana, Pune and Gandhigram) on 4608 women, received much media attention because the reported prevalence was as high as 48.2%, indicating that around one in two women in urban India was hypertensive. A cross sectional study on prevalence and determinants of diabetes in South India (Bharti et al 2011), reported the prevalence by studying 686 urban individuals to be as high as 54.4, implicating that more than half of the subjects were hypertensive.

An exploratory study on 453 administrative employees in a hospital in North India revealed a prevalence of 20.7% of hypertension in the subjects (Sharma et al 2012).

Pandey et al (2011) studied the determinants of urban-rural differences in the cardio-vascular risk factors in Indian women, in a multi centric study involving five rural and four urban (4624 women) locations across India. Among the results, it was found that age adjusted prevalence of hypertension in urban women was 37.5%, while the comparative figure for rural women was 29.3% ($p<0.01$).

The prevalence of hypertension and trends in blood pressure was studied by Kaur M (2012) in 600 Jat women from Haryana in North India. The reported findings revealed a prevalence of hypertension of 26.7% in urban women whereas in the rural women, the prevalence was 9%. Gupta et al (2013) studied the prevalence across 11 cities across all regions of India, evaluating 6106 urban subjects. The age adjusted prevalence of hypertension estimated in the study came out to be 30.4 for women; and that of pre hypertension was 30.1%. the awareness of hypertension in the study participants was found to be 55.3%.

The determinants and awareness about hypertension was studied by Meshram et al (2012) in a tribal population in Kerala in Southern India. The prevalence of hypertension in 4193 individuals studied was found to be as high as 40%. A

recent systematic review on hypertension in India by Devi et al (2012), which included 206 studies, concluded that prevalence of hypertension showed a significant ($p<0.001$) positive trend by region and gender.

The most large scale study on hypertension was reported to be conducted in Mumbai (Gupta et al 2004) on 88,653 individuals and estimated the prevalence of hypertension to be 48.4% in women, in addition to reporting that the mean SBP was found to be higher in females across all age groups compared to men.

Diabetes Mellitus

WHO (2000) estimates the global prevalence of Diabetes to be 171 million and India contributes 31.7 million cases. The World Health Statistics report estimated that one in ten individuals in the world is diabetic (WHO 2012). Wild et al (2004) have projected that he burden of diabetic individuals is purported to increase to 366 million in 2030. It has also been estimated that though the prevalence of diabetes was higher in men, there are more number of women with diabetes in the world as compared to men (Wild et al 2004). The National Health Interview Survey in US (2003) mapped diabetes prevalence and found a systematic increase in prevalence with age in both the sexes: 2.8% in 35-39 years, 6.5% in 45-49 years, 11.7% in 55-59 years and 15.1% in 65 years and above.

Khoo et al (2011) looked at the comparative prevalence of glucose dysregulation among Asian populations consisting of Chinese, Malays and Asian Indians ($n=4804$). The findings were indicative of the fact that prevalence of diabetes was significantly higher ($p<0.001$) in Asian Indians (18.6%), when compared to prevalence in Chinese (5%) or Malays (12.4%). Another inter-ethnic comparison of glucose homeostasis was done by Mente el al (2010) across South Asians, Chinese and Canadians and it was observed that the prevalence of newly diagnosed diabetes was 27% in South Asians compared to the 16% in Canadians and 12% in Chinese ($p=0.02$). Similarly, the prevalence of impaired glucose tolerance was found to be 17.7% in South Asians compared to the 11.2% in Canadians and 15.2% in Chinese ($p=0.11$).

In India, National Urban Diabetes Survey (Ramachandran et al 2001) reported the national prevalence of diabetes (FBS > 125) to be 8.5% in women aged 34-35 years, which increased to 19.7% in 45-49 years and 28.7% in 54-59 years. Jaipur Heart Watch 3 (Gupta et al 2004) estimated prevalence in Punjabi Bhatia community in urban as well as rural areas to be 1.6% in women aged 30-39 years, 12.2% in 40-49 years and 27.3% in 50-59 years and 37.8% in individuals aged 60 years or more. Thus a trend of a sudden rise in prevalence of diabetes after 40 years can be noticed in Indian women.

Prevalence of diabetes in 1320 individuals in Puducherry in South India was studied by Bharti et al (2011). The findings reflected a similar prevalence of 8% diabetes in both urban and rural sectors.

Diabetes burden was looked into in the urban wards in Odisha, Eastern India by Prasad et al (2012), where 1178 individuals were studied. The results revealed that crude rate of diabetes was 15.7% and the age standardized prevalence was 11.1%, while the same figures for impaired glucose tolerance were 8.8% and 6.7% respectively.

A large scale study on diabetes in 2227 urban residents, reported the prevalence of pre diabetes to be 13.2% and diabetes to be 11.1% (Zaman et al 2011). Similar study on 1370 rural counterparts, reported prediabetes to be present in 13.6% of women and diabetes to be present in 22% of women, which was higher than the burden in urban sector (Ravikumar et al 2011).

Insulin Resistance

Mente et al (2010) looked into the ethnic variations in the insulin resistance in 1176 individuals of a mixed group comprising of South Asians, Chinese and Canadians and aboriginals. The authors found that south Asians were most prone to increase in HOMA IR for same amount of decrease in the adiponectin levels, compared to Canadians and Chinese ($p<0.01$). The mean levels of HOMA IR were significantly higher ($p<0.001$) in South Asians (3.03) compared to Canadians (2.12) and Chinese (2.23).

Another large scale inter-ethnic study by Khoo et al (2011) on 4804 participants consisting of Chinese, Malays and Asian Indians, reported that the mean HOMA IR vales were significantly higher ($p<0.001$) in Asian-Indians (3.18), compared to Chinese (1.58) and Malays (2.28).

A yet another cross-population comparative study on insulin resistance by Petersen et al (2006) conducted on 482 individuals who were a mixed group of Eastern Asians, Asian Indians, Blacks, Caucasians and Hispanics. The authors reported that age and BMI adjusted insulin resistance as measured by HOMA IR values, was significantly higher in Asian Indian women (2.30), compared to other ethnic groups (Eastern Asians: 1.79, Caucasians: 1.95, Blacks: 1.97, Hispanics: 1.70).

With regard to Indian populations, Kumar et al (2005) studied the prevalence of insulin resistance in 350 individuals from Lucknow in Northern India, to find that the 11.8% of the subjects were insulin resistant and 40.7% had hyperinsulinemia.

Dyslipidemia

Dyslipidemia is defined as total cholesterol higher than 200mg/dl, LDL-C >100mg/dl, Triacylglycerols >150mg/dl (NCEP ATP III 2001). Dyslipidemia increases rapidly in menopausal age. Percent prevalence of hypercholesterolemia (TC > 200mg/dl) in US women, as reported by National Health and Nutrition Examination Survey (Center for Disease Control and Prevention 2002) was 16.2 in 35-39 years age group, which increased to 25.3% in 45-49 years and 31.1% in 55-59 years. In a study done in Jaipur (Gupta et al 2005), the prevalence of high total cholesterol (TC>200mg/dl) in urban and rural women was reported to be 22% in 30-39 years, 34% in 40-59 years and 42% in 60years and above.

Global trends in serum cholesterol (Farzadfar et al 2011) indicated that there was a fall in the total cholesterol levels in the high-income region, which consisted of North America, Australasia and western Europe while the regional declines in central and eastern Europe were about 0.2 mmol/L per decade. The mean total cholesterol increased in only in the south east and East Asia and Pacific by 0.08

mmol/L per decade in men and 0.09 mmol/L per decade in women. This might partly exaplain the increased prevalence of hypercholesterolemia in Asians.
Bharti et al (2011) reported the prevalence of high total cholesterol in 1320 individuals from Puducherry to be 33.9%. When the data was segregated depending upon urban and rural sectors, it was seen the prevalence was as high as 63.2% in the urban sector, indicating that more than two-thirds of the subjects had hypercholesterolemia, highlighting the role of urbanization and development of cardio-vascular diseases.

The age adjusted prevalence of elevated total cholesterol in a multi centric study on 4624 women all across four regions in India (Pandey et al 2011), was reported to be 27.7% in urban women and only 13.5% in rural counterparts, with the difference being statistically significant ($p<0.01$).

Another large scale cross sectional prospective study on 7000 individuals in Delhi, North India involving 1666 women and 5334 men, reported a prevalence of 21% of individuals who were found to be hypercholesterolemic (Padmavati et al 2011). Sharma and co-workers studied the distribution of cardio-vascular risk factors among 453 employees of a tertiary hospital in Delhi in North India (Sharma et al 2012); and observed that the subjects had a prevalence of hypercholesterolemia of 25.7% and elevated TAG of 34.5%.

The latest in the series of Jaipur Heart Watch studies (JHW 5), estimated the age adjusted prevalence of high TC to be 25.3% in women and 24.8 in males. The figures for low HDL in females was reported to be 35.1% and 32.2% in men; in case of elevated TAG, the age adjusted prevalence in women was found to be 31.5% as compared to 48% in men (Sharma et al 2012).

Prasad et al (2012) reported the prevalence of metabolic risk factors in 1178 subjects from Eastern Indian community. The extent of low HDL was seen in 84.5% of the females, compared to 9.5% in males ($p<0.0001$).

Metabolic Syndrome

Metabolic syndrome represents a grave situation because it is characterized by constellation of various risk conditions, thus aggravating the chances of an individual to develop adverse cardio-metabolic consequences. The prevalence of metabolic syndrome being reported in various parts of India are frighteningly high off late.

Sawant et al (2011) estimated the prevalence in the city of Mumbai in Western India on a sample of 548 subjects who attended a cardiac evaluation camp. The results revealed a prevalence of 19.5%. Prasad et al (2012) assessed the extent of cardiovascular risk factors in 1178 urban Eastern Indian individuals in Odisha. The study reported that 33.5% of the subjects had metabolic syndrome. What was alarming was that the prevalence in females (42.3%) was almost double than that in males (24%). The prevalence reported from Delhi by Sinha et al (2012) on a sample of 300 women recruited using multi stage systematic random sampling, came out to be as high as 30%. Das, Pal and Ghosh (2011) studied the burden of cardiovascular risk factors in 448 urban and rural individuals from Kolkatta in Eastern India. The estimates indicated a prevalence of 57.8% in urban females, implicating that every other female had the metabolic syndrome. The prevalence in the rural counterparts was reported to be 34.8%, which is comparable to the prevalence in other major cities in India. With regard to women from Western India, Pandey et al (2010) conducted a retrospective study on 498 middle aged women; and found that as high as 45% of the premenopausal women and 55% of the post menopausal women were diagnosed with metabolic syndrome, which raises quite an alarm, because it signifies half of the population is unhealthy and runs the risk of developing cardiovascular and metabolic conditions.

Hypothyroidism

Subclinical Hypothyroidism (SCH), defined as TSH > 4mU/l in presence of normal free T4 (FT4) [0.9 to 1.9 ng/dL], is emerging as a yet another co-morbid factor in the family of risk factors of chronic diseases. While clinical

Hypothyroidism has been known to adversely affect cardiovascular health, SCH is also argued to be associated with hypertension (Sharma et al 2005), responsible for 19.3mg/dl rise of total cholesterol in middle aged women and its prevalence runs as high as 7.6% in middle aged women belonging to Netherlands, as compared to only 1.9% in men of the same age group (Luboshitzky and Herer 1999).

According to the Rotterdam prospective cohort study (Alkazemi et al 2008), the prevalence (in middle aged women) was even higher: 10.8%. Badawy, State and Sherief (2007) reported the prevalence of subclinical hypothyroidism in Egyptian menopausal women to be 6% and overt hypothyroidism to be 5.1%.

Michalek, Mahoney and Calebaugh (2000) studied the prevalence of hypothyroidism in 892 Indians settled in America. The results reflected a hypothyroidism prevalence of 13% in the women compared to the 0.2% in men.

Ray et al (2009) looked at the iodine and non-iodine deficiency associated hypothyroidism in 101 women from West Bengal, India. It was reported that 37.62% of the subjects were found to be hypothyroid, out of which 76.3% suffered from iodine deficiency and rest of the 23.7% had hypothyroidism due to other causes.

A recent large-scale study on thyroid function in 4409 adults from Delhi in North India all of whom consumed iodized salt, revealed the prevalence of hypothyroidism to be 21.4% in women compared to 15.9% in men. It was also found that the prevalence of subclinical hypothyroidism was not correlated with iodine intake, measured by iodine excretion in the urine.

Thus, altered thyroid function is yet another endocrinological feature that presents in middle aged women, and is prevalent to the order of 13% to 37.6% in Indian population, which is higher than the figures for other populations.

Thus, transition into the middle-age coupled with menopausal transition, places women at increased risk of all adverse health conditions. These are: overweight and obesity; hypertension; Dyslipidemia; diabetes; insulin resistance,

hypothyroidism; iron deficiency anemia and osteoporosis & osteopenia. Figure 2.11 gives a summary of the burden of each of these conditions in Indian women.

Summary: From the evidence reviewed so far, it is evident that the hormonal changes in menopause results in substantially high cardio-metabolic derangements in middle aged women, and epidemiological trends also indicate that the burden of cardiovascular, metabolic and endocrinological risk conditions is disturbingly high in middle aged women. Specifically, the prevalence of hyperlipidemia, in various forms is the most prevalent. This calls for dietary management of hyperlipidemia which in turn will gear the chaotic metabolism favorably. Thus the dietary management should focus on incorporation of biomolecules like flavonoids, dietary fiber components, phytoestrogens, antioxidant vitamins, chlorophyll, etc. Therefore, functional foods containing such components have been attempted for management of hyperlipidemia.

MANAGEMENT OF HYPERLIPIDEMIA

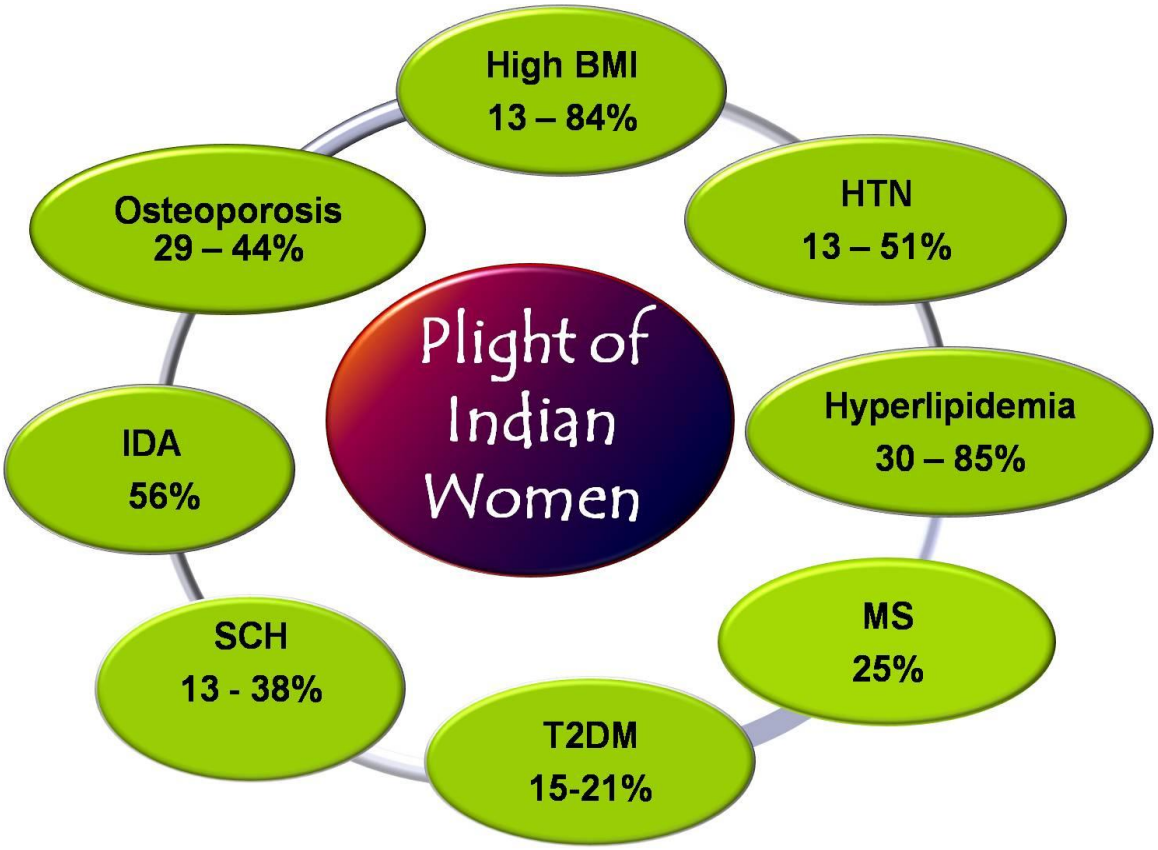
The medical nutrition therapy for hyperlipidemia involves 3 lines of therapy: pharmacological, dietary, and physical activity based.

[A] PHARMACOLOGICAL THERAPY

Statins (HMG-CoA Reductase Inhibitors)

Despite other pharmacological options available, statins continue to be the most popular line of drug therapy for hyperlipidemia. Statins are chemically HMG CoA Reductase inhibitors, meaning they inhibit the first step in cholesterol synthesis, by inhibiting the respective enzyme: HMG CoA redcutase (Witzum 1996).This class of drugs is the most effective in reduction of LDL levels in hyperlipidemic patients and involves minimal adverse side effects as observed in several large

FIGURE 2.11 BURDEN OF CARDIO-METABOLIC CONDITIONS IN INDIAN WOMEN*



* HTN: Hypertension, MS: Metabolic Syndrome, T2DM: Type 2 Diabetes Mellitus, SCH: Subclinical Hyothyroidism, IDA: Iron Deficiency Anemia

[Source: Based on summary of literature cited in text]

scale clinical trials (Shepherd et al 1995, Plehn et al 1999, Herd et al 1997, Downs et al 1998). The statins currently available are atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin and simvastatin. Though all the statins have been found to reduce LDL levels, only cerivastatin and atorvastatin and simvastatin are lableled by the USFDA for lowering TAG levels. Unlike other statins, atorvastatin has not been proved to reduce morbidity and mortality (Last et al 2011). . The only drawback of statins is that they have minimal effects on raising HDL (Hunninghake et al 1998). This puts the weight of lipid management on diet and physical activity to complement drug therapy for balancing the HDL levels, while taking statins. Other considerations for initiating statins for treatment of hyperlipidemia, include its cost, which depending upon the type of statin, varies between $42 to $200. Comparing the various statins, this comes out to be relatively highest among all the lipid lowering drugs (Last et al 2011).

Niacin (Nicotinic Acid)

The oldest hypolipidemic drug known is niacin. Niacin is inhibits adipose tissue lipolysis and results in decreased FFAs, leading to reduced VLDL, ultimately resulting in reduced LDL (Witzum 1996). It reduces serum triacylglycerols, LDL, total cholesterol and raises HDL levels. Niacin is not as effective as statins in reducing LDL values, extended release form of niacin increases 20% HDL and brings about 25% reduction in TAG levels (Canner et al 1986). Niacin is usually discontinued because of its adverse side effects which include flushing, nausea, abdominal pain and vomiting; though they are seen in less than 8% of patients (Gibbons et al 1995). These side effects require the patients to follow a specific diet regimen where they areto avoid taking the drug with alcohol or hot fluids. This leads to side effects and subsequent discontinuation of the treatment by the patient (Last et al 2011).

Fibrates (Fibric Acid Derivatives)

Fibrates are the class of drugs used to treat hypertriacylglycerolemia. This class of drugs includes clofibrate, gemfibrozil and fenofibrate. Fibrates are PPAR agonists, which aids in increased expression in LPL genes, eventually resulting in clearance of LDL, TAG and increase in HDL (Witzum 1996). According to the NCEP guidelines for patients with elevated triacylglycerol levels, initiation of drug therapy should be considered when a patient having hypertriacylglycerolemia also has confirmed CHD and/or pancreatitis (NCEP ATP III 2001). Drug therapy is not used for hypertriacylglycerolemia unless fasting triacylglycerol levels are greater than 400 mg/dl (4.50 mmol/l). Fibrates decrease triacylglycerol levels by 20% to 45% and increase HDL by 7% to 15% (Austin 1999). Adverse effects observed with fibrates include cholelithiasis in 1% (American Hospital Formulatory Service 1998). Contraindications for fibrates include severe renal or hepatic dysfunction (NCEP ATP III 2001).

Bile Acid Sequestrants (Resins)

As the name suggests, this class of drugs acts on lipids by sequestering bile acids and thereby affecting cholesterol absorption and further reduction by incorporation into bile acids (Witzum 1996). The bile acid sequestrants available include Cholestyramine and Colestipol. These drugs act mainly on LDL, resulting in 20% reduction and HDL, resulting in 5% increase. They do not have any effect on the TAG levels (American Hospital Formulatory Service 1998, Safer 2000). The most common side effects include indigestion, constipation, nausea, flatulence and diarrhea. Other than these, they also interfere with intestinal absorption of several nutrients, mainly minerals and vitamins (folate, zinc, magnesium, vitamin A, E, D and K). Therefore in this drug therapy, complimentary dietary inclusions and vitamin and mineral supplements also need to be considered.

Cholesterol Absorption Inhibitors

The only drug in this class is Ezetimibe, which as the name suggests, inhibits cholesterol-absorption in the gut. Once absorbed, ezetimibe undergoes glucuronidation in the liver, and localizes in the brush border of the intestinal cell. It lowers LDL by almost 20%, lowers TAG, and raises HDL slightly. Dosing studies show that it greatly augments LDL lowering when it is added to statin therapy. It also lowers plant sterol absorption from the gastrointestinal tract (Witzum 1996).

[B] Physical Activity

Ample evidence has demonstrated that modest lifestyle changes if sustained can substantially reduce cardiovascular morbidity and mortality (Smith et al 2001, Thompson et al 2003). Sustaining the activity is important because many of the beneficial effects of lifestyle changes accrue over time; long-term adherence improves individual and population benefits. Interventions targeting dietary patterns, weight reduction, and new PA habits often result in impressive rates of initial behavior changes, but frequently are not translated into long-term behavioral maintenance. The latest American Heart Association scientific statement (Artinian et al 2010) on dietary and lifestyle changes for cardiovascular risk reduction, recommends The AHA's 2020 Goals include a new concept of cardiovascular health that directly incorporates metrics of lifestyle behaviors, including diet and physical activity habits, as defining health.

TABLE 2.2 EFFECTS OF PHYSICAL ACTIVITY ON SERUM LIPIDS AND LIPID ENZYMES

Lipid Fraction	Single exercise session	Regular Exercise Participation
TG	Decreases of 7% to 69%; approximate mean change 20%	Decreases of 4% to 37% Approximate mean change 24%
Cholesterol	No change*	No change
LDL-C	No change	No change
Small dense LDL-C particles	No change	Can increase LDL particle size usually with TAG lowering
Lp(a)	No change	No change
HDL-C	Increases of 4% to 18% Approximate mean change 10%	Increases of 4% to 18% Approximate mean change 8%
Chylomicron and VLDL-C	Usually lower	Usually lower
Lp(a)	No change	No change
Postprandial lipemia	Reduced	Reduced
apoA1	No change	Increased
apoB	Parallels LDL changes	Parallels LDL changes
apoE $_2$, apoE$_3$, apoE$_4$	Varied response based on age, homozygote/ heterozygote phenotype	Varied response based on age, homozygote/heterozygote phenotype
LPL Activity	Delayed change (≥4 h)	Increased
HL Activity	No change	No change or reduced (may be reduced with weight loss)
LCAT Activity	Increased/no change	Increased/no change
CETP Activity	No change	No change/increased

Source: Artinian et al 2010

[C] DIETARY MANAGEMENT FOR REDUCTION OF HYPERCHOLESTEROLEMIA

Dietary Guidelines for Management of Hyperlipidemia

Dietary management of LDL-C is a major goal of CHD risk management. In addition, drug-induced reductions in LDL-C result in a concurrent reduction in the rates of coronary disease morbidity and mortality (NCEP ATP III). There is evidence from dietary studies that a marked reduction in LDL-C decreases the risk of CHD (Trichopoulou et al 2003). Nutritional factors that affect LDL-C levels are noted in Table 2.3. The principal dietary strategy for lowering LDL-C levels is to replace cholesterol-raising fatty acids (ie, saturated and *trans* fatty acids) with dietary carbohydrate and/or unsaturated fatty acids.

Increasing viscous (soluble) fiber (10 to 25 g/day) and plant stanols/sterols (2 g/day) to enhance lowering of LDL-C is recommended by AHA guidelines (Fletcher et al 2005). In addition, weight management and increased physical activity are recommended. An increase in viscous fiber of as little as 5 to 10 g/day is expected to reduce LDL-C by 3% to 5%. Inclusion of 2 g/day of plant stanols/sterols would be expected to reduce LDL-C by 6% to 15%. A 10-lb weight loss would be expected to decrease LDL-C by 5% to 8%. Among nutrients, the major determinant of elevated TGs in atherogenic dyslipidemia is dietary carbohydrate. In general, simple sugars and rapidly hydrolyzed starches have a greater glyceridemic effect than more complex carbohydrates and those consumed in conjunction with a higher intake of fiber. The recommended level of dietary fat is 25% to 35% of calories

The American Heart Association (Fletcher et al 2005) recommendations for maintaining a desired lipid profile (summarized in Table 2.4) includes limiting SAFA, trans fats, including more viscous fiber, PUFA, stanol and sterol esters fortified foods.

TABLE 2.3 NUTRITIONAL FACTORS TO BE REGULATED FOR MANAGEMENT OF HYPERLIPIDEMIA

Increased LDL	Saturated and *trans* fatty acids
	Dietary cholesterol
	Excess body weight
To Decrease LDL	Polyunsaturated fatty acids
	Viscous fiber
	Plant stanols/stenols
	Weight loss
	Isoflavone-containing soy protein (limited evidence)
	Soy protein

Source: Artinian Et al 2010

TABLE 2.4 AHA (2005) DIETARY RECOMMENDATIONS FOR ACHIEVING DESIRABLE LIPID PROFILE

Limit foods high in saturated fats
Replace saturated fats with lower-fat foods
Increase type of foods with unsaturated fat
Carefully monitor intake of food high in cholesterol
Severely limit foods containing *trans* fatty acids
Increase foods rich in viscous fiber
Increase foods containing stanol/sterol esters (special margarines, fortified orange juice, special cocoa/chocolate bars)

Source: Fletcher et al 2005

DIETARY INTERVENTIONS FOR MANAGEMENT OF HYPERLIPIDEMIA

Fruits

Fruits and vegetables have been advocated for cardiac and metabolic health. They contain a plethora of nutrients and non-nutrient complex carbohydrates and phytochemicals like flavonoids, carotenoids and polyphenols. A recent systematic review on the cardio-protective role of fruits reported that when various studies on impact of fruits supplementation on blood lipids were looked into, it was found that flavonones containing fruits appeared to impact blood lipids more than fruits containing other phytochemicals like anthocyanins and proanthocyanidins (Chong, Macdonalds and Lovegrove 2010). Fruits including oranges, grapefruit and marula were found to exert hypocholesterolemic effects.

Borochov-Neori et al (2008) studied the impact of marula juice consumption at the level of 200ml for a period of 21 days. The results indicated a reduction in the atherogenic lipoproteins and increase in the HDL fraction after the supplementation period.

Wilson et al (2008) investigated the effects of consuming either a red or a blond grapefruit per day on the blood lipids. It was observed that grapefruit significantly reduced TC, TAG and LDL. Red grapefruit appeared to have a greater effect on the lipid fraction compared to the blond grapefruit.

Franke et al (2005) and Kurowa et al (2000) investigated the cardio-protective effects of orange juice in hypercholesterolemic subjects. Franke et al reported a significant increase in HDL and a marginal decrease in LDL, while Kurowa et al observed a significant TAG and LDL reduction, with no changes in HDL fraction. However, in both studies the ration of LDL: HDL reduced significantly.

In a similar study, pomegranate juice flavonoids were shown to resist LDL oxidation and atherosclerotic changes in mouse model and human studies (Aviram et al 2002). The mechanism was speculated to be scavenging of reactive

oxygen species and nitrogen species by the polyphenols in pomegranate juice. These polyphenols were also demonstrated to augment the paraoxonase activity, leading to hydrolysis of the lipid peroxides found in the atherosclerotic lesions and lipid peroxides (Aviram et al 2002).

El-Beshbishy et al (2006) isolated from alcohol extracts of the Egyptian Mulberry, four active compounds: 5,7,2'-trihydroxyflavanone-4'-O-beta-D-glucoside, mulberroside and abanols A and B and studied the effect of these compounds on serum lipids in case of experimentally induced hypercholesterolemia in rat model. The results indicated that the supplementation resulted in favorable changes in the lipid profile and resisted atherogenic modifications: LDL retention by 33%, LDL oxidation by 44% and LDL aggregation by 30% and significantly reduced liver and plasma lipid peroxides.

Blackberries have been found to contain antioxidant molecules: anthocyanins, phenolic acids, flavonoids. In a controlled experiment, hypercholesterolemic hamsters were supplemented with 5ml of blackberry nectar for 98 days, at the end of which it was observed that there were significant reductions in TC (16%), TAG (31%) and LDL (44%). The intervention did not influence the HDL levels, but the LDL/HDL ratio did decrease non-significantly in the supplemented group (Ferreira de Araujo et al 2011).

Licorice

Fuhrman et al (2002) attributed inhibition of LDL oxidation and suppression of atherosclerotic lesions in mouse model to the dietary flavanoids in licorice root, by supplementation with ethanolic extract of licorice root at the rate of 0.1g/day for a period of one month, in a placebo controlled experiment. Also reported was reduction in TC (5%), LDL (9%), TAG (14%) and LDL oxidation (55%), LDL aggregation (28%) and LDL retention (25%).

Green Tea

The antioxidants in green tea have been reported to exert a hypocholesterolemic effect. The predominant polyphenols in green tea are catechins and theaflavins. In a double blind placebo controlled trial, 240 mildly hypercholesterolemic subjects were supplemented with theaflavin enriched extract of green tea for a period of 12 weeks. The findings indicated that there was 11.3% reduction in TC ($p=0.01$) and 16.4% in LDL ($p=0.01$), but the HDL levels and TAG levels remained unchanged (Maron et al (2003). The cardio-protective agent in tea is a monomeric flavan-3-ol called epigallocatechin gallate (EGCG). Supplementation with 100mg/kg of EGCG in hypercholesterolemic rats for 15 days significantly lowered levels of TC, LDL, TAG and increased levels of HDL (Ramesh et al 2008). Several other rat model studies have demonstrated similar hypolipidemic effect of EGCG after intervention with green, black, pu-erh tea (Huang and Lin 2012, Kim et al 2012).

Nuts

One category of foods that is rich in phytosterols is nuts. Apart from that, nuts have been reported to contain fiber and lignans, which have been implicated to exert hypolipidemic actions. A recent meta-analysis by Sabate et al (2010) reviewed and analyzed data from 25 nuts-based intervention trials conducted on 583 normolipidemic and hyperlipidemic individuals. The results indicated that an average 67g of daily nut consumption resulted in 5.1% reduction in TC, 7.4% reduction in LDL, 8.3% reduction in LDL/HDL ratio and 5.6% reduction in TC/HDL ratio, where all changes were significant at $p<0.001$. The reduction in TAG levels was 10.2%, which was significant at $p<0.05$. A recent systematic review by Grieland Kris-Etherton (2008) reviewed trials on interventions on nuts and reported that 10 of 17 studies on nuts established a reduction in LDL that was greater than that predicted using predictive equations for blood cholesterol. The predicted mean decrease in LDL for these 17 controlled feeding studies was

found to be 20.23 mmol/L, with an observed decrease of 20.29 mmol/L while comparing the nut rich diet to the control diet.

Beans

Fruhbeck, Monreal and Santidiran (1997) supplemented 40 healthy men with field bean (Vicia faba) flour, which contains bio-active compounds including saponins and complex carbohydrates, exerting hypocholesterolemic effects. The supplementation, which included 90g field bean flour per day for 30 days, resulted in significant reductions in mean LDL and VLDL levels compared to baseline ($p<0.0001$).

Oil Seeds

Flax seeds are the richest vegetarian source of alpha linolenic acid (ALA), with almost 22% of it being ALA. They are also a good source of lignans (0.2 – 13.3mg/g), and dietary fibre, which forms 28% of flax seeds by weight. Of this fiber content, one fourth is soluble fiber. Lucas et al (2011) evaluated the impact of supplementing flax seeds and flax oil in 24 ovariectomized golden Syrian hamsters for 90 days. The findings revealed that whole flax seed resulted in 12% reduction in TC while flax oil resulted in 4% reduction. The TAG and HDL concentrations did not vary much between the intervened and control groups. A departmental study by Mani et al (2011) investigated the outcome of supplementation with 10g flax seed powder 29 diabetic subjects in a controlled trial for a period of one month. The supplementation resulted in a 14.3% reduction in TC, 17.5% in TAG, 21.8% in LDL coupled with an 11.9% increase in HDL. Pan et al (2009) reported a meta analysis of 28 flax seed intervention human trials for management of hyperlipidemia. The analysis reflected that flax seeds were effective in reducing TC by 3.9mg/dl and LDL by 3.1mg/dl. The reductions were significant for whole flax seed (TC: 8.1 mg/dl, LDL: 6.2 mg/dl) but not for flax oil. Further, the hypolipidemic action was more prominent in postmenopausal females. The overall changes in TAG and HDL were not significant.

Another polyphenols-rich intervention is Nigella sativa seeds, which is found to contain tocopherols, trans-retinols, thymols, saponins, among other bio-active compounds. The impact of supplementation with Nigella sativa seeds for 28 days was investigated by Sultan et al (2011) in mouse model in a placebo controlled trial. The authors reported significant reductions TC (6.7%), TAG (4.5%) and LDL (24.8%) coupled with marginal increments in HDL fraction.

Cereal Grains and Products

Soluble fiber components β glucans, lignans, mucilage gums and ALA found in oats have also been reported to have protective role in cardiovascular health (Andersson and Hellstrand 2012), based on invitro assays, animal model and human trials, with possible mechanisms involved being antioxidant effects resisting LDL oxidation and lipid accumulation, in addition to the already establish hypolipidemic effects. Wolever et al (2012) compared the hypolipidemic action of oat β glucan in Caucasians and non Caucasians by supplementing 3-4g of β glucans in 345 mildly hyperlipidemic individuals for a period of 4 weeks. The intervention saw a 7mg/dl reduction in LDL in Caucasians which was not significantly different from the 14.3mg/dl reduction in non- Caucasians.

Departmental studies by Tuteja et al in 2003 and 2008 (unpublished M.Sc. dissertations) investigated impact of supplementation with bakery products incorporated with barley and guargum at the level of 25g barley/day and 4% guargum per product per day for a period of 6 weeks on mildly hyperlipidemic subjects. The interventions resulted in significant reduction in the TC, TAG, and LDL,

Garlic

Another popular hypolipidemic food substance that has been used in alternative healing systems is garlic. The principal lipid lowering component in garlic is claimed to be Allicin, which is the component in various active molecules in garlic including the water soluble *S*-allylcysteine (SAC) and lipid soluble diallyl

sulphides, all of which have been demonstrated to inhibit cholesterol synthesis (SAC 40-60%, diallyl sulphides 10-15%). A recent systematic review on hypolipidemic action of garlic indicated that aged garlic extract effectively reduced TC by 7% and LDL by 10% in 34 subjects with hypercholesterolemia, in a randomized placebo controlled trial (Yeh et al 1997, Yeh et al 2001).

Pectin and Polyphenols

Metzger, Barnes and Reed looked at the comparative effect of pectin, polyphenols and phytosterols in reducing the cholesterol content in hypercholesterolemic swine. It was found that all of the supplements, except pectin, reduced total cholesterol by 71 mg/dl compared to the control diet (53 mg/dl) and lovastatin (143 mg/dl) during the 8 week supplementation period. All in all, phytosterols was found to be the most effective intervention.

Departmental study by Sheth et al in 2009 (unpublished M.Sc. dissertation) evaluated the effects of pectin incorporated (10g) food supplementation on 15 dyslipidemic and diabetic subjects for 4 weeks. The results indicated significant declines in TC (1.44%, $p<0.01$), TAG (1.16%, <0.05), LDL (1.61%, $p<0.01$) and non significant reduction in FBS (1.44%).

Leaves and Grass

Leaves or grass are the part of the plant that also pack excellent amounts of antioxidant enzymes, fiber and pigment chlorophyll, among other bioactive components. A number of studies have been conducted on leaves and grass of various indigenous plants and crops in the department which have yielded promising results.

Departmental study on supplementation with curry leaves (*Murraya koenigi)* powder (12g/day) on 30 diabetic patients for a period of 1 month revealed a transient change in the fasting and post prandial blood sugar levels (Iyer et al 1990). Marginal reduction in total cholesterol was also seen. Rai et al (1997)

evaluated effect of Tulasi leaf (*Ocimum sanctum*) powder supplementation at 1% level for a 1 month on normal and diabetic rats, to find that the supplementation resulted in significant reductions in FBS, TC, TAG, phospholipids and tissue lipids in kidney and heart. Samuels et al (2002) looked into the effect of supplementation with Spirulina (1g/day) for 2 months in 23 patients with nephritic syndrome. The results reflected a significant decline in TC (by116.3mg/dl), LDL (by 94mg/dl) and TAG (by 67mg/dl).

Nambiar et al (2010) investigated the impact of drumstick leaf (*Moringa oleifera)* tablets supplementation (4.6g/day) in 20 hyperlipidemic subjects for a period of 50 days, and found a significant reduction in non-HDL values and an overall favorable impact on the lipid profile. Kumar et al (2010) studied the benefits of *Gymnema sylvestre* supplementation (500mg/day) for 3 months among diabetic subjects. The authors reported reduction in polyphagia, fatigue, blood glucose and glycated hemoglobin following the intervention. The results also indicated favorable shifts in the lipid profile of the subjects. Venugopal et al (2010) investigated the role of subatmospheric dehydrated barley grass (*Hordeum vulgare*) supplementation (1.2g/day) on 59 stable diabetic subjects for a period of 2 months. The findings indicated significant declines in the FBS, HbA1c, TC, LDL, non-HDL and a significant increase in HDL levels following the intervention.

Thus, it can be seen from the literature on natural product interventions which contain bioactive molecules, that, natural food and plant based products can exert clinically relevant reduction in serum cholesterol levels in hyperlipidemic, mildly hyperlipidemic and even in normolipidemic subjects.

One such plant based intervention is Wheatgrass, which has been purported to contain many diverse antioxidant, hypolipidemic, hypoglycemic, anti inflammatory, anticarcinogenic properties.

WHEATGRASS (*TRITICUM AESTIVUM* L.) – THE WONDER HERB OF AYURVEDA

HISTORY

The domestication of wheat has been speculated to have occurred in the fertile crescent of the Middle East nine thousand years ago (Simmons 1987). However, certain accounts describe wheat cultivation in Indian subcontinent as early as 9000 BC (Gupta 2004). The oldest documented references to use of wheat for therapeutic uses, has been in ancient 2800 year old Indian texts on surgery and medicine, the Sushruta Samhita and Charaka Samhita, written around 800 and 600 BC respectively (History of Medicine 2013). The ancient Indian system of healing, the Ayurveda, believed wheat to be effective in treatment of gastrointestinal disorders, among others. The reference to wheat (called *'Godhuma'* in Sanskrit) can be found in the following verse 21 from chapter 27 of the first Section '*Sutrasthana'* (meaning 'Summary')

सन्धानकृद्वातहरो गोधूमः स्वादुशीतलः
जीवनो बृंहणो वृष्यः स्निग्धः स्थैर्यकरो गुरुः २१

The above verse denotes the properties of wheat to be a stabilizer and that alleviates disorders of *vaatha*, which include heart disorders.

TAXONOMY

The grass of common wheat plant (*Triticum aestivum L*.) is an annual grass, and taxonomically belongs to the family *Poaceae*, subfamily *Pooideae* and tribe *Triticeae*. It survives a wide range of conditions and hybridizations on account of its hexaploid nature (6x), as opposed to grains like barley which are diploid (Department of Health and Ageing 2008). It grows to be an erect, hollow, flat,

narrow grass. The spikes are generally long, slender and dorsally compressed and more or less flattened.

CHEMISTRY

It is a popular traditional belief that eating wheatgrass confers the benefits of consuming large amounts of vegetables in a day. The composition of wheatgrass accounts for this notion: 3.5g of wheatgrass itself has 15mg chlorophyll, 1g dietary fibre, 1mg Lutein and 29mcg Lycopene, 2-8% RDA of all essential amino acids. Wheatgrass has been shown to exhibit excellent antioxidant properties as well (Kulkarni et al 2006).

Wheatgrass is found to be rich in all major three classes of bioactive compounds: Phytosterols, Viscous Polysaccharides and Polyphenols. Phytosterols, namely beta-sitosterol, campesterol, and stigmasterol were found in hexane extracts of wheatgrass, with beta-sitosterol accounting to 74% of the total phytosterols in the extract, which ranged from 834-1206 mg/kg (Dunford, Irmak and Jonnala 2009, Dunford and Edwards 2010). Polyphenol tests revealed the presence of flavonoids, triterpenoids, anthranol, alkaloids, tannins, saponins and sterols in fresh grass juice (Kothari et al 2011).Aqueous extracts of wheatgrass were found to contain gums and mucilages also, which belong to the family of viscous polysaccharides (Shirude 2011).

It has been found that wheatgrass has a lysine arginine ratio of 0.7, considered to be low compared to animal protein, with the value for casein being 1.2; and also a low methionine content of 15mg per 3.5g of wheatgrass, which is abysmally low compared to 86mg of 100ml cow's milk or other proteins of animal origin. A low lysine-arginine ratio and low methionine content have been found to exert hypocholesterolemic effects (Kritchevsky 1979).The underlying mechanisms seem to be reduced absorption of cholesterol, increase in glucagon secretion and inhibition of insulin production (Sanchez 1991). The other mechanism can be suppressing the HMG CoA reductase and 7-α-hydroxylase

activities through regulating hepatic glutathione concentrations (Potter and Kies 1990).

PHYSIOLOGICAL EFFECTS OF WHEATGRASS

Effects on Hematological Parameters

Wheatgrass has been extensively researched for its ability to improve iron status in patients with Thalassemia and anemia**.** Marwaha et al (2004) studied the impact of 10ml wheatgrass juice supplementation on 38 B-thalassemia major patients for a period of 6 months to find that 50% of the patients experienced beneficial effects on transfusion treatments. Mukhopadhyay et al (2009) investigated the impact of 30ml fresh wheatgrass juice supplementation on 200 thalassemia intermedia patients for 6 months and found that wheatgrass juice was an effective alternative to blood transfusion in patients with thalassemia intermedia. Singh et al (2010) evaluated the effect of wheatgrass tablets (2-8 tablets/ day) on children suffering from thalassemia major. The study saw increases in the Hb, interval between blood transfusions and decline in the amount of blood transfused. Choudhary et al (2009) on the other hand, observed no beneficial effect of 100mg/kg wheatgrass tablets for 6 months on experimental rats with B-thalassemia major.

Apart from this, departmental research by Sharma et al in 2001 (unpublished M.Sc. dissertation) investigated the impact of 100ml wheatgrass juice supplementation on 80 adult women for a period of 30 days and found a significant increase 0.85 g/dl ($p<0.05$) in the mean hemoglobin levels of the supplemented group.

Effects on Cancer

On account of it antioxidant properties, some research has also gone into the cancer alleviating properties of wheatgrass. Bar Sela et al (2007) supplemented

16ml of wheatgrass juice on 60 breast cancer patients taking chemoptherapy on the first 3 cycles of the chemotherapy sessions, and found that the intervention reduced myelotoxicity and resulted in decrease of the dosage of chemotherapy in the subjects. Wheat et al (2006) studied the impact of supplementation with wheatgrass extract on breast cancer patients undergoing radiotherapy and observed that it reduced the skin toxicity which occurred as an offshoot of radiotherapy. Dey et al (2006) investigated the impact of wheatgrass juice supplementation on 400 cancer patients ranging from lung cancer, to breast cancer, to esophageal, to colon to ovarian to hepatocellular carcinomas. The patients were supplemented with 30ml fresh wheatgrass juice everyday for a period of 6 months. The authors reported a 20% improvement in the Karnofsky performance scores in the patients, after the supplementation. The hemoglobin, total protein and albumin levels improved significantly ($p<0.05$) and the patients requiring blood transfusion at the end were drastically reduced.

Antioxidant and Anti-Inflammatory Effects

The anti-inflammatory effect of wheatgrass can be attributed partly to the presence of beta sitosterol which has been found to exert protective effects against endothelial inflammation. Specifically, beta-sitosterol has been found to prevent inflammatory changes by suppressing vascular adhesion molecule 1 and intracellular adhesion molecule 1 expression in Tumor Necrosis Factor alpha (TNF-α)-stimulated human aortic endothelial cells in addition to inhibiting binding of U937 cells to TNF-α-stimulated human aortic endothelial cells. It also attenuates the phosphorylation of nuclear factor-kappa B (Loizou 2010). Ben-Arye et al (2002) investigated the effect of 100ml wheatgrass juice supplementation on 23 ulcerative colitis patients for a period of 1 month. The results reflected a positive impact of the supplementation on inflammation in the colon, with reductions in rectal bleeding and disease severity index.

Pant et al (2013) investigated the effect of wheatgrass supplanted with 10mg wheatgrass powder per 10ml diet on Drosophila melanogaster flies for one

lifecycle of the fly till mortality. The authors reported decreased SOD and Catalase activity in the flies after wheatgrass supplementation, indicating decreased oxidative stress. The effects of wheatgrass on oxidative stress can be attributed to its high antioxidant activity as reported by Kulkarni et al (2006). Wheatgrass extracts have been found to significantly inhibit lipid peroxidation induced by ascorbate and Fe2+ in liver mitochondria in rat model and its free radical scavenging ability is reported to be higher than those of many natural extracts or vegetables, as indicated by ORAC values of 39.9 and 48.2 for aqueous and ethanol extracts respectively. The antioxidant activity, reported in terms of FRAP values, were found to be 0.463 and 0.573 mmol of ascorbic acid and Trolox equivalents/100 g fresh wheatgrass, for aqueous and ethanol extracts respectively. Shyam et al (2007) investigated the efficacy of 500mg wheatgrass for 30 days in attenuation of oxidative stress in adult subjects. The findings reflected that wheatgrass supplementation resulted in significant decline in the malondialdehyde levels ($p<0.05$), which is a marker for oxidative stress; and a parallel increase in the antioxidant ascorbic acid levels and superoxide dismutase levels.

Hypolipidemic and Hypoglycemic Effects

Results from a recent mouse model study (Kothari et al 2011) on wheatgrass were similar to found in this research, wherein wheatgrass juice was administered at 5 mL/kg and 10 mL/kg in hypercholesterolemia induced Wistar rats for a period of 14 days. The supplementation resulted in dose dependent significant ($p<0.05$) decline in TC, TAG, LDL, VLDL and FBS levels. The researchers also looked at the fecal cholesterol excretion which was significantly enhanced ($p<0.05$) upon wheatgrass supplementation.

Another study in rabbit model (Das, Hakim and Mittal 2012) evaluated the effect of ethanol extract of wheatgrass hyperlipidemic as well as normal animals. The experimental animals were fed 500mg/kg/day of wheatgrass extract orally for a period of 12 weeks, after which the authors found a significant ($p<0.05$) decline in

the serum FBS, TC, TAG, LDL and MDA levels of the animals in both the normal and hypercholesterolemic groups. Interestingly, the HDL cholesterol had increased in the normal group but decreased in the hypercholesterolemic group. In the present study too, the supplemental group, all of whom were hypercholesterolemics, saw a decline in the HDL levels.

Experiments on the glycemic and lipemic index of wheatgrass containing recipes done in the department (Iyer et al 2010) have reported that incorporation of wheatgrass into recipes reduced the glycemic index and the TAG level response of the recipes as compared to without addition of wheatgrass.

Acceptability trials of wheatgrass incorporated recipes have been conducted in the department. Iyer et al in 2001 (unpublished M.Sc. dissertation) evaluated the acceptability of five common Indian recipes (*Paratha, Dhebra, Cutlet, Samosa* and *Muthia*) incorporated with fresh wheatgrass at the level of 10g, 15g and 20, using sensory evaluation employing 5 point hedonic rating scale after. The results revealed that both 10g and 15g levels were equally acceptable, however, at 20g the mean scores decreased slightly, indicating a maximum of 15glevel of incorporation using fresh wheatgrass. Apart from this, another acceptability trial in the department involving a health drink incorporating freeze-dried wheatgrass and other antioxidant foods: gooseberry and cocoa and cereal pulse combination, was carried out by Iyer et al (2011) and it was found that till the level of 0.5g per 100ml, incorporation of freeze dried wheatgrass powder was quite acceptable and did not adversely affect the sensory attributes, or result in any gastrointestinal or allergic problems on consumption for 14 days.

The cardio-protective effects and beneficial effects on inflammation have been summarized in Table 2.5.

TABLE 2.5 EFFECTS OF WHEATGRASS ON CARDIOMETABOLIC HEALTH & CANCER

Author, Year	Duration	Subjects	Form	Dosage	Effects
Hyperlipidemia					
Kothari et al 2011	14 days	Hyper-cholesterolemic rats	Juice	5ml/kg, 10ml/kg per day	TC ↓, TAG ↓, LDL ↓, VLDL ↓, FBS ↓
Das et al 2012	12 weeks	Hyperlipidemic rabbits	Ethanol extract	500mg/kg per day	TC ↓, TAG ↓, LDL ↓, MDA ↓, FBS ↓, HDL ↑
Shirude 2011	14 days	Hyperglycemic rats	Juice	100mg/kg per day	FBS ↓
Kothari et al 2008	21 days	Normolipidemic rats	Juice	5ml/kg, 10ml/kg per day	TC ↓, TAG ↓, LDL ↓, VLDL ↓, HDL ↑
Oxidative Stress					
Pant et al 2013	1 lifecycle	Drosophila flies	Powdered	10mg per 10ml diet per day	SOD ↓, Catalase ↓
Shyam et al 2007	30 days	30 healthy adults	Powdered	1g	Vit C↑, MDA ↓, SOD ↑
Inflammation					
Ben-Arye et al 2002	1 month	23 ulcerative colitis patients	Juice	100ml per day	↓ rectal bleeding, ↓ disease severity
Cancer					
Bar Sela et al 2007	3 sessions of chemo-therapy	60 breast cancer patients	Juice	16ml per day	Chemotherapy dosage ↓, myelotoxicity ↓
Wheat et al 2006	1 session of radio therapy	Breast cancer patients	Aqueous extract	25ml per day	Skin toxicity↓
Dey et al 2006	6 months	400 cancer patients	Juice	30ml per day	Hb↑, Total Protein↑, Albumin↑

Thus it is evident that wheatgrass is a treasure trove of bioactive molecules that exert diverse health benefits, as seen in experimental, molecular and animal model studies. It is also apparent that there are no human trials in this regard to establish the above reviewed benefits of wheatgrass in humans.

SUMMARY

- Menopause endocrinology and physiology, alters the metabolic equilibrium in female biology
- The changes in metabolic and cardiovascular systems puts women at increased risk of clinic biochemical changes occurring in the cardio-metabolic systems
- The epidemiological trends confirm that the burden of metabolic and cardiovascular risk condition, which are more often that not, the consequences of menopausal changes, is high in Indian population.
- The problem that was most commonly seen in Indian middle aged women was found to be aberrations in the lipoprotein fractions (dyslipidemia)
- The prevalence of menopausal symptoms also runs high in Indian population
- Food and plant based interventions that contains bio-active phytonutrients and nutraceuticals compounds, have resulted in favorable changes in the lipoprotein distribution in dyslipidemic populations.
- Wheatgrass has been reported to contain a wide variety of nutraceutical molecules that have been implicated to exert hypolipidemic effects in animal model studies.

RATIONALE

The different level of saturation of risk factors in women, together with their interaction with female hormones, plays an important role in the development of cardiovascular disease; and given that middle women form a sizeable part of the Indian demography, the health expenses incurred towards chronic disease

alleviation by this huge segment of the population would be a cause of grave concern for the stake holders. However, to sketch conclusive decisions on the interventions and the extent of coverage, comprehensive studies spanning the complete picture of the metabolic and cardio-vascular risk factors across a significant part of the Indian population is a pre-requisite.

But in this regard, most of the studies are on the western population and data in the regional context is lacking. Moreover, the review suggests that Indian studies even though documented, are scattered and do not provide an all-encompassing portrait of the situation.

In this context, a wide range of nutraceuticals and functional foods have been tried as has been reviewed, but discreetly designed trials on the Indian ethnic population groups are scarce and fail to provide any conclusive evidence. On the other hand, the benefits of the wonder herb of Ayurveda- Wheatgrass has been scientifically shown to possess a variety of vitamins, essential minerals, phytochemicals, antioxidants and other bioactive molecules which render wheatgrass to be a promising natural substance to be considered for reducing serum cholesterol and lipid peroxidation due to oxidative stress. Therefore, a scientifically designed trial in this regard is justified to separate myths from facts and to assess whether wheatgrass can be promoted as a functional food for the management of hyperlipidemia.

Hence a need was felt to undertake a set of studies which would address all these queries and the details of the research questions addressed therein are described in the subsequent section.

METHODS AND MATERIALS

Escalating chronic disease burden in women has links to complex underlying metabolic and endocrine disturbances. On one hand, there is plausible biological evidence about ovarian steroids having a protective effect on not only the cardiac health but a host of other body systems in a woman. Also menopause (mainly loss of the ovarian steroids due to decline in ovarian function) has been observed to disrupt the harmony in the cardio-metabolic and related systems. On the other hand, the disturbingly high statistics of cardio-metabolic events occurring in women worldwide raises a concern to address it promptly with utmost gravity*.* Hence this research was undertaken for studying the clinico-biochemical derangements in middle-aged menopausal women; describing the major risk factors, identifying most pressing risk factor and furnishing a natural remedy for its management. The initial formative research paved the way for identification of the most prevalent problem (hyperlipidemia) and the subsequent intervention research was directed at the management of this problem.

The outline of the study and the detailed experimental design are given below.

PHASES IN THE STUDY

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 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.

DETAILED EXPERIMENTAL DESIGN

I. FORMATIVE RESEARCH: CLINICO-BIOCHEMICAL CHANGES IN PRE-PERI AND POST MENOPAUSAL WOMEN IN A] FREE-LIVING POPULATION IN VADODARA AND B] THOSE ATTENDING A HEALTH CHECK-UP FACILITY IN AHMEDABAD.

DESIGN: By employing a cross-sectional & analytical study design, the formative research was conducted to study the distribution of clinico biochemical changes across pre, peri and post-menopausal women, with regard to the presence of cardio-metabolic risk factors. The experimental plan for Part A of Phase I is shown in Figure 3.1 and part B is shown in Figure 3.2. The study design was approved by the Institutional Ethics Review Committee of the Department of Foods and Nutrition, The Maharaja Sayajirao University of Baroda (No. Fc Sc/FND/ME 59 dated 30/9/2010).

SAMPLE-SIZE ESTIMATION: Since the objective of the present study was to study a number of major risk conditions, the sample size was estimated using the prevalence of metabolic syndrome, which represents clustering of major risk factors. Recent cross-sectional studies in literature conducted on Indian population suggest that the prevalence of metabolic syndrome is 25%. The sample size was determined employing the following formula (Daniel 1999):

Figure 3.1 EXPERIMENTAL DESIGN FOR PHASE I- PART A

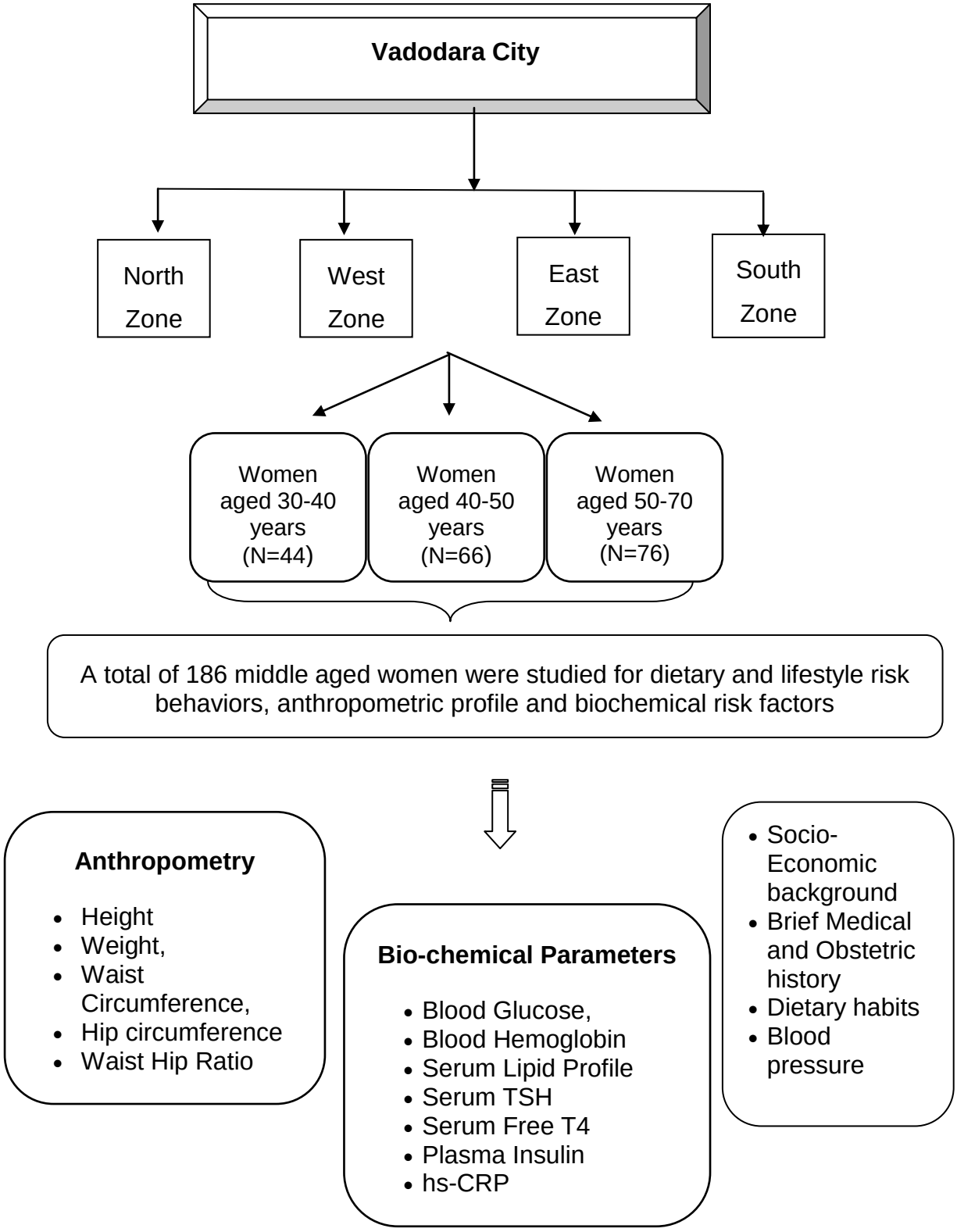
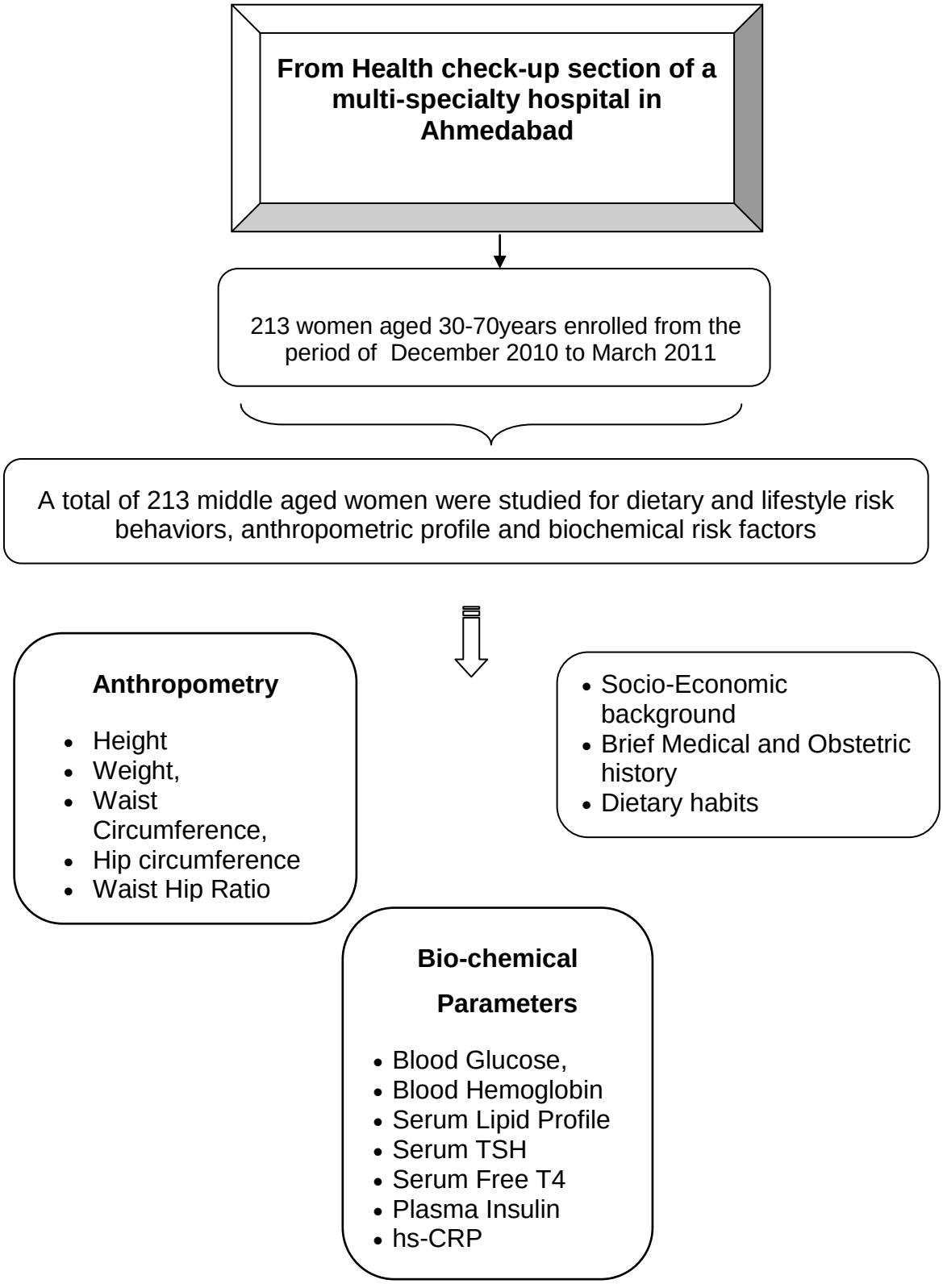


Figure 3.2 EXPERIMENTAL DESIGN FOR PHASE I - PART B



$$n = \frac{Z^2 \times p(1-p)}{d^2}$$

Where, n=estimated sample size,

Z=confidence interval, which was taken as the Z-score corresponding to 95% on the Gaussian distribution curve, therefore, Z=1.39,

P=reported prevalence of the condition under study (obesity-50%, MS-25%), d=error of estimation/ precision, which was taken as 0.05.

Fitting the above values into the equation, gave the sample size to be 288.

PART A - WOMEN FROM FREE-LIVING POPULATION IN VADODARA

SUBJECTS AND SAMPLE SELECTION

Thus, to study both the problems of concern described above and numerous other cardio-metabolic risk factors, a sample of 399 women aged between 30 and 65 years of age were enrolled for this phase, which was conducted in two parts: Part A and Part B.

In Part A, 186 women (44 women in the age group of 30-40 years, 66 women in the age group of 40-50 years and 76 women in the age group of 50-70 years) were enrolled from free-living population during the time span of October 2009 to April 2010 from each four zones of Vadodara city namely, north zone, south zone, east zone and west zone (zonal map outlined in Appendix I).

The information about the enrollment in the study was passed on employing the snowballing technique, wherein one or couple of individuals in each zone of the city was informed about the enrollment of subjects for the study. The potential subjects were notified that the incentives for participation in the study included a free health check-up at their households and provision of

the health report following the health check up. It was made clear to the subjects that they would not incur any cost for participating in the study. All the consenting individuals were asked to read and sign the consent form (Appendix II), those who were not able to read, were explained clearly, the objectives of the study, the information required to be provided by the subject upon enrollment, the fact that a fasting blood sample will be required and that it will be done using safe disposable syringes by a trained lab technician. When the required number of subjects from a particular zone was achieved (roughly 50), the enrollment was stopped.

Data Acquisition Process

Once the subjects were enrolled after obtaining the written consent, an appointment was scheduled for one or two hours as per their convenient time and during this allotted time period, the collection of information pertaining to socio-economic status, medical obstetric history, dietary habits & intake, lifestyle habits, physical activity, anthropometric measurements and blood pressure measurements were conducted at the subjects' place of residence. Following this, another appointment was scheduled for drawing of blood for biochemical estimations within the next few days, which was also done at the subjects' place of residence. The subjects were adequately informed about the level of fasting required (12 hours) before the blood sample was to be drawn and information on what all fluids were allowed to be ingested during the fasting hours was also provided. On the day of the scheduled appointment, a trained lab technician was taken along with the researcher during the morning to obtain a fasting blood sample from the subject, while care was taken to see that the blood sample was maintained at low temperature during the transportation till it reached the lab. The disposable syringe used for the collecting the blood sample was disposed off immediately after use.

Parameters Studied

The parameters that were studied included biochemical ones, biophysical, physical and reported data. These were as follows

1. Reported Data

- Information on socio-economic status
- Medical obstetric history
- Dietary habits & intake
- Lifestyle habits
- Physical activity

2. Physical Parameters

- Height
- Weight
- Waist circumference
- Hip circumference

3. Bio-physical Parameters

- Blood pressure
- Bone Mineral Density

4. Biochemical Parameters

- Blood Hemoglobin
- Plasma Glucose
- Serum Lipid profile
- Serum Thyroid Stimulating Hormone (TSH)
- Serum Free Thyroxine (FT4)
- Plasma Insulin

The methodological details of data collection techniques and the parameters studied are explained in the detailed **Methods, Tools and Techniques** section on page number 121.

PART B - WOMEN ATTENDING A HEALTH CHECK-UP FACILITY IN AHMEDABAD

In part B, the formative research was conducted in a clinical setting as opposed to part A where the subjects were from a free living population. The clinical site here was the health check-up facility of the multi-specialty 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. After being explained the objectives and nature of the research and the information that they will be required to divulge for the purpose of the research, the potential participants were asked for their consent in writing (Appendix III) for enrolling in the study.

DATA ACQUISITION PROCESS

The consenting people were interviewed in the hospital premises itself for obtaining reported data and the measurements for the physical and biophysical parameters under study were done on the same day. The subjects then provided a fasting blood sample in the laboratory associated with the health check up section for estimation of the biochemical parameters. On an average, 5 females had OPD appointments daily and the average response rate was 83.3%. In total, we interviewed 213 subjects aged 20-65 years.

PARAMETERS STUDIED

The parameters that were studied included biochemical ones, biophysical, physical and reported data. These were as follows

1. **Reported Data**

    - Information on socio-economic status
    - Medical obstetric history
    - Dietary habits & intake
    - Lifestyle habits
    - Physical activity

2. **Physical Parameters**

    - Height
    - Weight
    - Waist circumference
    - Hip circumference

3. **Bio-physical Parameters**

    - Blood pressure

4. **Biochemical Parameters**

    - Blood Hemoglobin
    - Fasting Plasma Glucose
    - Post Prandial Plasma Glucose
    - Serum Lipid profile
    - Serum Thyroid Stimulating Hormone (TSH)
    - Plasma Insulin

The methodological details of data collection techniques and the parameters studied are explained in the detailed **Methods, Tools and Techniques** section on page number 121.

II. FOLLOW-UP STUDY: THE IMMEDIATE AND LONGITUDINAL OUTCOMES OF THE HEALTH CHECK-UP ON WOMEN'S HEALTH-SEEKING 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.

Parameters Studied

The parameters that were studied included biochemical ones, biophysical, physical and reported data. These were as follows

1. **Reported Data**
    - Information on the action taken after the subjects got the results of the health check up
2. **Physical Parameters**
    - Height
    - Weight
    - Waist circumference
    - Hip circumference
3. **Bio-physical Parameters**
    - Blood pressure

The methodological details of data collection techniques and the parameters studied are explained in the detailed **Methods, Tools and Techniques** section on page number 121.

III. TRANSLATIONAL RESEARCH: EVALUATION OF NUTRITIVE VALUE OF WHEATGRASS POWDER, PRODUCT DEVELOPMENT BY INCORPORATION OF WHEATGRASS POWDER IN SELECTED INDIAN RECIPES AS A FUNCTIONAL FOOD AND EVALUATION OF ACCEPTABILITY OF THESE PRODUCTS

Freeze-dried nitrogen packed wheatgrass powder was procured from an exporting firm based in Vadodara. The nutrient component analysis (conducted by Analytical & Environmental Sciences, Vadodara) included quantitative testing of energy, protein content, total fat, fibre, iron, moisture, ash, carbohydrate & sugar content, ascorbic acid, and β carotene, the methodological details of which have been described in the Methods, Tools and Techniques section on page number 121.

Product development was carried out by incorporating freeze-dried wheatgrass powder in selected Indian recipes The various recipes tested included Khakhra, Thepla, Muthiya, Dal and Buttermilk. All the recipes were standardized first (Appendix V) and then 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. Except Khakhra, Thepla & Muthiya, rest of the recipes did not involve heat application to wheatgrass powder in order to serve the objective preserving the heat-sensitive antioxidant compounds in the freeze-dried wheatgrass powder. Method of preparation of Khakhra involved roasting, with minimal amount of water content, Thepla was also prepared by roasting, but had higher water content than Khakhra; whereas Muthiya was steam cooked.

Following the product development, all the developed products were evaluated for their acceptability depending on the sensory attributes, by conducting a sensory evaluation using composite rating test (Appendix VI). The evaluation panel included 12 semi-trained members comprising of post-graduate and doctoral students of the department. The procedure required the panel members to be present for the testing atleast an hour after the previous meal. The evaluation criteria for all the products included aroma, appearance, flavor, color, aftertaste, texture and overall acceptability. To rate the products, the panelists were asked to score each product on a 10 point scale based on degree of liking/dislike for the attributes of the product.

IV. INTERVENTION RESEARCH: IMPACT OF WHEATGRASS POWDER SUPPLEMENTATION ON LIPOPROTEIN STATUS IN PRIMARY HYPERLIPIDEMIC WOMEN – AN OPEN LABEL STUDY

The intervention was targeted at alleviating hyperlipidemia which was found to be the most prevalent problem among the women studied in the formative research phase.

DESIGN: The study was conducted using an open label randomized design. The study had a control group, which was not given any intervention and an experimental group which was given the intervention: wheatgrass capsules; and the data was collected prior to and after the intervention (pre and post data). The study design was approved by the Institutional Ethics Review Committee (No. Fc Sc/FND/ME 158 Dated 30/9/2010)

PREPARATION OF THE TREATMENTS

***Source of Wheatgrass*:** Freeze-dried wheatgrass powder (subjected to dehydration at 0-5°C and ground using cold water jacketing) was procured from Aum Agri Freeze Foods, a local exporting firm based in Vadodara. The

powdered wheatgrass was nitrogen-packed so as to ensure minimal moisture accumulation and contamination.

***Encapsulation*:** The powdered wheatgrass was encapsulated into 350mg gelatin capsules of size 0, courtesy Centurion Laboratories, Vadodara. Prepared capsules were hermetically packed in sterile plastic jars, each having a capacity to contain 100 capsules.

Sample-Size Estimation. Since the intervention was targeted at management of hyperlipidemia, the major outcome that was used to estimate the required sample size was the mean triacylglycerol levels. The targeted percent change in TAG levels was taken as 15%, attrition rate was considered 20% and the standard deviation in the TAG levels in the population was 45mg/dl. With the above mentioned specifications and at 80% power, the sample size was estimated using an online statistical model (Length 2006-2009) adapted for an open label controlled trial and it came out to be 28 in each arm.

Subjects and Sample Selection: For the supplementation study the major inclusion criteria was the subject to be a primary hyperlipidemic, therefore the subjects were to be selected from a population of primary hyperlipidemic women. For this reason, the subjects who were found to be hyperlipidemic in the formative research phase were approached for this trial, in addition to other women who had previously been found to have either high TC or high TAG levels subjects, were approached for this trial. All in all, there were 78 women who were identified as being mildly hyperlipidemic in the formative research phase. The potential participants were explained the nature and purpose of the research in a language comprehensible to them, and also the risks and benefits involved in the research, following which a written consent (Appendix IV) was obtained from those willing to participate. They were also explained clearly the fact that

they would not incur any costs for participating in the trial. There were 69 women who gave consent for participation in the study, rest 9 did not. The consenting women were subject to scrutiny by following a set of inclusion and exclusion criteria (Figure 3.4). Those meeting the inclusion criteria & not falling in the exclusion criteria were enrolled in the study, which included 64 women. After enrollment of the subjects, the baseline blood tests were conducted to ascertain hyperlipidemia, following which, 61 subjects were still hyperlipidemic. After this the subjects were randomized into the two study groups: control and experimental group. Randomization was carried out by following the chit method, the specifics of which have been delineated by Giesbrecht and Gumpertz (2004). Following the baseline data collection, 61 women were confirmed to be still mildly hyperlipidemic and after randomization, 31 women were allotted to the experimental group and 30 into the control group.

Data Acquisition Process

Data for all the parameters studied was collected in a fashion similar to the formative research phase. After the enrollment, an appointment was scheduled for one or two hours as per a time convenient to the subjects' time and during this allotted time period, the collection of information pertaining to socio-economic status, medical obstetric history, dietary habits & intake, lifestyle habits, physical activity, anthropometric measurements and blood pressure measurements were conducted at the subjects' place of residence. The collection of blood sample for biochemical estimations was done at another visit which was scheduled within the next 2-3 days of the first visit, & was done at the subjects' place of residence. As with the drawing of the previous blood samples, this time too, the subjects were adequately informed about the level of fasting required (12 hours) before the blood sample was to be drawn and information on what all fluids were allowed to be ingested during the fasting hours was also provided. A trained

lab technician collected the venous blood samples from the subjects in the presence of the researcher on the morning of the scheduled appointment and care was taken to see that the blood sample was maintained at low temperature during the transportation till it reached the lab. The disposable syringes used for drawing the blood samples were disposed off immediately after use. The detailed experimental plan, sample selection process and the inclusion criteria for the supplementation study are shown in Figures 3.3, 3.4 and 3.5.

Parameters Studied

The parameters that were studied included biochemical ones, biophysical, physical and reported data. These were as follows

1. Reported Data

- Medical obstetric history
- Dietary habits & intake
- Lifestyle habits
- Physical activity

2. Physical Parameters

- Height
- Weight
- Waist circumference
- Hip circumference

3. Bio-physical Parameters

- Blood pressure

FIGURE 3.3 SELECTION OF SUBJECTS FOR THE WHEATGRASS SUPPLEMENTATION STUDY

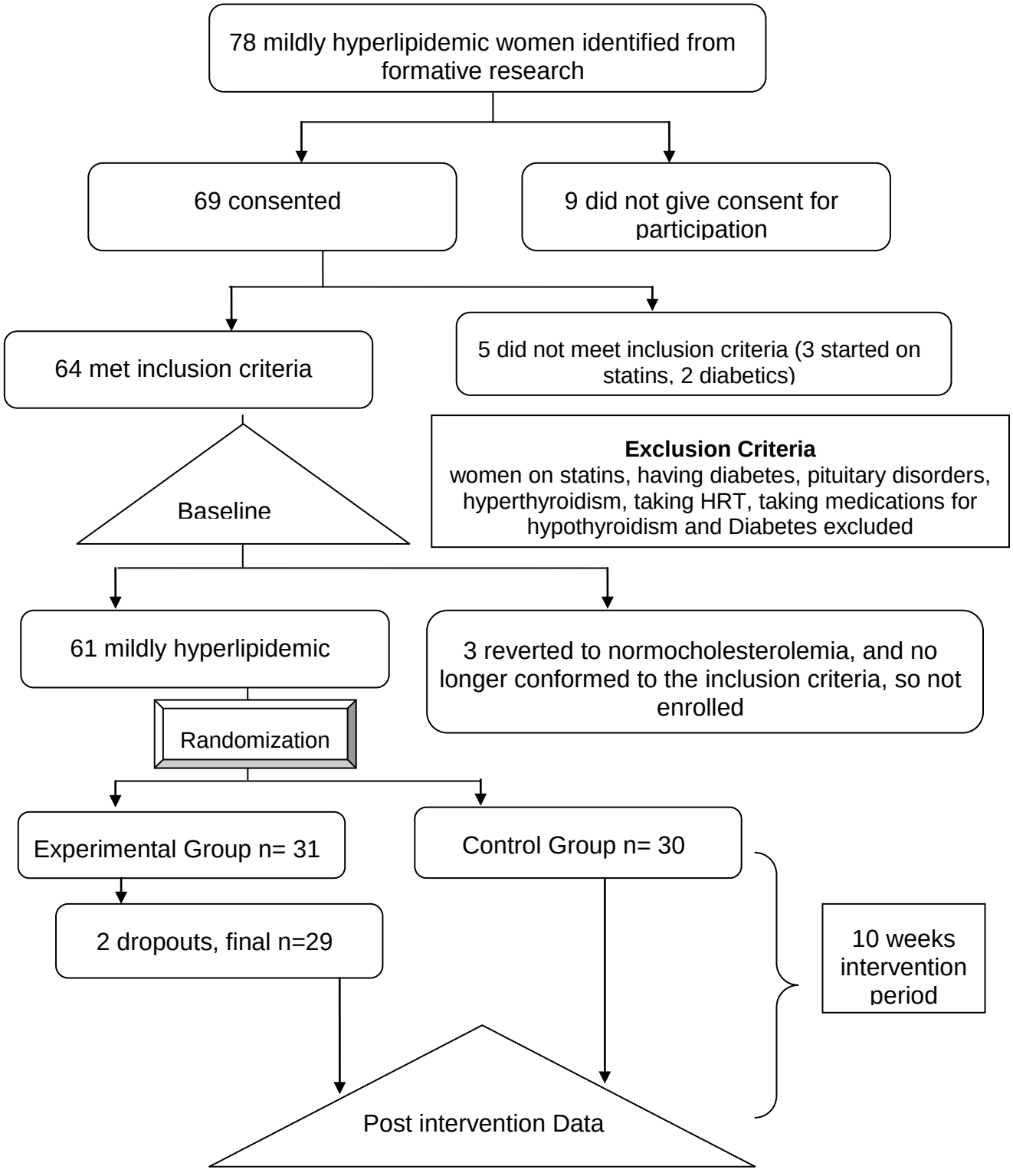


FIGURE 3.4 INCLUSION AND EXCLUSION CRITERIA FOR THE SUBJECT SELECTION FOR THE WHEATGRASS SUPPLEMENTATION TRIAL

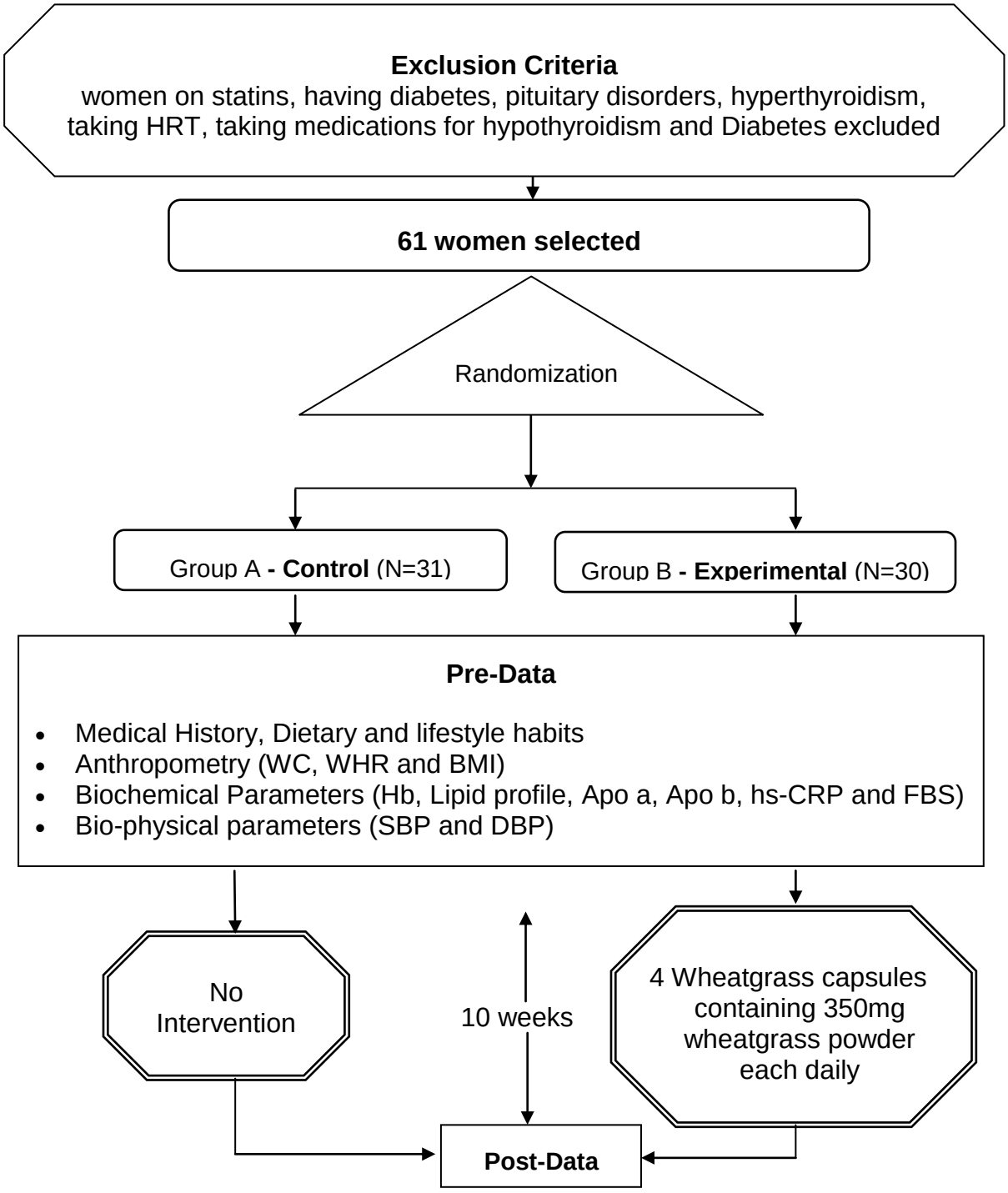
Inclusion Criteria

- Females
- Age between 30-60 years
- Hyper-cholesterolemic defined either by serum TAG ≥200mg/dl and/or serum AG≥150mg/dl and/or serum LDL≥100mg/dl
- Willingness to participate in the trial

Exclusion Criteria

- Diabetics
- Taking Hormone Replacement Therapy
- Statins initiated <3 months before trial
- Pituitary disorders
- Hypothyroidism
- Genetic traits of Hyperlipidemia
- Polycystic Ovarian Disease (PCOD)
- Not willing to participate in the trial

FIGURE 3.5 EXPERIMENTAL DESIGN FOR THE OPEN LABEL RANDOMIZED CONTROLLED SUPPLEMENTATION STUDY WITH WHEATGRASS CAPSULES IN PRIMARY HYPERLIPIDEMIC WOMEN



4. Biochemical Parameters

- Blood Hemoglobin
- Plasma Glucose
- Serum Lipid profile
- Serum Apo A
- Serum Apo B
- Serum hs-CRP

The methodological details of data collection techniques and the parameters studied are explained in the detailed **Methods, Tools and Techniques** section on page number 121.

TABLE 3.1 METHODS AND PARAMETERS FOR DATA COLLECTION

	Conditions & Parameters Studied	Data Collected	Tools and Techniques used
Bio-physical			
1	Overweight/Obesity, Abdominal Obesity	Height, weight, Waist Circumference (WC)	Non-elastic fiberglass measuring tape, Salter research grade weighing scale
2	Hypertension	SBP, DBP	Sphygmomanometer
3	Osteoporosis	Bone Mineral Density at Calcaneus bone (quantitative Ultrasound-qUS)	Achilles EXP III (Lunar, General Electric, China) Ultrasonometer
Bio-chemical			
4	T2DM and Insulin Resistance	Fasting plasma Glucose and Plasma Insulin	Glucose Oxidase, Chemiluminescent assay
5	Dyslipidemia	Serum Fasting Lipids, Apo a, Apo b	Cholesterol oxidase, HDL direct precipitation, Friedewald equation, Nephelometry
6	Anemia	Blood hemoglobin	Cyanmethemoglobin
7	Sub-clinical Hypothyroidism	Serum Fasting TSH & FT4	Sandwich Chemiluminescent assays
8	Arterial Inflammation	Serum hs-CRP	Nephelometry
Dietary and Lifestyle Factors			
9	Dietary & Lifestyle Risk Behaviors	Semi structured pretested questionnaire	One to one Interview
10	Food Intake	24hour dietary recall method	
11	Nutrient Intake	Calculated from food intake	Nutritive Value of Indian Foods' Food Composition Tables

METHODS, TOOLS AND TECHNIQUES

A. BACKGROUND INFORMATION, MEDICAL HISTORY AND DIETARY & LIFESTYLE HABITS

Information pertaining to demographic details, medical history, use of medication, family history of chronic diseases, dietary practices, physical activity patterns and smoking status was elicited from each participant in an interview method using a pretested semi-structured questionnaire (Appendix VII). Physical activity level was assessed using an open ended questionnaire which was modified from the Behavioral Risk Factor Survey following standardization and pre-testing. The questions collected information on the number of days per week that the subjects devoted to physical activity and also the duration of activity per day were obtained.

All the questions were asked either in English, Hindi or Gujarati. Collection of all the data including standardized cardiometabolic risk factor screenings such as height, weight, BMI, WC, blood pressure, and estimations of blood hemoglobin, HDL cholesterol, triglycerides, fasting plasma glucose, serum thyroid stimulating hormone, plasma insulin and serum creatinine were carried out by trained health care professionals, including the researcher, who collected all the non-invasive data. The biochemical examinations were carried out at the national accredited diagnostic lab facility, Thyrocare.

B. BODY COMPOSITION PARAMETERS

1) Height and Weight

Height was measured by a non-elastic fiber-glass tape. Body weight was taken by research-grade, portable Salter scales standardized using 5 kg standard weights and readings were recorded to the nearest 0.1kg. BMI

was calculated directly by the standard formula: weight (kg)/height (m^2). Then the patient's Body Mass Index (BMI) was computed with the standard formula: BMI = Weight (kg)/ Height (m^2)

For defining overweight and obesity the Asia-Pacific high cut-off points delineated by the WHO expert consultation (2004) were used in this study (Table 3.2).

2) Waist and Hip Girth

The waist and hip circumferences (WC and HC) were measured with a non-elastic fiberglass tape by trained examiners using a standard protocol (National Health Institute 1998). For WC, the participants were asked to stand with their feet together and arms placed on the side. The tape was placed through the midpoint between the inferior margin of the last rib and the crest of the ileum in the mid - auxiliary plane and the measurement was taken to the nearest 0.1 cm. For HC, the subjects were asked to stand straight and the fibre glass tape was placed through the hips and the measurement at the widest point of the hip was recorded to the nearest 0.1cm. The cut points used for increased WC was defined >80cm for women, as per the WHO 2000 recommendations (Table 3.3).

Indices of Abdominal Obesity

- ***Waist: Hip Ratio (WHR)***

One of the widely used measures for abdominal obesity, WHR separates gynoid (pear shaped) obesity from android (apple shaped) obesity (Baldwin, 2010). As the name suggests, it's the ratio of the waist and the hip circumferences

WHR = Waist girth (cm) / Hip girth (cm)

TABLE 3.2 CLASSIFICATION OF ADULTS ACCORDING TO BMI (WHO, 2004)

Classification	BMI (Kg/m^2)
Underweight	< 18.5
Normal	18.5 – 22.99
Overweight	23.0 - 24.99
Pre-obese	25.0 – 26.99
Obese	≥27.0

TABLE 3.3 CUT OFFS FOR CENTRAL OBESITY

Cut-offs (WHO 2000)	Men	Women
Waist to hip ratio	more than 0.95	more than 0.80
Waist circumference	more than 90 cm	more than 80 cm
Waist Stature Ratio	more than 0.5	more than 0.5

- ***Waist-stature Ratio (WSR)***

Another anthropometric index that has been found to correlate well with cardio-vascular mortality is the waist stature ratio. We used the WHO 2000 cut offs for waist hip ratio waist-stature ratio and waist circumference, the same are given in Table 3.3.

C. BIO-PHYSICAL PARAMETERS

- ***Blood Pressure***

Systolic and diastolic blood pressure was assessed by a sphygmomanometer using standard protocol by trained personnel. The patient was asked to be seated for the measurement, relax the arm muscles and rest for 5 minutes prior to the measurement. Other things that were ensured before the measurement were that the arm did not have any clothing, was supported at heart level and the palm was facing up; also care was taken that the subjects' legs were uncrossed. All readings were taken in triplicate. In this study, high blood pressure taken to be a systolic blood pressure value of 140 mm Hg or more, or a diastolic blood pressure 90 mm Hg based on JNC VII guidelines (Chobanian et al 2003), which are outlined in Table 3.4.

TABLE 3.4 JNC VII CRITERIA FOR DIAGNOSING HYPERTENSION

	Optimal	Pre-hypertension	Stage I Hypertension	Stage II Hypertension
SBP (mmHg)	<120	120-139	140-159	≥ 160
DBP (mmHg)	<80	80-89	90-99	≥ 100

- ***Bone mineral density***

The bone mineral mass of the subjects was measured through Quantitative Ultrasound (qUS) technique using the Achilles EXP III (Lunar, General Electric, Shanghai, China) ultrasonometer. The site of measurement was the heel bone *Calcaneus.* Despite the gold standard being Dual Energy X-ray Absorptiometry (DEXA) technique, qUS is widely used for the measurement of bone density because it does not involve the use of ionizing radiation, is relatively inexpensive (especially compared DEXA), correlates well with DEXA, is relatively simple to implement and process and the most importantly, it is portable and perfect for large-scale field surveys.

qUS can provide information about the density and elasticity of bone by measuring the velocity of sound through bone, and about the structure of bone by measuring the attenuation of the signal. Bone tissue can be characterized in terms of speed of sound and broadband ultrasound attenuation (BUA). Speed of sound and attenuation of a sound wave are affected by the density, compressibility, viscosity, elasticity, and structure of the material it is travelling through. The primary assumption made while using this technology is that bones with varying biomechanical properties have different attenuation and velocity values as determined by ultrasound. The propagation of the US wave through bone is affected by bone mass, bone architecture, and the directionality of loading among other factors.

It proves to be somewhat difficult to use ultrasound to measure common fracture sites (hip & vertebrae) because the depth of soft tissue surrounding these bones attenuates too much of the ultrasound signal so a reading cannot be obtained. However, such issues are not encountered for calcaneus. There is very little soft tissue which makes it easy to measure bone. It has a relatively flat surface which ensures good

contact between the heel and the transducers. It is similar in composition to the main fracture sites (approx 90% trabecular bone). It is easily accessible and requires very little patient preparation (Evans 2006). This makes calcaneus the most popular ultrasound measurement site.

Principle: Ultasound imaging devices offer a parametric image of what is called as the Broadband Ultrasound Attenuation (BUA) at the calcaneus. For a given material the attenuation of the ultrasound wave will be constant, known as its BUA index. This is a measure of the increase in attenuation of the ultrasound wave as a function of increasing frequency. An ultrasound wave covering a range of frequencies is passed through a known thickness of sample. The amplitude spectrum of the received signal is then compared to the spectrum of a reference material. The difference between the two spectra is plotted against frequency and the slope is the BUA index (dB/MHz). If it is then divided by the thickness of the measured sample, it gives a volumetric parameter in (dB/MHz)/cm. Along with the BUA, the Achilles EXP III also gives the T-score, which is used to classify the individual as having adequate bone mineral density or not, using the WHO (2007) classification suggested by the WHO scientific consultation group on assessment of osteoporosis, as outlined in table 3.5.

TABLE 3.5 WHO CRITERIA FOR DIAGNOSING OSTEOPOROSIS AND OSTEOPENIA

Category	T-Score
Normal	> -1
Osteopenia	-1 to -2.5
Osteoporosis	< -2.5

The precision of this technique was found to be 1.4 to 3.3 percent and at the calcaneus qUS and DEXA measurements have been found to have a correlation of approx. 0.8 to 0.85 (Gluer 1997). However there are also studies which found that the precision of qUS is not as good and changes in qUS of the heel may not reflect changes in BMD at the spine or hip. But, more importantly, studies also suggest that qUS is useful in determining fracture risk of hip and spine independently of BMD measurements, with qUS devices demonstrating significant age-adjusted odds ratios regarding the association with fractures and the diagnosis of osteoporosis (Hans, Hartl and Krieg 2003).

D. BIOCHEMICAL PARAMETERS

- **BLOOD HEMOGLOBIN**

  Hemoglobin was estimated in whole blood using the cyanmethemoglobin method (Gowenlock 1988). The hemoglobin cut-offs used for diagnosing various severities of anemia was as per the WHO criteria, outlined in Table 3.6.

TABLE 3.6 WHO CRITERIA FOR DIAGNOSING SEVERITY OF ANEMIA

Severity	Range (g/dl)
Mild	11.0-11.9
Moderate	8.0-10.9
Severe	<8

Principle: The popular cyanmethemoglobin method makes use of the fact that when the ferrous ions in the hemoglobin molecule are oxidized to ferric form, it results in formation of a compound called methemoglobin, which when made to react with cyanide ions, forms cyanmethemoglobin which is a colored compound proportional to the content of hemoglobin in the test sample and can be measured spectrophotometrically at the absorbance of 540nm. To remove the cell membrane barrier of the erythrocytes, the whole blood is combined with the Drabkin's reagent which results in hemolysis of the erythrocytes and the hemoglobin is released into the solution containing potassium ferricyanide and the oxidation reaction of the ferric ions begins resulting in formation of cyanmethemoglobin.

- **PLASMA GLUCOSE**

Glucose was estimated in blood spectrophotometrically on an EM200 (Erbannhein, Germany) fully automated autoanalyzer using the glucose oxidase method (Trinder 1969), for which the diagnostics kits were obtained from Aggape Diagnostics, India. Diagnosis of diabetes and impaired glucose metabolism was made according the ADA 2011 guidelines, given in table 3.7.

Principle: In the glucose oxidase method, the aldehyde group of glucose molecule is acted upon by the enzyme glucose oxidase to form gluconic acid and hydrogen peroxide. Peroxide further undergoes a condensation reaction, catalyzed by horseradish peroxidase, wherein along with the colorless chromogen compound 4-amino phenazone, forms a red colored compound which can be photometrically read at 505nm.

TABLE 3.7 ADA 2011 GUIDELINES FOR DIAGNOSIS OF IMPAIRED GLUCOSE TOLERANCE AND DIABETES

	Fasting Blood Sugar levels (mg/dl)		**Blood Sugar levels After 2 hr of meal or 75g oral glucose load (mg/dl)**
Impaired Fasting Glucose (IFG)	100-125	*and*	<140
Impaired Glucose Tolerance (IGT)	<100	*and*	140-199
Pre-diabetes	100-125	*And/or*	140-199
Diabetes	≥126	*or*	≥200 (or random blood sugar)

- **PLASMA INSULIN**

  Plasma Insulin after a 12 hour fast was assessed using the Insulin Chemiluminescent immune assay (CLIA). Briefly, Chemiluminescence is another technique employed to follow antigen–antibody combination.

  β-D-glucose $+H_2O+O_2 \xrightarrow{\text{Glucose oxidase}}$ D-gluconic acid $+ H_2O_2$

  H_2O_2+ 4-amino phenazone $\xrightarrow{\text{Peroxidase}}$ red colored product $+4\ H_2O$

  Principle of Chemiluminescence makes use of emission of light caused by a chemical reaction, typically an oxidation, which gives rise to an excited molecule that emits light while decaying back to its ground state. A large number of molecules are capable of chemiluminescence, but the most common substances used are luminol, acridinium esters, ruthenium derivatives, and nitrophenyloxalates. When these substances are oxidized, typically using hydrogen peroxide and an enzyme for a catalyst, intermediates are produced that are of a higher energy state. These intermediates spontaneously return to their original state, giving off energy in the form of light. Light emissions range from a rapid flash of light to a more continuous glow that can last for hours. Acridinium esters, for example, emit a quick burst or flash of light, while the light remains for a longer time with luminol and dioxetane (Stevens 2010).

  This type of labeling is used for heterogeneous and homogeneous assays, because labels can be attached to either antigen or antibody. In heterogeneous assays, competitive and sandwich formats are the ones most often used. Smaller analytes such as therapeutic drugs and steroid hormones are measured using competitive assays, while the sandwich format is used for larger analytes such as protein hormones.

Chemiluminescent assays have an excellent sensitivity, comparable to ELISA and RIA, and the reagents are stable and relatively nontoxic. The sensitivity of some assays has been reported to be in the range of attamoles (10-18mol) to zeptomoles (10-21mol). Because very little reagent is used, they are also quite inexpensive to perform. The relatively high speed of detection also means a faster turnaround time.

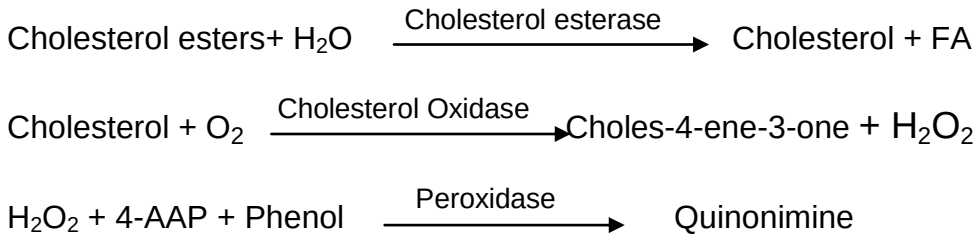
Principle: The insulin CLIA is a solid phase enzyme linked immunosorbent assay. The assay system utilizes one anti-Insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme conjugate solution, which has horseradish peroxidase. The standards and serum are added to the Insulin antibody coated microtiter wells. Then anti-Insulin antibody labeled with horseradish peroxidase conjugate solution is added. The Insulin present in the serum combines with the antibody on the well and the enzyme conjugate resulting in the Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After an hour of incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of Insulin in the sample. By reference to a series of Insulin standards assayed in the same way, the concentration of Insulin in the unknown sample is quantified.

The cut-point used for identifying hyperinsulinemia in this study was an insulin concentration of >12mU/L (McAuley et al 2001).

- **SERUM TOTAL CHOLESTEROL (TC)**

  The total serum cholesterol was estimated photometrically on Olympus AU2700 (Beckman Coulter) chemistry analyzer using Aggape cholesterol oxidase kits. The cut-points used for detecting hypercholeseterolemia were used from the NCEP ATP III guidelines (2001) given in Table 3.8.

  Principle: Estimation was done by the cholesterol oxidase method, (Allain et al 1974) which makes use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the first step, CE separates cholesterol esters in to cholesterol and fatty acids. In the second step, CO oxidizes the cholesterol into a ketone and hydrogen peroxide, which in the presence of peroxidase condenses the mixture of phenol and 4-aminoantipyrine (4-AA) are to form a red colored quinoneimine in what is called the Trinder's reaction. This quinoneimine dye is proportional to the concentration of cholesterol in the sample and is read colorimetrically at 505nm (green filter) to estimate the cholesterol in the sample.



- **SERUM TRIACYLGLYCEROLS (TAG)**

  Triacylglycerols in serum were estimated using the glycerol kinase (Buccolo and David 1973) diagnostic kits (Aggape) employing photometry on an Advia1800 (Siemens) chemistry analyzer.

  Principle: Serum TAG were estimated using in a four step method , where first the TAG are split using lipoprotein lipase and the resulting

TABLE 3.8 NCEP ATP III (2001) CRITERIA FOR DIAGNOSING DYSLIPIDEMIA

Total Cholesterol -TC (mg/dl)	
<200	Desirable
200-239	Borderline high
>240	High
LDL Cholesterol (mg/dl)	
<100	Optimal
100-129	Near optimal/above optimal
130-159	Borderline high
160-189	High
>190	Very high
HDL Cholesterol (mg/dl)	
<50 (for women)	Low
>60	High
Triacylglycerols- TAG (mg/dl)	
<150	Normal
150-199	Borderline high
200-499	High
500	Very high

glycerols which are further phosphorylated using glycerol kinase. The glycerol phosphates are oxidized in to form Dihydroxy Acetone Phosphate (DHAP) and hydrogen peroxide, which in turn, following the Trinder's reaction generates the red colored quinoneimine dye, which is colorimetrically read at 505 nm using the green filter.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{FFA}$$

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

$$\text{Glycerol-3-phosphate} + O \xrightarrow{\text{Glycerol-Phosphate Oxidase}} \text{DHAP} + H_2O_2$$

$$H_2O_2 + \text{4- Aminoantipyrine} + \text{Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine}$$

- **SERUM LOW DENSITY LIPOPROTEIN (LDL) CHOLESTEROL**

The LDL levels were estimated directly on an Olympus AU2700 (Beckman Coulter) chemistry analyzer using the Rapid Diagnostics' LDL direct measurement diagnostic kit.

Principle: The principle for estimation employs two steps: first where the total lipoproteins are eliminated by cholesterol oxidase and in the second step, employing the cholesterol oxidase-peroxidase method, LDL is estimated as in the estimation of total cholesterol.

Step 1- Elimination of lipoproteins other than LDL

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{FA}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

Step 2 Measurement of LDL

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + FA$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + 4\ H_2O_2$$

:

- **SERUM HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL**

  The HDL cholesterol assay was performed using the HDL cholesterol plus second generation assay for direct measurement using diagnostic kits (Aggape) on an Olympus AU2700 (Beckman Coulter) chemistry analyzer.

  Principle: Briefly, The apo B containing lipoproteins in the specimen namely, very low density lipoprotein (VLDL) and the low density lipoproteins (LDL), are made to react with a blocking reagent that makes them non-reactive with the cholesterol enzyme reagents. Hereafter, running the cholesterol esterase detects only the HDL in the specimen.

- **SERUM VLDL CHOLESTEROL**

  The very low density lipoprotein cholesterol was arrived at using the Friedewald (1972) formula using the triglycerides, total cholesterol and LDL concentrations. The standard assumption made while using this formula is that there are no chylomicrons present in the serum while estimating other lipoprotein fractions to be used in the formula.

VLDL = TC – LDL - HDL

- **SERUM APOLIPOPROTEIN A (APO A) AND APOLIPOPROTEIN B (APO B)**

  The present apolipoprtoein assays were done using a BN II automated nephelometer (Dade Behring/Siemens), which uses the N Antiserum to Human Apolipoprotein A-I and Apolipoprotein B as the reagent.

  Principle: Serum concentrations of apolipoproteins a and b were estimated using Nephelometry. Nephelometry measures the light that is scattered at a particular angle from an incident beam as it passes through a suspension. The amount of light scattered is an index of the solution's concentration. Beginning with a constant amount of antibody, if the amount of antigen is increased, it results in an increase in antigen–antibody complexes. Thus the relationship between antigen concentrations approaches linearity, as indicated by antigen–antibody complex formation and light scattering. Light scatter may be recorded in arbitrary units of "relative light scatter," or it may be directly extrapolated by a computer to give actual concentrations in milligrams per deciliter (mg/dL) or inter-national units per milliliter (IU/mL), based on established values of standards. Nephelometers measure light scatter at angles ranging from 10 degrees to about 90 degrees (Stevens 2010).

- **SERUM THYROID STIMULATING HORMONE (TSH) AND**

  The TSH estimation in serum was carried using a chemiluminescent two-site or 'sandwich' type assay. The cut-off used to detect low TSH levels was > 4µIU/ml (Baskin et al 2002).

  Principle: This CLIA uses two specific antibodies. The microtiter wells are coated with a monoclonal antibody specific for TSH and another monoclonal antibody specific for a different region of TSH is conjugated to horseradish peroxidase (HRP). In this one-step capture assay, the TSH from the test specimen and standards are allowed to bind simultaneously to the antibody coated wells and to the HRP conjugate.

The HRP conjugate is removed by washing after incubation and decantation. Following this, a luminescent substrate is added to the system, which leads to the phenomenon of light emission and the luminosity in terms of relative luminescence units (RLUs) are measured in a microtiter plate luminometer. The RLUs formed by the enzymatic reaction are directly proportional to the enzyme conjugate bound to the solid phase in the samples. A set of standards is used to plot a standard calibration curve which is used to determine the concentration of TSH in samples.

- **SERUM FREE THYROXINE (FT4)**

The unbound or free Thyroxine (FT4) was estimated in serum using the competitive CLIA. The cut-off level used to determine low FT4 levels was <0.9ng/dl (Baskin et al 2002).

Principle: The solid phase is the T4 antibody coated on the microtiter wells. To this, a conjugate of T4 and horseradish peroxidase along with the test specimen, in this case the subject's serum, are added. Hereafter, the fT4 in the test specimen and the fT4 in the conjugate compete for the limited T4 antibody on the microtiter wells. After an incubation period of 60min at 37 degrees Celsius, the unbound T4-enzyme conjugate is washed off and the RLU is measured, as is it is inversely proportional to the fT4 in the test specimen.

- **SERUM C- REACTIVE PROTEIN HIGH SENSITIVITY ASSAY (hs-CRP)**

The high sensitivity assay of C - reactive protein (hs-CRP) in the serum was done using nephelometry on a BN II automated nephelometer (Dade Behring/Siemens).

Principle: Here a soluble analyte and corresponding antibodies that are bound to polysterene particles are made to react. The test specimen is mixed with latex particles coated with monoclonal antibodies (anti-CRP antibodies), so the CRP present in the specimen will bind with the latex bound antibodies.

BIOCHEMICAL INDICES

- **HOMA 2**

The index used for assessing insulin resistance was the modified version of Homeostasis Model Assessment or HOMA put forth by David Jonathan Levy et al (1998) called as HOMA 2. This new version is different from the first version (published by David Matthews et al in 1985), because it took into consideration the hepatic and peripheral glucose resistance variations and the increases in the insulin secretion curve for glucose levels higher than 180mg/dl, and also the contribution of circulating pro-insulin. It was calculated using the software HOMA 2 Calculator version 2.2, released in 2004 by the Oxford Centre for Diabetes, Endocrinology and Metabolism, The University of Oxford. The cut-point used for diagnosing insulin resistance using in this study, a HOMA 2 value greater than 1.4 was used (Geloneze , Vasques & Stabe 2009).

E. NUTRIENT CONTENT ANALYSIS OF WHEATGRASS POWDER

A sample of the freeze-dried nitrogen packed wheatgrass powder that was procured from an exporting firm in Vadodara was sent for evaluation of nutritional value. The nutrient component analysis (conducted by Analytical & Environmental Sciences, Vadodara) included quantitative testing of energy, protein content, total fat, fibre, iron, moisture, ash, carbohydrate & sugar content, ascorbic acid, and β carotene. For this, the

sample of wheatgrass powder was homogenized and a representative sample was taken after confirming that it was free from insect infestation and fungal growth, also that it conformed to the general PFA rules (1995). The estimation of nutritive value was conducted by employing methods conforming to the BIS standards. These methods have been described in Table 3.9.

STATISTICAL ANALYSES

- Statistical analysis has been carried out using the software packages Microsoft Excel 2007, Epi Info 3.4.1 and SPSS 17.
- Data has been described using descriptive statistics (means and standard deviations) for continuous variables. Data has also been described using proportions: percentages in case of categorical variables and prevalence rates for continuous variables by using cut-off points.
- Wherever relevant, the 95% confidence intervals in which the means and proportions lied have been indicated.
- The two sample Students' t test and Chi-square test have been performed to compare continuous and categorical variables, respectively.
- Bivariate analyses including Odds ratios and Correlation analyses (Spearman for non parametric variables) have been performed to estimate associations between two variables.
- Partial Correlation and binomial logistic regression (for odds ratios) were computed to estimate true associations between variables/conditions, while adjusting for covariates to rule out residual confounding.
- Data has been segregated based on quintiles of anthropometric indices to observe the distribution of prevalence of risk factors across quintiles of BMI, WC and WSR.
- In multivariate analysis, Stepwise backward Multiple Linear regression has been applied to find out the set of variables that contributed significantly to

TABLE 3.9 METHODS USED FOR NUTRITIONAL ANALYSIS OF WHEATGRASS POWDER

Test	Method
Moisture	Dehydration using hot oven
Ash	Dry Ashing using Muffle furnace
Fat	Soxhlet method
Protein	Kjeldahl method
Carbohydrates	Difference method
Energy	Calculation
Ascorbic acid	Titremetry
Fibre	Colorimetry
Iron	Colorimetry
β-carotene	Spectrophotometry

▪

to the variation in blood pressure and blood sugar levels.

- Paired ‘t’ test has been used to compare the difference between the pre and post intervention values of the outcome variables in the intervention study.
- One way analysis of variance (ANOVA) was computed to find the difference in the sensory evaluation scores across the categories of varying wheatgrass concentration of the wheatgrass incorporated recipes.
- All statistical analyses were considered significant at a 2 tailed significance level of $P<0.05$.

RESULTS AND DISCUSSION

The results of the research embodied in this thesis will be presented and discussed in this chapter under the following heads

- **Phase 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.

- **Phase II - Follow-up Study**

  The Immediate and Longitudinal Outcomes of Health Check-Up on Women's Health Care Practices.

- **Phase III –Translational Research**

  Analysis of Nutritional Quality of Wheatgrass Powder and its Incorporation of it in Different Recipes as a Functional Food and its Acceptability.

- **Phase IV - Experimental Research**

  Impact of Wheatgrass Powder Supplementation on Lipoprotein Status and Antioxidant Status in Hyperlipidemic Women – An Open Label Randomized Controlled Trial.

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

In an age where obesity, cardiovascular and metabolic diseases are rampant across all populations, a group that is vulnerable to all these conditions: menopausal women, undeniably needs special focus. Women have been found to suffer from higher comparative prevalence of obesity and cardio-metabolic disorders as compared to men (Vlassof 2007, Silander et al 2008, Ghosh et al 2010, Abbasi 2012). The repeated hormonal variations at various stages of reproductive life, for e.g. puberty, pregnancy and finally menopause, leads to changes in body composition, redistribution and expansion of adipose tissue, especially the metabolically active abdominal fat deposition, all of which partly explains the higher occurrence of obesity in women (Chen, Brown and Russo 2009, Kulkarni et al 2010). This is accompanied by a number of other clinical-biochemical changes that are reflective of the metabolic disturbances underneath and can become the reason for development of adverse cardio-vascular outcomes. Now, because of absence of an organized health check-up and surveillance system in the country, it makes sense to explore what all risk factors lie and go undetected in a free-living population vis-à-vis a population that goes for a health-check up.

As a consequence this study was planned to examine the distribution of clinical, biochemical risk factors that are pre-disposing factors towards development of altered lipid and glucose metabolism across various stages of menopause in women and compare the findings among women hailing from a free-living population with those attending a health check- up facility. For this 186 pre, peri and post menopausal women were enrolled from four zones of Baroda city, essentially from free-living population. For studying women from a health check-up facility, 213 pre, peri and post menopausal women from the health-check up section of a multi specialty hospital in Ahmedabad were enrolled.

I. BACKGROUND INFORMATION OF THE SUBJECTS

Background information on the subjects was collected using a pre-tested questionnaire through a one-to-one interview and included information on regionality, religion, education, occupation and type of family. Information on the ethnicity (Table 4.1) revealed that majority of the subjects studied were Gujaratis (83.5%), with the group being a bit more diverse in case of Baroda where apart from the 76% of the Gujaratis, 8% were Punjabis, almost 6% were Maharashtrians. The study sample from Ahmedabad was more or less Gujaratis (88%), with a small fraction of people from various other states, as shown in Table 4.1. A vast majority of the subjects in both Baroda and Ahmedabad were Hindus (93.5% and 95.3% respectively), with only a minute fraction of the subjects belonging to religious minorities (Table 4.1).

The data on education revealed that almost half of the subjects studied were graduates (54%), with more of them being so in Baroda sample (64%), as compared to Ahmedabad (45%). The proportion of uneducated people was relatively insignificant (2.5%), so was highly educated people (0.5% doctorate holders), however the group in Baroda was slightly more educated as indicated by higher number of post graduates (15% versus 11% in Ahmedabad) and significantly higher proportion of graduates (64% versus 45% in Ahmedabad, $p<0.001$). When categorized occupation wise, it was found that more of the subjects were housewives (77%) as compared to those who worked (22.3%), with the proportion being significantly skewed in case of Baroda, where 89% of the subjects were home makers, as compared to Ahmedabad where this figure was lower: 67% ($p<0.001$). More than half of the subjects studied belonged to a nuclear family (66%), with the percent being significantly higher ($p<0.001$) in Baroda (76%) than in Ahmedabad (58%).

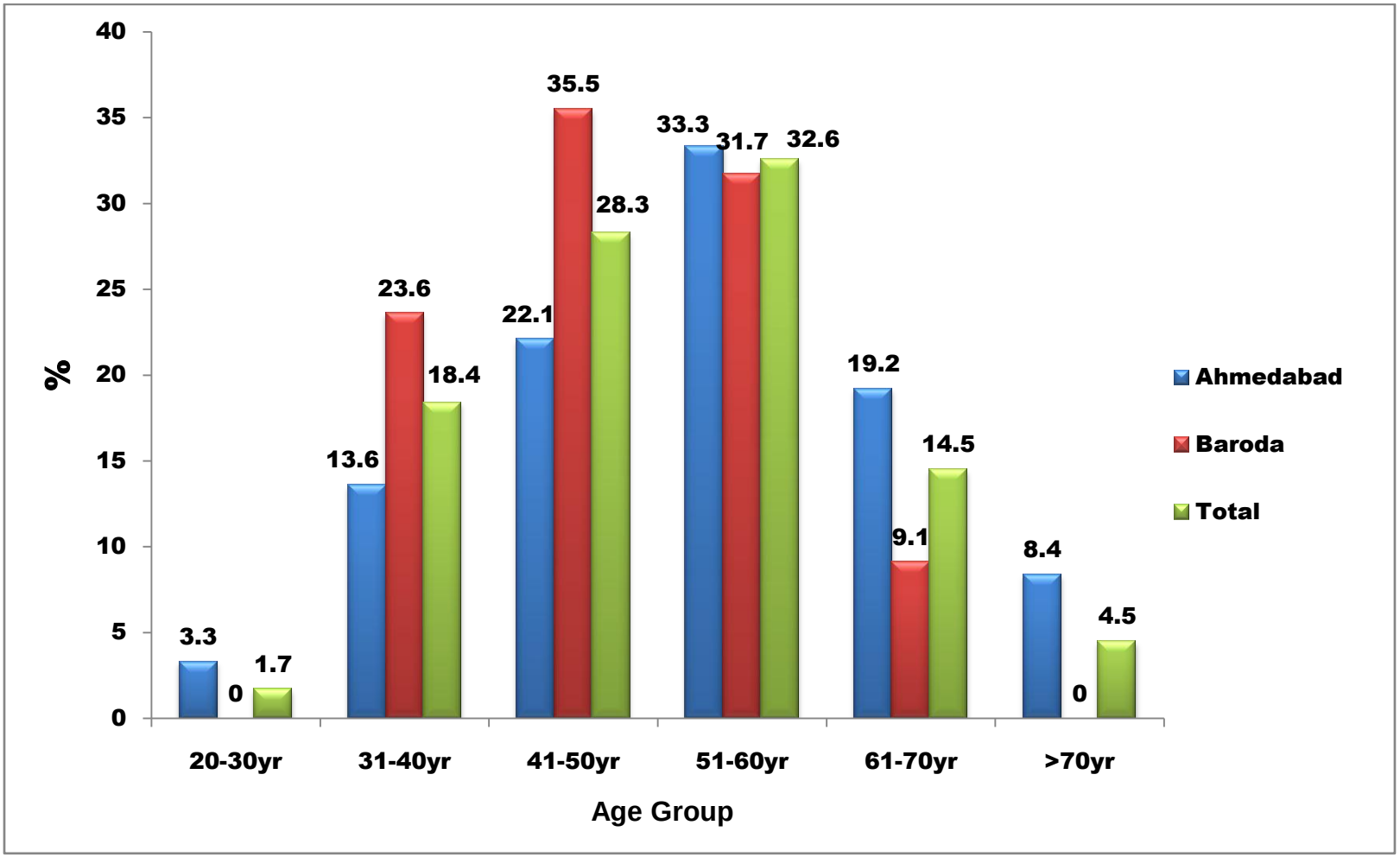
The subjects who came for health checkup had a wide age range, ranging from 20 years to 80 years with maximum being in the age group of 51 to 60 years (Figure 4.1). The maximum number of subjects belonged to the age-range of

TABLE 4.1 BACKGROUND INFORMATION OF THE SUBJECTS

	Ahmedabad		Baroda		Total	
	N=213	%	N=186	%	N=399	%
Regionality						
Gujarati	**191**	**89.7**	**142*** **	**76.3**	333	83.5
Rajasthani	8	3.7	4	2.1	12	3.0
Punjabi	**0**	**0**	**15** **	**8.1**	15	3.8
Malayali	2	0.9	5	2.7	7	1.8
Tamil	0	0	2	1.1	2	0.5
Kannada	1	0.5	0	0	1	0.3
Maharashtrian	5	2.3	11	5.9	16	4.0
UP	3	1.4	0	0	3	0.8
Telugu	1	0.5	4	2.1	5	1.3
Sindhi	1	0.4	0	0	1	0.3
Bengal	0	0	3	1.6	3	0.8
Delhi	1	0.4	0	0	1	0.3
Religion						
Hindu	203	95.3	174	93.5	377	94.5
Islam	8	3.7	8	4.3	16	4.0
Christian	2	0.9	1	0.5	3	0.8
Sikh	0	0	2	1.1	2	0.5
Education						
Illiterate	6	2.8	4	2.1	10	2.5
Primary	**25**	**11.7**	**7** **	**3.8**	32	8.0
SSC	**41**	**19.2**	**13*** **	**7.0**	54	13.5
HSC	13	6.1	11	5.9	24	6.0
Diploma	6	2.8	3	1.6	9	2.3
Graduate	**97**	**45.5**	**119*** **	**64.0**	216	54.1
Postgraduate	24	11.2	28	15.0	52	13.0
Doctorate	1	0.4	1	0.5	2	0.5
Occupation						
Housewife	**144**	**67.6**	**166*** **	**89.2**	310	77.7
Desk Job	69	32.4	20	10.7	89	22.3
Type of family						
Nuclear	**125**	**58.6**	**141*** **	**76.2**	266	66.7
Joint	9	4.2	12	13.4	21	5.3
Extended	**79**	**37**	**33** **	**17.4**	112	28.1

***Significantly different from Ahmedabad at p<0.01, *** p<0.001**

FIGURE 4.1 AGE DISTRIBUTION OF THE SUBJECTS (%)



51-60 years. Subjects from Ahmedabad were more diverse with respect to age; with 3% being in the 21-30 years age group and 8% aged more than 70 years; whereas subjects from Baroda were confined mostly to the 41-50 year age group (35%) and the 51-60 year age group (31%).

Distribution of Menopausal Status

Data on the menopausal status (Figure 4.2) revealed that majority of women were 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%). This distribution was more or less even in subjects from Baroda, 32.8% being pre menopausal and 37% being post menopausal. In Ahmedabad however, the most number of subjects were in post menopausal category (56.3%).

Clinical Characteristics and Biochemical Profile of the Subjects

The mean values of the anthropometric and biochemical variables are depicted in Table 4.2. The mean age differed slightly among subjects of both the cities (Ahmedabad 53 years and Baroda 48.2 years). The BMI values for both cities were almost similar (27.6Kg/m^2 Ahmedabad and 26.7Kg/m^2 for Baroda). Same was the case with indicators of abdominal obesity. The mean WC was 93cm which is way above the Asia Pacific cut point of 80cm for women. The mean blood pressure values were in the pre hypertension category (SBP 127mmHg and DBP 80mmHg).

The mean hemoglobin level was in the anemic category for Ahmedabad (11.9g/dl), but was well above the cut point of 12g/dl in case of Baroda (12.5g/dl). In case of the mean FBS levels, again the mean for Ahmedabad lied in the pre-diabetes category (103.5mg/dl), unlike Baroda (91mg/dl). The mean PP2BS, Insulin, HOMA2 IR, TC, TAG, HDL, TAG/H were also in the normal category for subjects both the cities. Mean LDL and AIP were high in both Ahmedabad (117mg/dl and 0.3 respectively) and in Baroda (109mg/dl and 0.3 respectively). The Mean TSH value was normal for Ahmedabad (4.2µIU/ml), but was elevated for Baroda (5.1µIU/ml).

FIGURE 4.2 PERCENT DISTRIBUTION OF THE MENOPAUSAL STATUS OF THE SUBJECTS

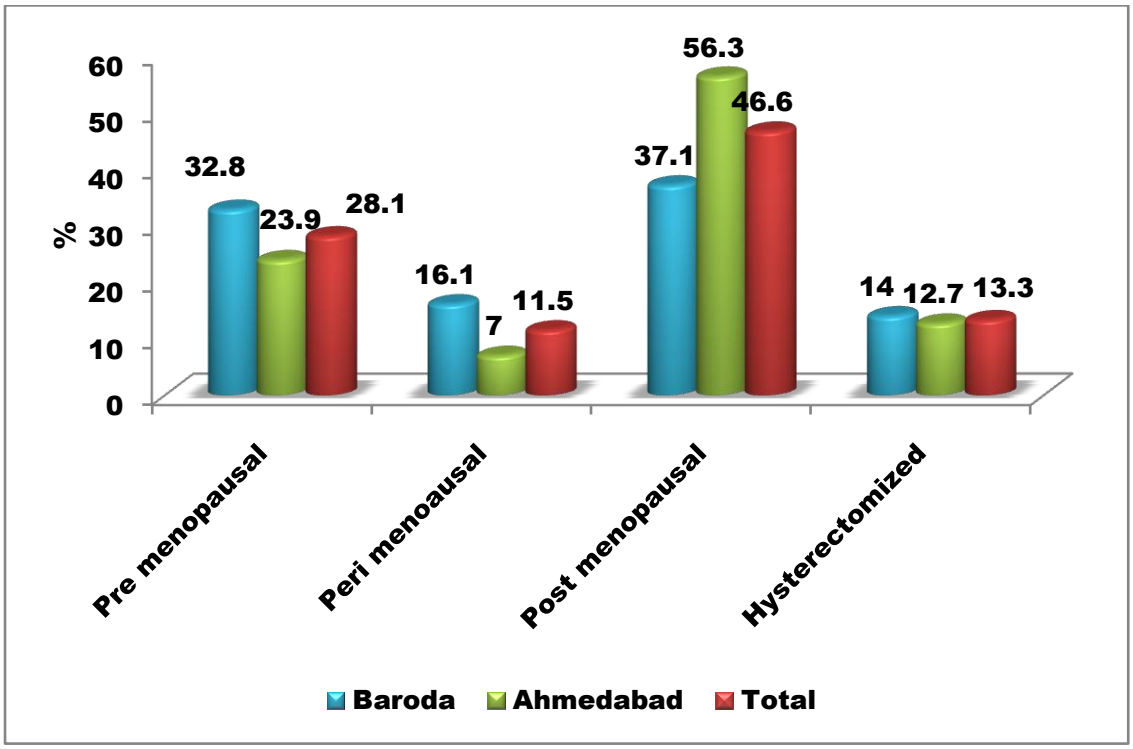


TABLE 4.2 CLINICAL CHARACTERISTICS OF THE SUBJECTS

Variables	Ahmedabad		Baroda		Total	
	N	(mean± S.D.)	N	(mean± S.D.)	N	(mean± S.D.)
Age (Years)	213	53±12.0	186	48.2 ± 9.4	399	51 ± 11.2
Height (cm)	213	151.9±6.4	186	153.6 ± 5.4	399	153 ± 6.0
Weight (cm)	213	63.7±11.2	186	63.1 ± 11.2	399	63 ± 11.2
BMI (Kg/m^2)	213	27.6±4.7	186	26.7 ± 4.6	399	27 ± 4.6
WC (cm)	184	91.7±9.7	186	94.2 ± 9.2	370	93 ± 9.5
HC (cm)	184	103.2±9.8	186	103.3 ± 9.3	370	104 ± 9.6
WHR	184	1.3±0.1	186	0.9 ± 0.1	370	0.9 ± 0.1
WSR	184	0.6±0.1	186	0.6 ± 0.1	370	0.6 ± 0.1
SBP (mmHg)	213	129 ± 19.9	186	124.4 ± 16.4	399	127 ± 18
DBP(mmHg)	213	80.9 ± 11.8	186	78.7 ± 8.1	399	80 ± 10
Hb (g/dl)	213	11.9±1.4	186	12.5 ± 7.6	399	12 ± 5.3
FBS (mg/dl)	213	103.5±24.7	186	91.6 ± 25.5	399	98 ± 26.7
PP2BS(mg/dl)	193	117.9±42.5	-	-	193	117.9±42.5
Insulin (µIU/ml)	148	10.5±9.7	186	9.0 ± 3.9	334	9.7 ± 7.1
HOMA IR	148	1.47±1.16	186	1.15 ± 0.5	334	1.3 ± 0.9
TC (mg/dl)	211	192.1±37.4	186	182.4 ± 40.1	399	187 ± 38.9
TAG (mg/dl)	211	105.0±51.8	186	116.4 ± 58.6	399	110 ± 55.3
LDL(mg/dl)	211	117.3±31.8	186	109.5 ± 32.6	399	114 ± 32.3
HDL(mg/dl)	211	54.8±13.6	186	49.1 ± 10.4	399	52 ± 12.5
TAG/HDL	211	2.2±1.7	186	2.6 ± 1.8	399	2 ± 1.8
LDL/HDL	211	2.7±5.4	186	2.3 ± 0.7	399	2 ± 3.9
AIP (log10 TG/H)	211	0.30 + 0.2	186	0.3 ± 0.2	399	0.3 ± 0.2
TSH (µIU/ml)	208	4.2+9.6	186	5.5 ± 14.2	399	5 ± 12.0
FT4 (µIU/ml)	-	-	186	1.1 ± 0.8	186	1.1 ±0.8

Prevalence of Overweight and Obesity

The prevalence rates of overweight, obesity, especially abdominal obesity, calculated based upon the Asia Pacific Cut-offs, were found to be staggeringly high in both Ahmedabad and in Baroda (Table 4.3), with the proportion of normal subjects being as low as 12.5%. Highest number of subjects was in the obese grade I category (42.1%), followed by obese II (25.3%), which was followed by overweight category (17.5%). The observation throws light on the fact that the Indian female population is increasingly becoming predominantly obese. Despite so many subjects being in the obese category, there were also subjects who were in the underweight category (2.5%), thus reflecting the dual burden of malnutrition in the society. The abdominal obesity indicators seemed to fare worse than BMI, with 90% of the subjects having a high WC, 86.7% having a high WHR and as high as 93.8% having a high WSR.

Family History of Diseases

The subjects were also interviewed for information on the family history of major illnesses (Table 4.4). The data revealed that hypertension was the most common condition the subjects' family members suffered from (42.3%). This was closely followed by diabetes which was reported by 41% of the subjects. The next most prevalent condition among subjects' family members was CHD which was reported by 20.5%. The least prevalent problem reported was Thyroid dysfunction (5.5%), which was close to Asthma (5.8%). Cancer was also reported by quite a few respondents (14.2%).

Medical history

The medical history of the study subjects (Table 4.5) reflected a high prevalence of most of the major diseases (Table 4.5 and Figure 4.3). Ahmedabad especially, had hypertension prevalence as high as 34.7% and a hypothyroidism prevalence of 19.2%, Baroda by comparison had 16.7% of hypertension and 4.3% of hypothyroidism. The second most prevalent

TABLE 4.3 PREVALENCE OF OVERWEIGHT AND OBESITY IN THE SUBJECTS

Category	Criteria	Ahmedabad N=213	Baroda N=186	Total N=399	95% CI
Based on BMI					
Underweight	BMI<18.5	2 (0.93)	8 (4.3)	10 (2.5)	0.94 – 4.1
Normal	BMI:18.5 – 22.9	29 (13.6)	22 (11.8)	50 (12.5)	9.2 – 15.8
Overweight	BMI:23 – 24.9	30 (14.1)	39 (21.0)	70 (17.5)	13.7 – 21.3
Obese I	BMI:25 – 29.9	89 (41.8)	81 (43.5)	168 (42.1)	37.2 – 47.0
Obese II	BMI≥30	63 (29.6)	36 (19.3)	101 (25.3)	20.9 – 29.6
Abdominal Obesity		**N=184**	**N=186**	**N=370**	
WC	>80	161 (87.5)	174 (93.5)	335 (90.5)	87.5 – 93.5
WHR	>0.8	140 (76.1)	181 (97.3)	321 (86.7)	83.2 – 90.2
WSR	>0.5	169 (91.8)	178 (95.7)	347 (93.8)	91.3 – 96.3

Values in parenthesis indicate percentage

TABLE 4.4 FAMILY HISTORY OF DISEASES*

Diseases	Ahmedabad		Baroda		Total	
	N=213	%	N=186	%	N=399	%
DM	94	44.1	71	38.2	165	41.3
HTN	92	43.2	77	41.4	169	42.3
Dyslipidemia	13	6.1	11	5.9	24	6.0
Asthma	13	6.1	10	5.3	23	5.8
Cancer	32	15.0	25	13.4	57	14.2
Thyroid	13	6.1	9	4.8	22	5.5
CHD	42	19.7	40	21.5	82	20.5

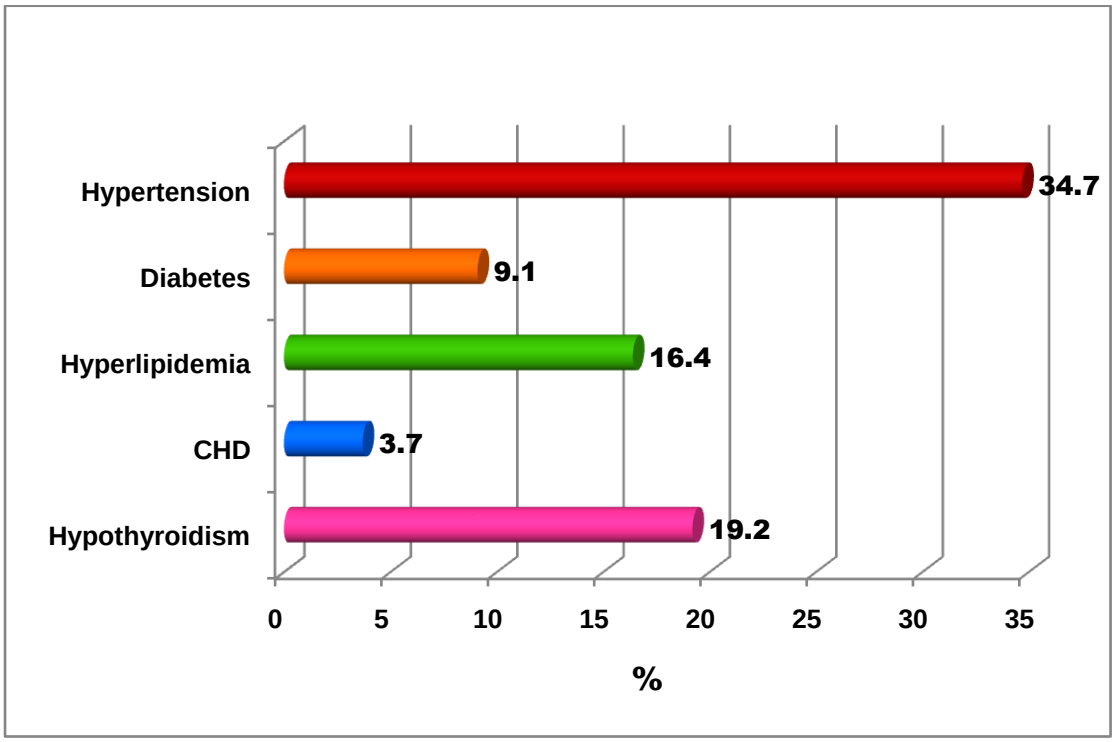
**** Disease categories are not mutually exclusive***

TABLE 4.5 PERCENT PREVALENCE OF CLINICAL CONDITIONS BASED ON MEDICAL HISTORY

Known Cases	Ahmedabad N=213 (%)	Baroda N=186 (%)	Total N=399 (%)
Hypertension	76 (34.7); 95% CI: 28.3 – 42.1	31 (1226.7); 95% CI: 11.3 – 22.1	107 (26.8) 95% CI: 22.1-31.2
Diabetes	20 (9.1): 95% CI: 5.2 – 13.0	9 (4.8); 95% CI: 1.7 – 7.9	29 (7.3); 95% CI: 4.7 – 9.9
Hyperlipidemia	36 (16.4); 95% CI: 11.4 – 21.4	15 (8.1); 95% CI: 4.1 – 12.1	51 (12.8); 95% CI: 9.5 – 16.1
CHD	8 (3.7); 95% CI: 1.1 – 6.3	8 (4.3); 95% CI: 1.4 – 7.2	16 (4.0); 95% CI: 2.1 – 5.9
Hypothyroidism	41 (19.2); 95% CI: 14.5 – 23.9	8 (4.3); 95% CI: 1.4 – 7.2	49 (12.3); 9.1 – 15.5

Values in parenthesis indicate percentage

FIGURE 4.3 PERCENT PREVALENCE OF CLINICAL CONDITIONS BASED ON MEDICAL HISTORY



condition after hypertension in the pooled data came out to be hyperlipidemia which was present in 12.8% of the subjects. **None of the subjects who were undergoing or already had undergone menopausal transition reported the use of HRT.**

Prevalence of Menopausal Symptoms

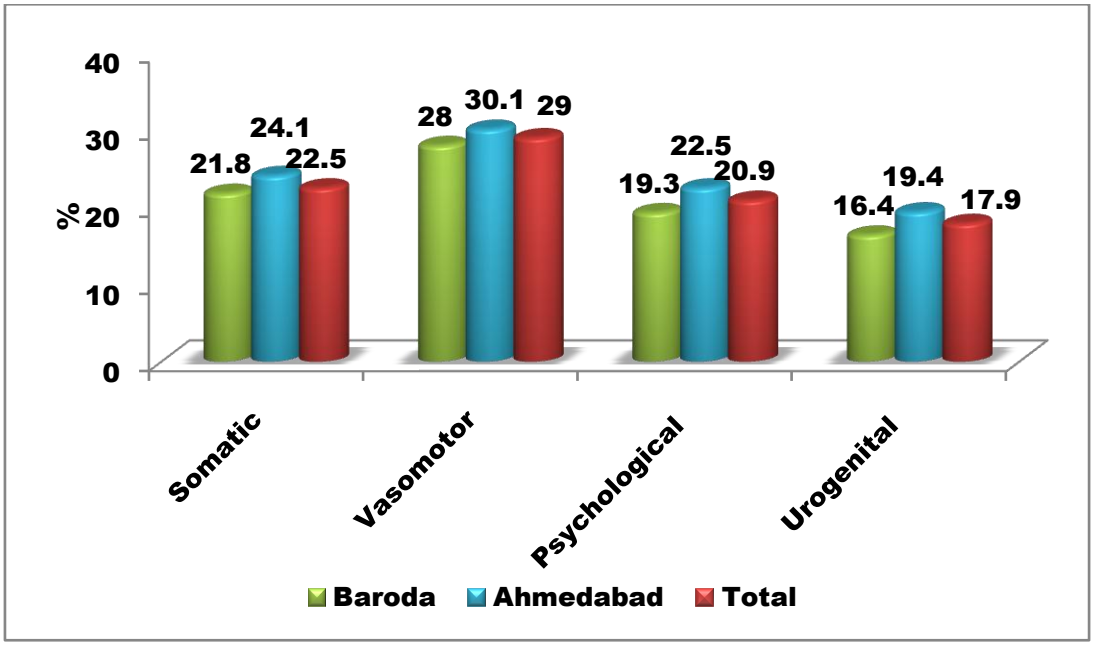
Another problem that is not a disease but is a cause of concern among a lot of middle age women across the globe is menopausal symptoms. In the present study, the prevalence of menopausal symptoms came up to be in the range of 16-30% (Figure 4.4). The most prevalent symptoms were headaches and body aches in the somatic symptoms category whose prevalence was as high as 29% in the pooled data. This was followed by hot flashes and night sweats in the vasomotor symptom category, whose prevalence came up to be 22.5%. The prevalence of urogenital symptoms, mainly urine incontinence, was found to be 17.9% and is the most uncomfortable problem as described by the subjects. Psychological symptoms were reported by 20.9% of the respondents. In general, all the symptoms were more prevalent in the subjects from Ahmedabad. These figures are indicative of the fact that estrogen withdrawal was more in the women who presented for a health check-up.

PREVALENCE OF CLINICAL CONDITIONS

Anemia

One of the most prevalent conditions that is yet to get adequate attention is iron deficiency anemia, the prevalence of which (Table 4.6) is astoundingly high (46.4%), both in Baroda (46.2%) and Ahmedabad (46.5%). This is indicative of the fact that almost every other woman in our population is anemic. This raises huge concerns about supplementing iron along with the dietary intake in the middle aged group as well. Table 4.6 depicts the prevalence of anemia.

FIGURE 4.4 PREVALENCE OF MENOPAUSAL SYMPTOMS IN THE SUBJECTS*



**menopausal symptoms categories are not mutually exclusive*

TABLE 4.6 PERCENT PREVALENCE OF IRON DEFICIENCY ANEMIA AMONG SUBJECTS

	N	n (%)	95% CI
Ahmedabad	213	99 (46.5)	39.7 - 53.3
Baroda	186	86 (46.2)	38.9 – 53.5
Total	399	185 (46.4)	41.5 – 51.3

Values in parenthesis indicate percentage

Hypertension

Apart from the pre-existing hypertension reported by the subjects in the medical history, a considerable number of individuals were also newly diagnosed with hypertension in this study (Table 4.7). Almost as high as 47% of the individuals attending the health check up facility in Ahmedabad were diagnosed as being pre hypertensives and 18.2% as hypertensives, which leaves hardly any participant with normal blood pressure. In Baroda however, the figures were not as bad, with 25.8% diagnosed as pre hypertensives and 17.2% diagnosed as hypertensives. This implicates a population level problem and hence dietary and behavioral approaches to reduce blood pressure need to be advocated more robustly so that the population sodium intake can be reduced in addition to other lines of therapy.

Diabetes

Comparing the prevalence of pre-existing diabetes with the newly diagnosed ones (Table 4.8), it was seen that Ahmedabad sample had significantly higher number of individuals with pre-existing diabetes (9.1%) as compared to Baroda (2.1%). In addition to this, as high as 28% individuals were diagnosed with pre-diabetes and 4.7% with diabetes in Ahmedabad. In case of Baroda, 8.6% of the subjects were diagnosed with pre-diabetes and 6.4% were diagnosed as diabetics. In addition to this, for Ahmedabad subjects, the post prandial glucose load challenge, helped diagnose an additional 2.6% of individuals who appeared to have impaired glucose tolerance. A stark disparity in the glucose metabolism in both the cities was that a significant proportion of the subjects in Ahmedabad had impaired fasting glucose which was more than twice that in Baroda.

Dyslipidemia

Among all the clinical conditions affecting middle aged women, dyslipidemia seemed to be the most pressing problem, with the most prevalent aberration being in the LDL fraction (65.2%). The figures for Ahmedabad again were far

TABLE 4.7 PREVALENCE OF HYPERTENSION (NEWLY DETECTED CASES)

	Ahmedabad N=213 (%)	**95% CI**	**Baroda N=186 (%)**	**95% CI**	**Total N=399 (%)**	**95% CI**
Old Cases	76 (34.7)	27.7 – 36.7	60 (32.2)	25.4 – 39	136 (34.1)	29.4 – 38.8
Newly Detected Cases						
Pre hypertension	65 (47.4)	40.7 – 52.3	48 (25.8)	19.4 – 32.2	113 (28.3)	23.8 – 32.8
Hypertension	25 (11.7)	17.3 – 27.1	32 (17.2)	11.7 – 22.7	57 (14.2)	10.8 – 17.6

Values in parenthesis indicate percentage

TABLE 4.8 IMPAIRED GLUCOSE METABOLISM AMONG THE SUBJECTS

	Ahmedabad	**Baroda**	**Total**
	N=193 (%)	**N=186 (%)**	**N=379 (%)**
Diabetes (Old Cases)	20 (9.1); 95% CI: 5.2-13.0	4 (2.1); 95% CI: 0-4.2	24 (6.3); 95% CI: 3.9-8.7
Impaired Fasting Glucose (IFG)	49 (25.4); 95% CI: 19.2-31.6	16 (8.6); 95% CI: 4.5-12.7	65 (17.1); 95% CI: 13.3-20.9
Impaired Glucose Tolerance (IGT)	5 (2.6); 95% CI: 0.4-4.8	-	-
Diabetes (FBS>125mg/dl)	9 (4.7); 95% CI: 1.7-7.7	12 (6.4); 95% CI: 2.9-9.9	21 (5.5); 95% CI: 3.2-7.8
Hyperinsulinemia (>12μIU/ml)	42 (28.4); 95% CI: 22.0-34.8	26 (14.0); 95% CI: 9.1-18.9	68 (20.3); 95% CI: 16.2-24.4
Insulin Resistance (HOMA2 >1.4)	47 (31.7); 95% CI: 25.1-38.3	39 (21.0); 95% CI: 15.1-26.9	86 (25.7); 95% CI: 21.3-30.1

Values in parenthesis indicate percentage

higher (72.9%) than those for Baroda (57.5%). Almost one-third of the subjects (Table 4.9) had elevated TC levels (33.8%), the prevalence being far greater in Ahmedabad (39.3%) than in Baroda (27.9%). Elevated TAG levels were seen in 17% of the subjects (Figure 4.5). Yet another common anomaly in the lipoprotein fractions was reduced HDL, which was found to the order of 47.3% in the subjects (Ahmedabad 40%, Baroda 55%). The ratio of TAG to HDL, an index of highly atherogenic small dense LDL particles, was also found to be in the higher than normal range in more than one-fifth of the participants (22.4%). **This draws attention to the imperative need for alternative adjunct therapies to keep the lipid levels under control.**

Metabolic Syndrome (MS)

The metabolic syndrome spells danger mainly because it represents a highly risky situation due to clustering of a number of interlinked risk factors that lead to different clinical conditions, namely obesity, hypertension, diabetes and dyslipidemia. In the present study, the prevalence (Figure 4.6) of metabolic syndrome was particularly high: 35.8%, meaning roughly one in every three study participants had metabolic syndrome. The prevalence was higher in subjects from Baroda (41.9%) as compared to Ahmedabad (30.5%), which is upsetting due to the fact that the sample from Baroda was a free-living population which is not expected to have a high prevalence of cardio-metabolic risk factors compared to individuals who seek health care at a health check-up facility. This means a notable amount of undetected risk is present in the free-living population also and is a cause of concern due to lack of appropriate surveillance measures to detect the development of these risk factors so that it is not too late to avail therapy.

Atherogenic Index of Plasma (AIP)

An improvisation of the indicator of small dense LDL, TAG/H, is its log transformed values, known as the Atherogenic Index of Plasma (AIP), is an efficient quantitative indicator of atherogenicity. In the present study population, the prevalence of aberrations in this indicator (Table 4.10) was to the order of almost 60% (Table 4.10), indicating increasingly high levels of

TABLE 4.9 PERCENT PREVALENCE OF DYSLIPIDEMIA AMONG SUBJECTS

Dyslipidemia	Ahmedabad		Baroda		Total	
	N=213 (%)	95% CI	N=186 (%)	95% CI	N=399 (%)	95% CI
TC ≥200 mg/dl	83 (39.3)	32.7 – 455.9	52 (27.9)	21.4 – 34.4	135 (33.8)	29.1 – 38.5
TAG ≥150mg/dl	29 (13.7)	11.5 – 15.9	38 (20.4)	14.5 – 26.3	67 (17.0)	13.3 – 20.7
LDL ≥100 mg/dl	154 (72.9)	66.9 – 78.9	107 (57.5)	50.3 – 64.7	261 (65.2)	60.5 – 69.9
HDL ≤50mg/dl (F)	84 (39.8)	33.2 – 46.4	102 (54.8)	47.6 – 62.0	186 (47.3)	42.4 – 52.2
TAG/HDL≥3	43 (20.2)	14.8 – 25.6	46 (24.7)	18.4 – 31.0	89 (22.4)	18.3 – 26.5

FIGURE 4.5 PERCENT PREVALENCE OF DYSLIPIDEMIA AMONG SUBJECTS

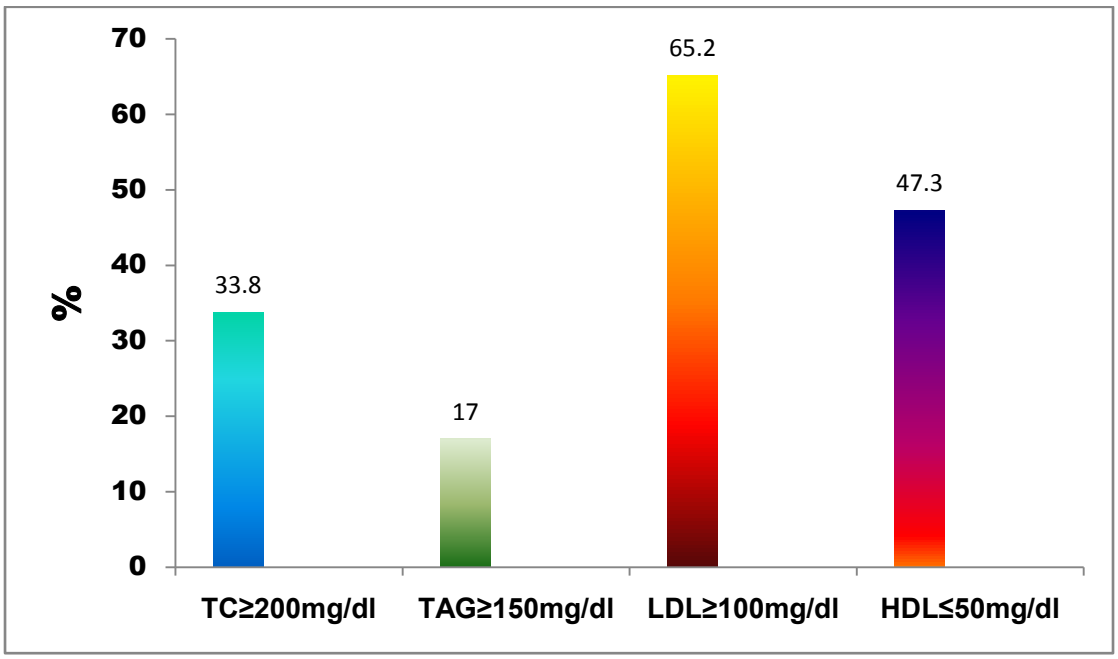


FIGURE 4.6 PREVALENCE OF METABOLIC SYNDROME AMONG THE STUDY POPULATION

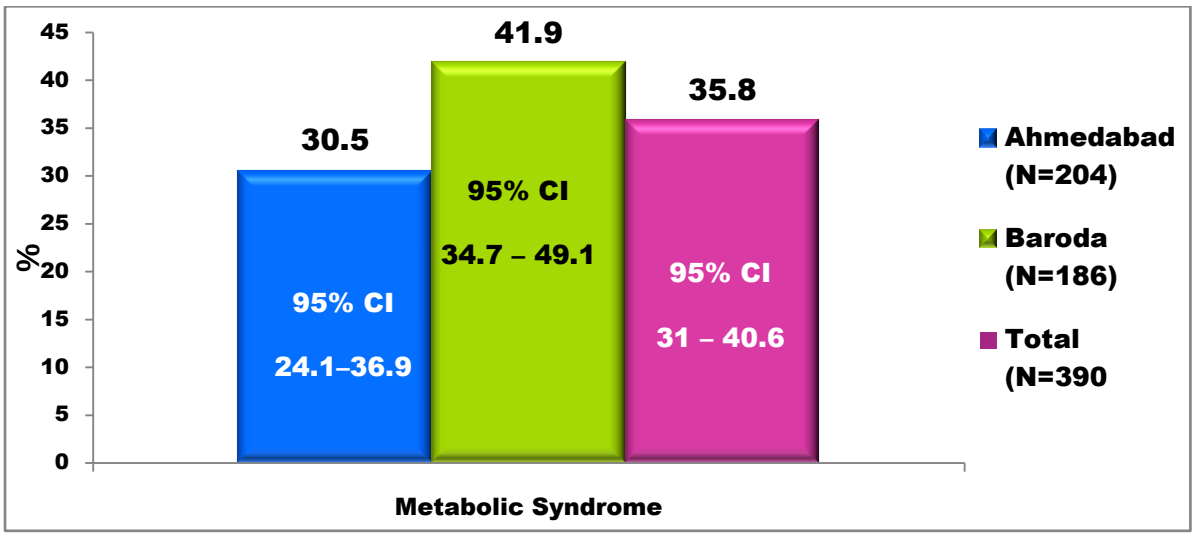


TABLE 4.10 EXTENT OF ABERRATIONS IN THE AIP LEVELS IN THE SUBJECTS

	Ahmedabad N=213	95% CI	Baroda	95% CI	Total	95% CI
AIP - Low risk (<0.11)	83 (32.3)	25.9 – 38.7	**26 *** (13.9)**	8.9 – 18.9	109 (27.3)	22.9 – 31.7
Intermediate risk (0.11 – 0.21)	29 (13.7)	9.1 – 18.3	39 (20.9)	15.0 – 26.8	68 (17.0)	13.3 – 20.7
High risk (≥0.22)	115 (54.0)	47.2 – 60.8	**121 * (65.0)**	58.1 – 71.9	236 (59.1)	54.2 – 64.0

* Significantly different from Ahmedabad at p<0.05, *** p<0.001

atherogenicity in the population. Here again, the free-living population in Baroda had higher levels of elevated AIP (65%) than the Ahmedabad population (54%) which was population attending a clinic and is logically purported to have higher risk.

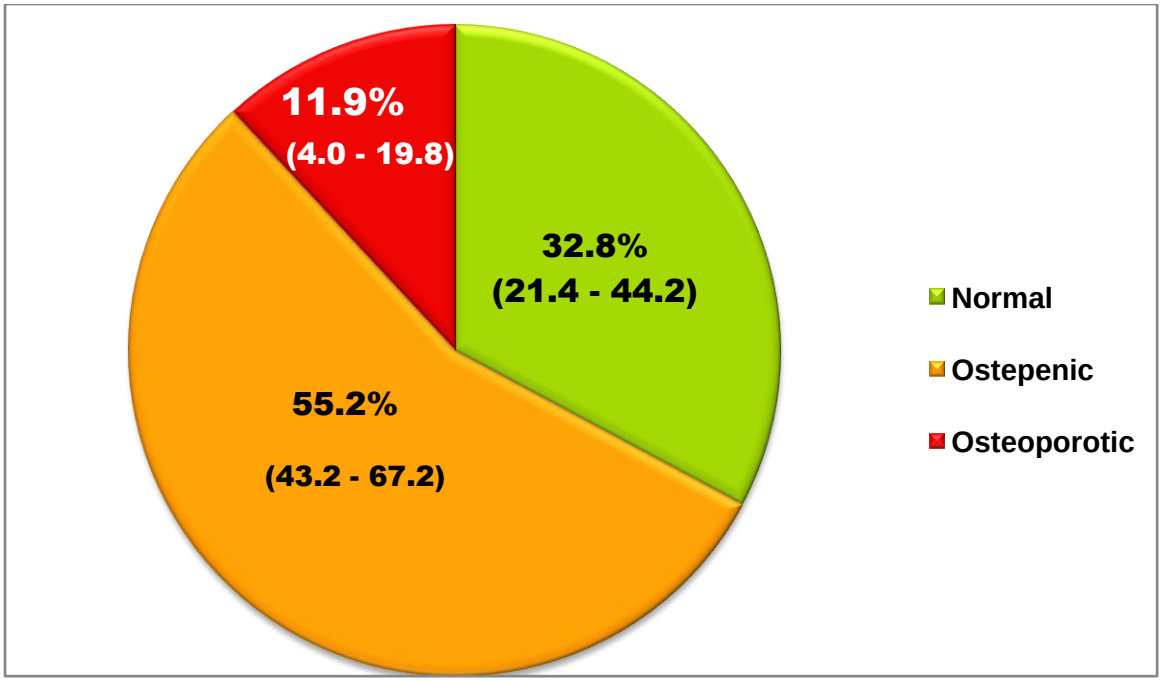
Low Bone Mass

The bone mineral density at calcaneus bone (heel bone) was collected in a subsample of 67 women in Baroda to study the bone health in the subjects (Figure 4.7). For a small sample size, it was observed a fairly high prevalence of osteoporosis (11.9%) was found in the subjects. The prevalence of the condition representing a stage that would advance to osteoporosis, osteopenia, was found to be as high as 55.2% in the subjects, indicative of the fact that a major proportion of the population has sub-optimal bone mass.

Comparative Prevalence of Cardio-Metabolic Conditions in Ahmedabad v/s Baroda

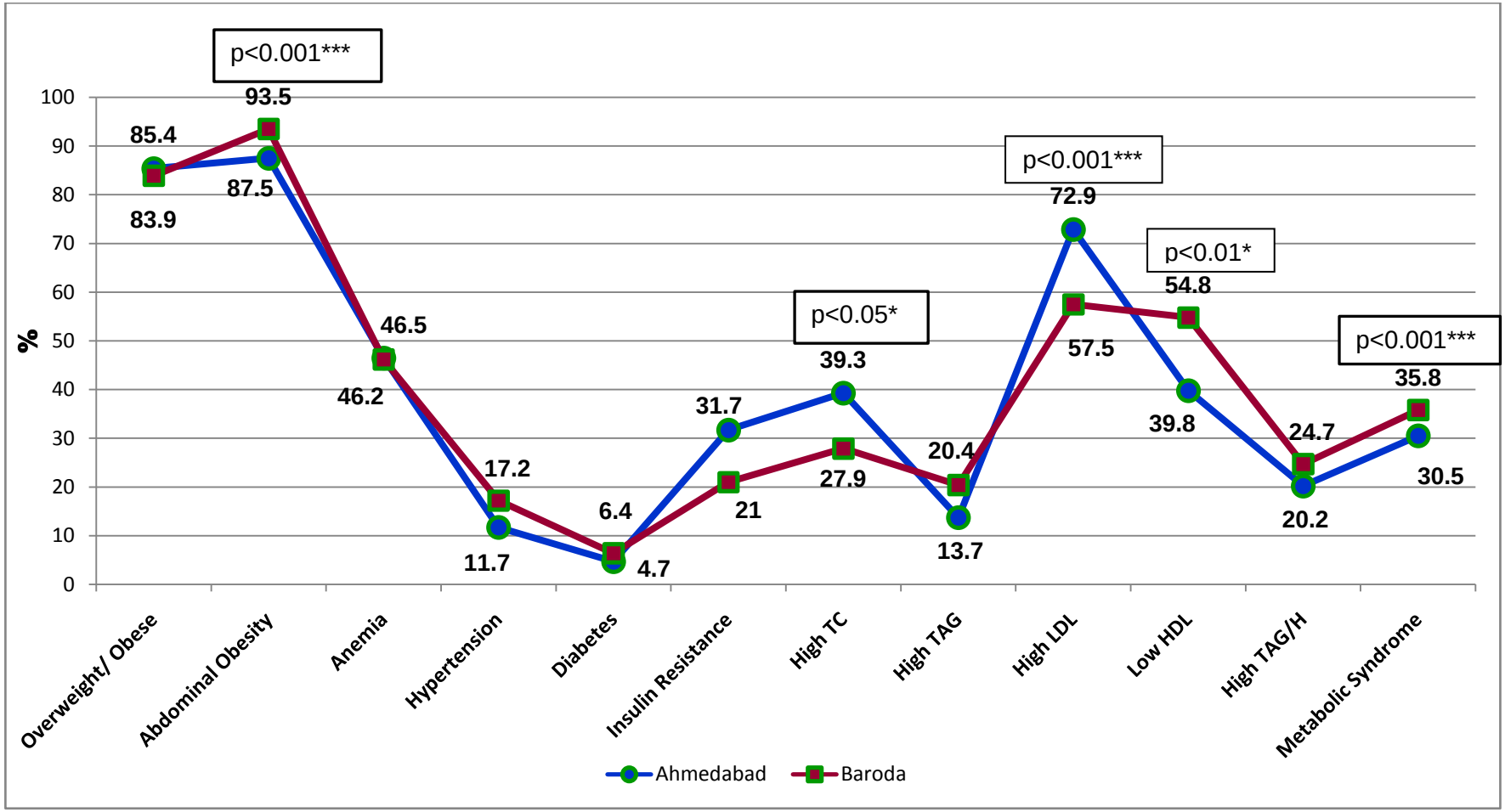
The comparison of cardio-metabolic risk scenario was made between Ahmedabad and Baroda (Figure 4.8). The prevalence of most of the risk factors and conditions was more or less similar in both the cities. The only differences were in case of prevalence of increased waist circumference, which was significantly high ($p<0.001$) in subjects in Baroda (93.5%) compared to Ahmedabad (87.5%); subnormal HDL (54.8% in Baroda versus 39.8% in Ahmedabad, $p<0.01$) and metabolic syndrome, which was prevalent to the order of 35.8% in Baroda, compared to 30.5% in Ahmedabad $p<0.001$). In Ahmedabad, the prevalence of elevated TC was significantly higher than in Baroda (39.3% versus 37.9%, $p<0.05$), in addition to prevalence of elevated LDL (72.9% versus 57.5% in Baroda, $p<0.001$). The prevalence of overweight/obesity, anemia and Diabetes was strikingly similar in both the cities.

FIGURE 4.7 PREVALENCE OF LOW BONE MASS IN THE SUBJECTS (BARODA, N=67)



Values in parenthesis indicate 95% CI limits

FIGURE 4.8 PREVALENCE OF CARDIO-METABOLIC RISK CONDITIONS IN AHMEDABAD V/S BARODA#



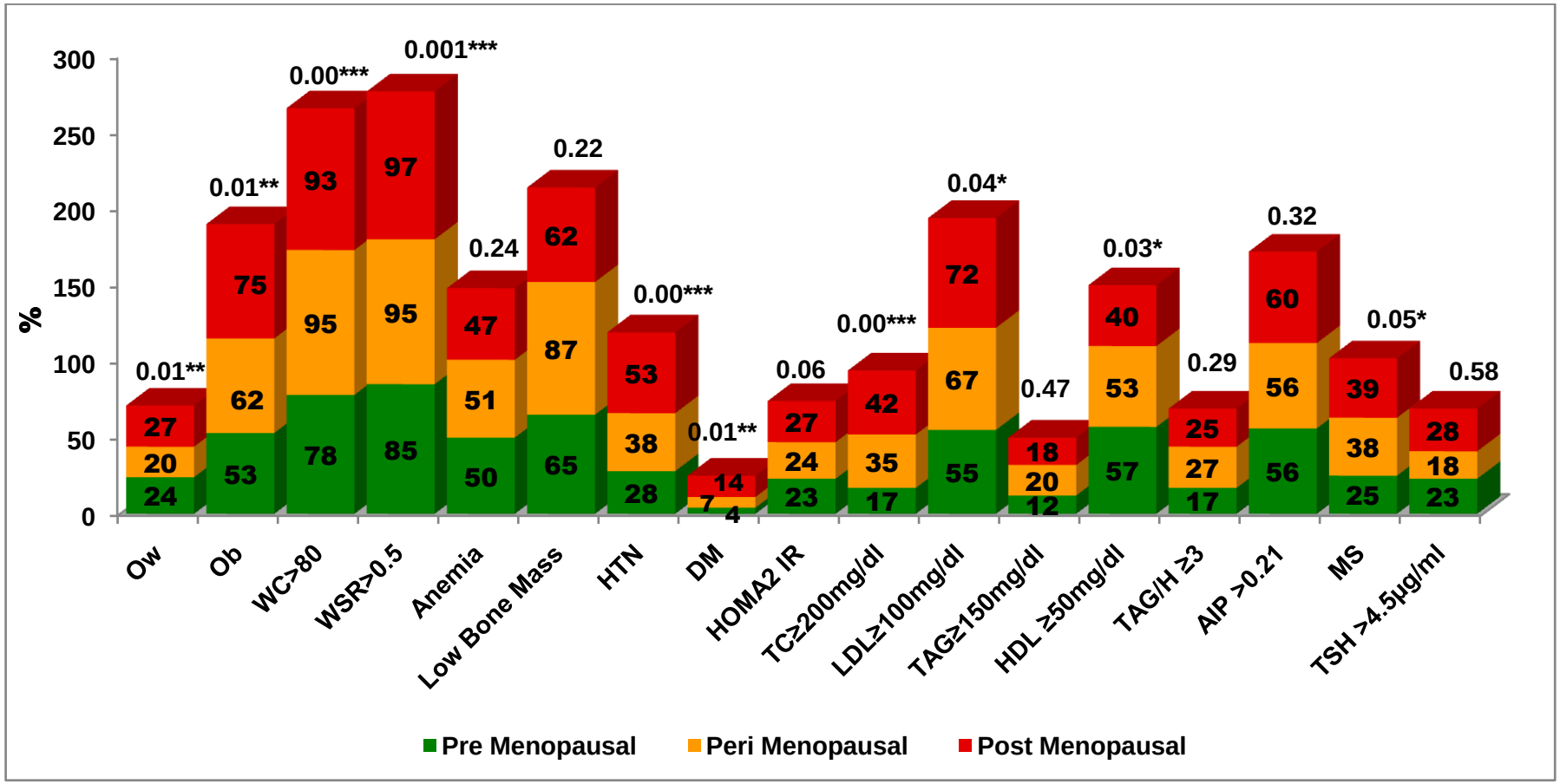
 # p values depicted are for Mantel-Haenszel Chi-square test between prevalence values for Ahmedabad and Barod

VARIATIONS IN CLINICO-BIOCHEMICAL CHANGES ACROSS MENOPAUSAL STAGES

One of the main objectives of the study was to track the prevalence of clinico-biochemical changes across various stages of menopause in the women studied. Therefore, proportion of women suffering from various cardio-metabolic risk factors was compared across pre, peri and post menopausal categories of women (figure 4.9). It was observed that the prevalence of elevated wsr was the highest (92.3%) among all other risk factors and conditions. The prevalence of all conditions varied widely across the menopausal categories. **The risk factors and conditions that had significantly higher prevalence in the pre and post menopausal categories were found to be overweight, obesity, abdominal obesity, high WSR, hypertension, diabetes, elevated TC, elevated LDL, low HDL and metabolic syndrome**. These statistics point out quite an evident role of menopausal transition in introducing imbalances that affect body composition, cardiovascular health and metabolic functions in women.

Among the conditions that were more or less equally distributed among pre, peri and post menopausal women and still were high, included anemia, low bone mass, and elevated AIP. **This draws attention to the fact that hemoglobin and bone mass are consistently low across all ages in Indian women and is a cause of serious concern.** Consistently increased levels of the atherogenicity marker, AIP, also is alarming since one would not expect such high levels of atherogenicity in pre menopausal group. A moderate prevalence of insulin resistance and high TSH levels were also found equally across the menopausal stages, which paints a grave picture of underlying metabolic chaos.

FIGURE 4.9 CLNICO-BIOCHEMICAL CHANGES ACROSS PRE, PERI AND POST MENOPAUSAL WOMEN (TOTAL N=399)



*Abreviations: Ow-Overweight, Ob-Obese, WC-Waist Circumference, WSR-Waist Stature Ratio, HTN-Hypertension, DM-Diabetes Mellitus, TC-Total Cholesterol, LDL-Low Density Lipoprotein, TAG-Triacylglycerols, HDL-High Density Lipoprotein, AIP-Atherogenic Index of Plasma, MS-Metabolic Syndrome and TSH-Thyroid Stimulating Hormone, * MaentelHanszel Chi square 2 tailed p values significant at p<0.05, **p<0.01, ***p<0.001*

AGE WISE PREVALENCE OF IMPAIRED GLUCOSE CONTROL AND IMPAIRED BLOOD PRESSURE CONTROL

Impaired Glucose Control

Given the high prevalence of diabetes and the wide age range of the subjects in the study sample in Ahmedabad, the distribution of the data across age decades was seen so as to detect any trends in the period of development of the disease during the lifespan (Figure 4.10). As expected, the prevalence of IFG (Figure 4.10a) kept doubling in each decade till 41-50 years and reached its maximum (37.5%) in the 61-70 years decade. Regarding IGT (Figure 4.10b), the prevalence was low and more or less the same during 31-40, 41-50 and 51-60 years, after which it peaks to 15% in the highest age decade. In case of diabetes (Figure 4.10c), there was a steady increase in the prevalence across all age groups until it peaked (24.2%) in the oldest age decade of 71-80 years, thus clearly implicating the increasing risk of impaired glucose metabolism with increase in age.

Thus the data on glucose challenge in a clinical setting cross tabulated with age showed that

1. IFG was seen across ages of 20 – 60 years. Prevalence of nearly 12% in the age group of 20 – 40 years is of great concern.
2. Though IGT was not seen at younger ages (20 – 30 years) the prevalence of 3.5% at higher ages merits attention.
3. The prevalence of confirmed diabetes increased with advancing age, with 2.6% in the age group of 20 – 30 years.
4. An alarming 8.4% of the subjects were diagnosed as diabetics.
5. Prediabetes condition i.e. abnormal FBS and PG2BS was seen peaking at 51 - 60 years and 71 – 80 years.

FIGURE 4.10 AGE WISE PREVALENCE OF IMPAIRED FASTING GLUCOSE AND IMPAIRED GLUCOSE TOLERANCE AND DIABETES (AHMEDABAD, N=213)

Figure 4.10a IFG

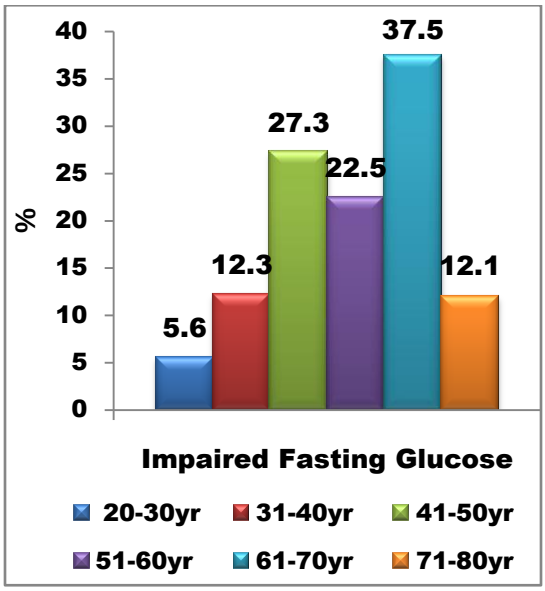


Figure 4.10b IGT

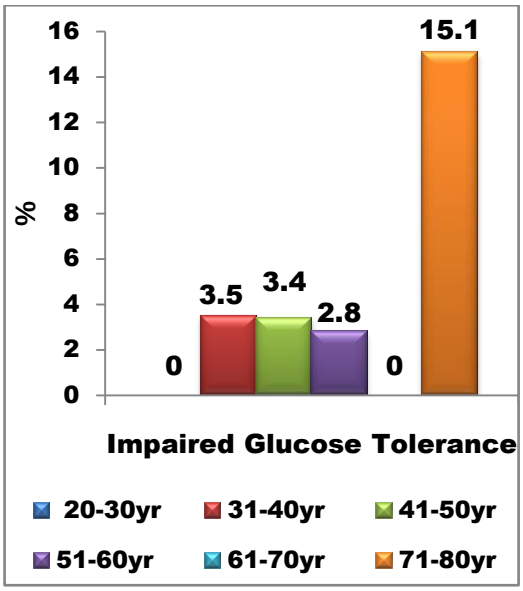
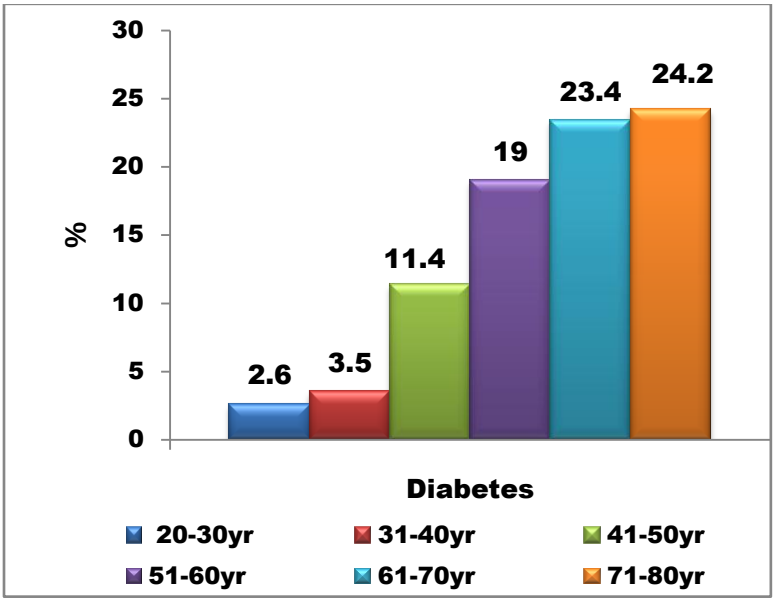


Figure 4.10c Diabetes



Impaired Blood Pressure Control

As with the case with diabetes, prevalence of pre-hypertension and hypertension was also compared across age decades (Figure 4.11). The data of pre-hypertension (Figure 4.11a) in Ahmedabad showed that the highest prevalence (44.7%) was in the decade of 20-30 years, which then fell as the age increased, which could be indicative of the fact that the individuals who had pre–hypertension in the younger years have developed hypertension in the older decades, hence the decline in the prevalence of pre-hypertension. However, regarding hypertension, the prevalence tends to increase across age decades (Figure 4.11b). The prevalence was 26.3% in the age group of 21-30 years, which increased to 80.7% in the age range of 31-40 years, before dropping to 31.8% in the next decade and then climbing a steady rise till 66.7% in the 71-80 years age group. In case of Baroda, the prevalence of pre-hypertension (Figure 4.11a) was highest in 41-50 years and more or less around 23% in rest of the decades. Regarding hypertension, the prevalence in Baroda was 38.6% in 31-40 year age group which increased to 64.4% in the 51-60 year decade, before declining to 58.8% in the 61-70 age group. Thus early prevention measures to deal with the increasing prevalence of hypertension in the older age groups needs to be worked out.

DIETARY AND LIFESTYLE HABITS AMONG THE SUBJECTS

Physical Activity

The information on lifestyle habits revealed that the physical activity levels of the subjects were not adequate (Table 4.11). Almost half of the subjects in both Ahmedabad and Baroda did not engage in regular physical activity, with only 56.4% of the subjects doing regular physical activity. Even among those who did engage in regular physical activity, the proportion of those who exerted for more than 3 hours per week was 61.9%, meaning for the one third of them, the physical activity did not even last for 3 hours for an entire week, which was far from adequate.

.FIGURE 4.11a AGE WISE PREVALENCE OF PRE-HYPERTENSION

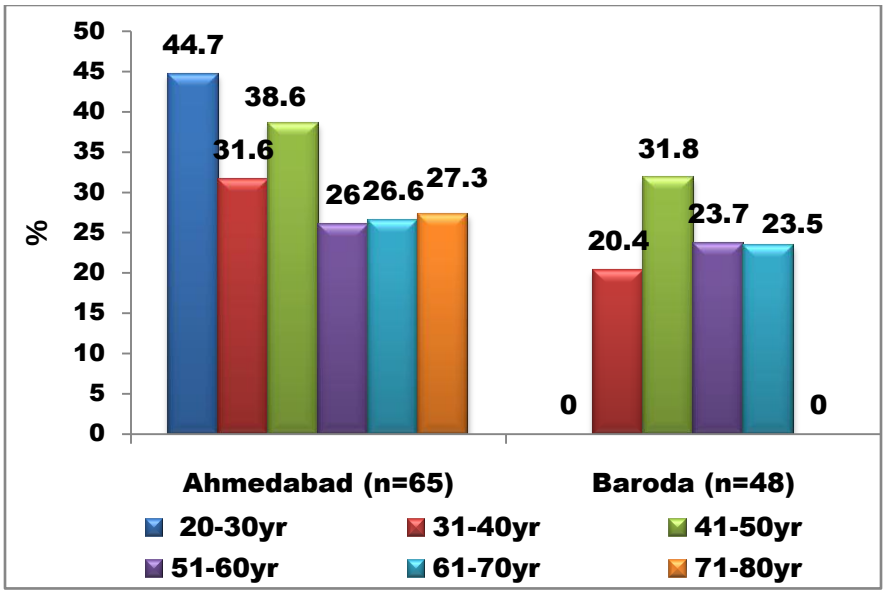


FIGURE 4.11b AGE WISE PREVALENCE OF HYPERTENSION

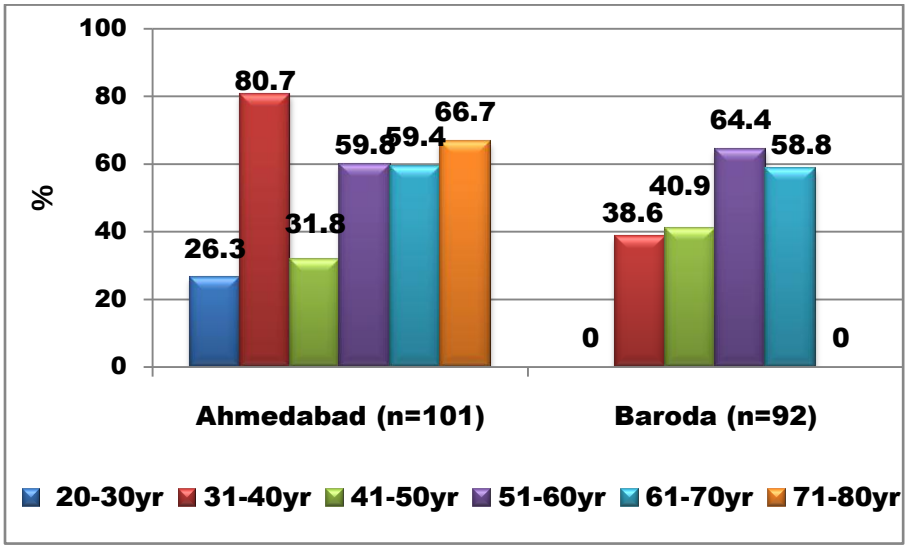


TABLE 4.11 PHYSICAL ACTIVITY LEVELS OF THE SUBJECTS

	Ahmedabad N=213	Baroda N=186	Total N=399	95% CI
Regular Physical Activity	118 (55.4)	107 (57.5)	225 (56.4)	51.5 – 61.3
<3hours/week	73 (61.9)	85 (79.4)	158 (70.6)	64.6 – 76.6
>3hours/week	45 (38.1)	22 (20.5)	67 (29.3)	23.3 – 35.3

Values in parenthesis indicate percentage

Type of the Oil used by the subjects

The type of oil used by the subjects gives an idea about the atherogenicity of the diet consumed by the subjects. In the present study (Figure 4.12), almost half of the subjects consumed groundnut oil on a daily basis, which if consumed in moderation is good for the cardiac heath because of the monounsaturates present in it. This was followed by cottonseed oil (19-22.6%) and sunflower oil (18.5%), A host of other oils including mustard, soybean, olive, sesame and corn were also consumed by a small fraction of the subjects.

Frequency of Having High Risk Foods

The food frequency data indicated that the frequency of consumption of foods having a high saturated and tans fat content and foods containing large amounts of refined sugar and carbohydrates, typically fried snacks & packaged snacks and sweets and bakery/confectionery items, was considerably high in the study subjects. Almost 41% of subjects in Ahmedabad consumed fried snacks (Figure 4.13) more frequently than once a week, while 38% of those in Baroda did the same. Similarly, 40% of the participants in Ahmedabad consumed bakery/confectionery items (Figure 4.14) more frequently than once a week, while 33% of those in Baroda did so. This is reflective of a high risk dietary behavior, which might be contributing to the thrifty phenotype of Indians.

Frequency of Having Healthy Foods

Now as far as healthy behavior is concerned most of the respondents in both Ahmedabad and Baroda reported that they have fruits (Figure 4.15) as frequently as more than a week (Ahmedabad 68%, Baroda 71%). Similarly 69% of respondents in both Ahmedabad and Baroda reported having salads and raw vegetables more frequently than once a week (Figure 4.16). The reported behavior however does not explain the high risk factor prevalence among the subjects, implicating a fair degree of over reporting.

FIGURE 4.12 TYPE OF OIL USED BY THE SUBJECTS

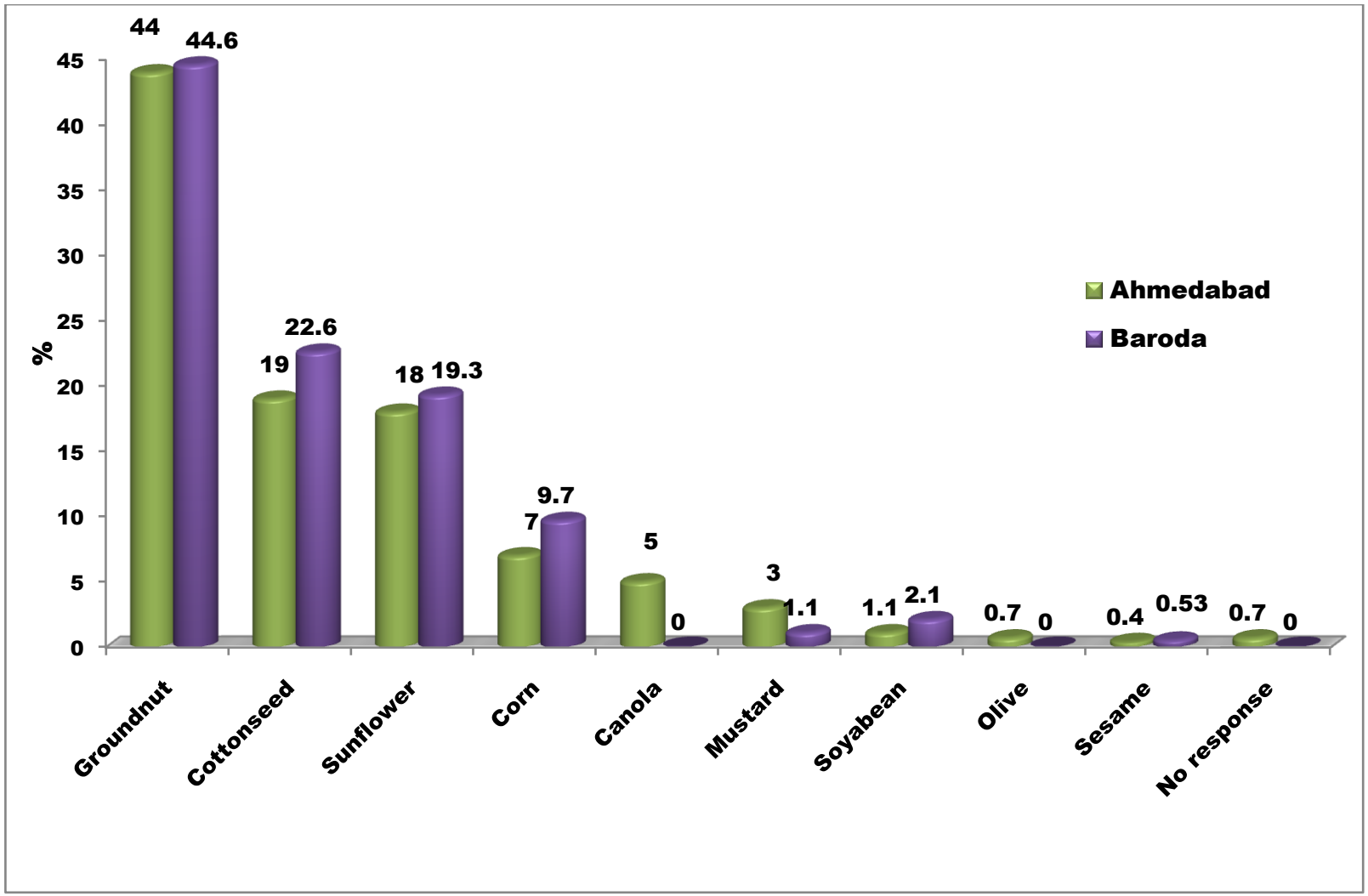


FIGURE 4.13 FREQUENCY OF HAVING FRIED SNACKS

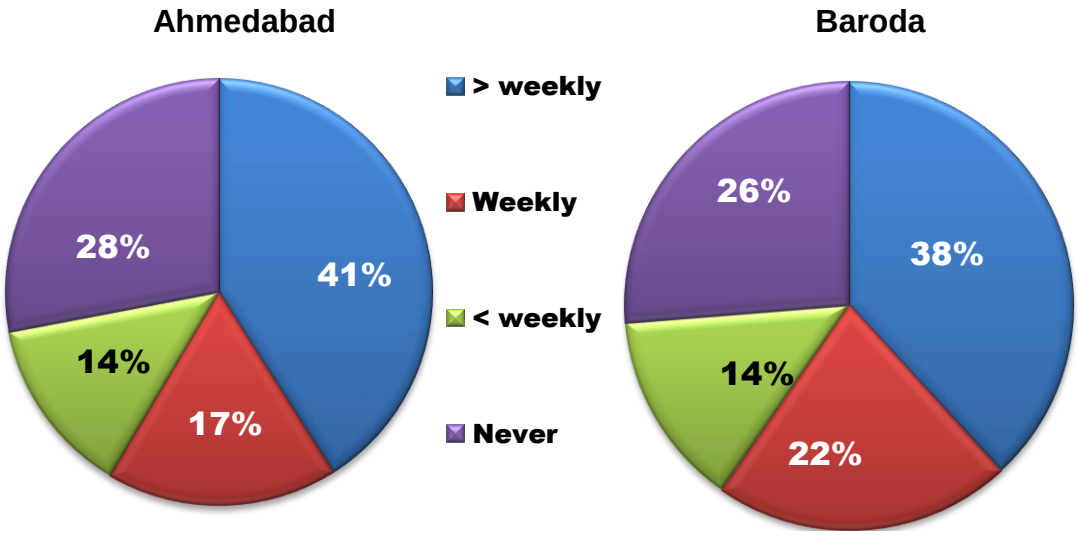


FIGURE 4.14 FREQUENCY OF HAVING BAKERY/CONFECTIONERY ITEMS

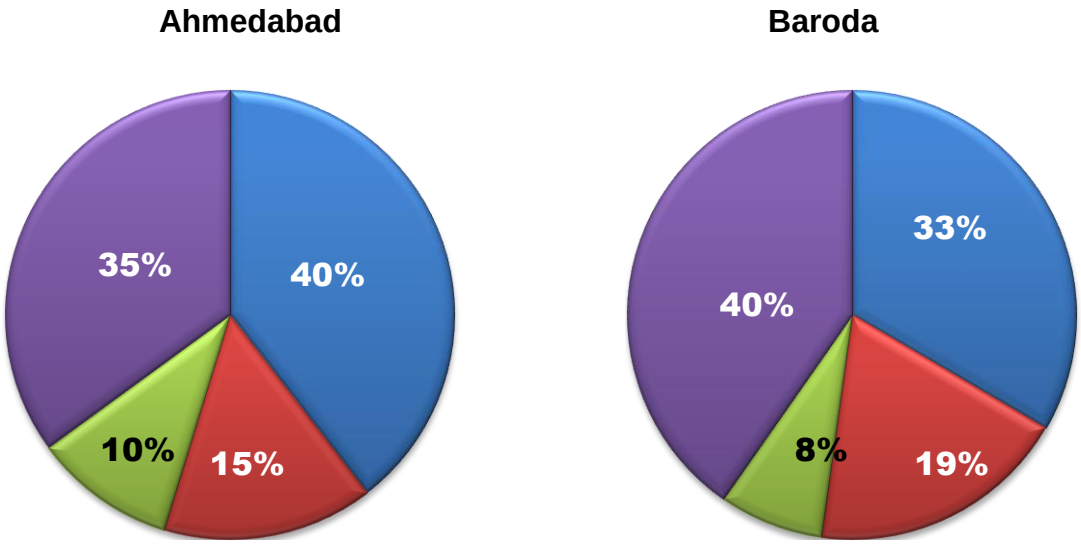


FIGURE 4.15 FREQUENCY OF HAVING FRUITS

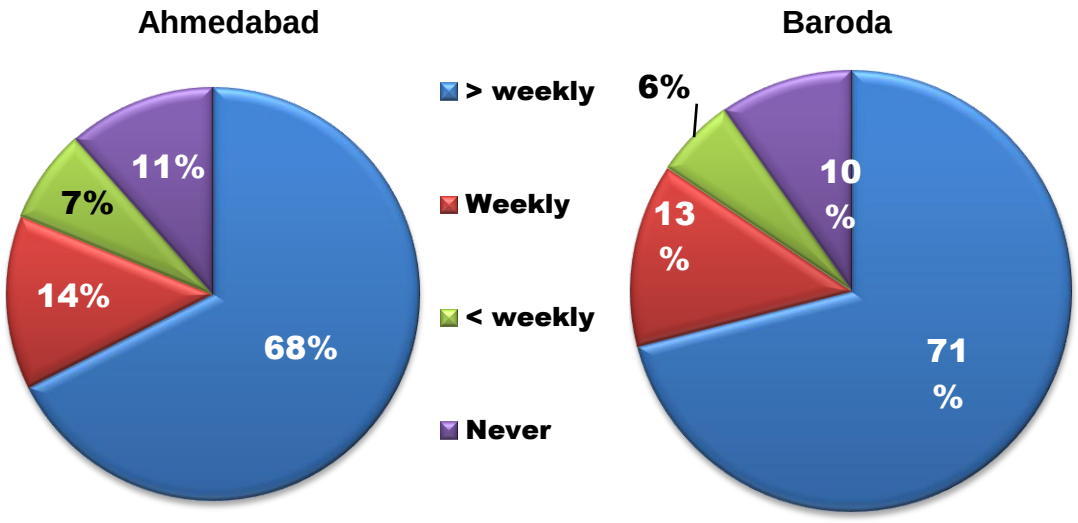
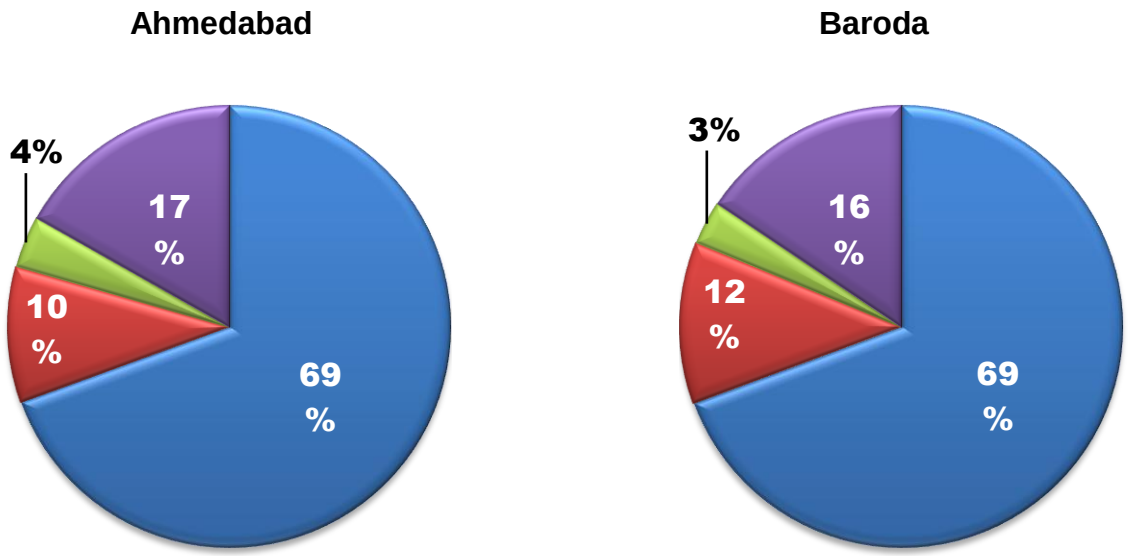


FIGURE 4.16 FREQUENCY OF HAVING SALADS



Nutrient Intakes

The information on nutrient intake was collected by 24 hour dietary recall method. The mean value for each nutrient and its corresponding % RDA as per 2009 guidelines is given in Table 4.12. The mean value for protein (55g Kcal/d), and Vitamin C (95 mg/d) met the requirements as per RDA for Ahmedabad. However the mean values for fat (66g/d) were far exceeding the percent daily requirements given by RDA and on the other hand, β carotene values (1863µg) met only a small percent of the daily requirements (39.5%). In case of Baroda, lower amount of energy requirements were met (82%), as well as protein (80.4%), iron (48%) and β carotene (30.8%). Only the vitamin C values fulfilled the daily requirements (123%), while the fat intake was quite high (153%). The nutrient intakes clearly indicate that the diet needs to incorporate more of micronutrients and less of fat. The percent distribution of calories from various nutrients is given in Figure 4.17. The contribution of the macronutrients to the calorie intake was observed to be 57.4% from carbohydrates, 32.8% from fats and 9.8% from proteins. It is evident that the energy intake from fat exceeded the recommended allowance of 20% energy.

Percent Calories from Fat

As the mean value for fat was considerably higher than the recommended daily allowance, the percentage of subjects consuming more than 30% of calories from fat were calculated (Table 4.13). It appeared that more than 70% of the subjects consumed more than 30% of calories from fat. This pattern of fat consumption is bound to increase the future cardio-vascular health risk and development of adverse metabolic and clinical conditions.

Correlation between fat intake and various variables

Bivariate analysis of extent of relation between fat intake and different variables was carried out in order to see the impact of high consumption of fat on other parameters. However a clear trend was not evident as none of the anthropometric indices or lipoprotein fractions showed a significant correlation with fat intake (Table 4.14).

TABLE 4.12 NUTRIENT INTAKES OF THE SUBJECTS

Nutrients	Ahmedabad (N=199)	% RDA	Baroda (N=186)	% RDA
Energy (Kcal)	1733±370	91.0	1562 ± 233	82.0
CHO (gms)	203±44.2	-	189 ± 30	-
Fat (gms)	66±20.9	176.2	61.4 ± 15	153.5
Protein (gms)	55±21.1	100.0	44.2 ± 12	80.4
Iron (mg)	17±20.9	81.7	10.1 ± 4.3	48.1
Vitamin C (mg)	95±38.5	158.0	74 ± 21	123.3
Crude Fibre (gms)	8±2.1	-	6.35 ± 1.2	-
β carotene (µg)	1863±1932	39.5	1447 ± 788	30.8

Values in parenthesis indicate percent RDA

FIGURE 4.17 PERCENT DISTRIBUTION OF CALORIES FROM VARIOUS NUTRIENTS (AHMEDABAD AND BARODA)

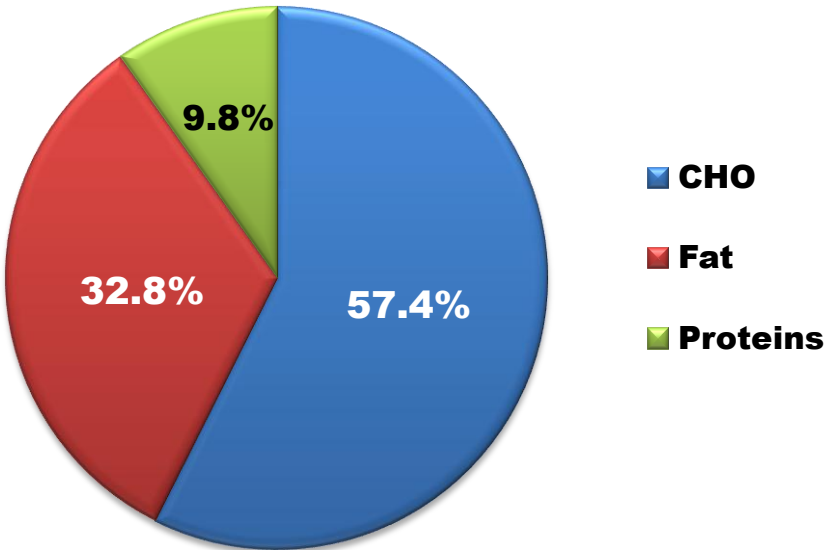


TABLE 4.13 PERCENT DISTRIBUTION OF SUBJECTS BASED ON FAT INTAKE

	Ahmedabad (N=184)	95% CI	Baroda (n= 186)	95% CI	Total (n= 370)	95% CI
	n (%)		n (%)	n	n (%)	
≥30% kcal from fat	134 (69.1)	62.4 – 75.7	161 (86.5)	81.5 – 91.5	295 (79.7)	75.6 – 83.8
<30% kcal from fat	60 (30.9)	24.2 – 37.5	25 (13.5)	8.5 – 18.5	85 (23.0)	18.7 – 27.3

TABLE 4.14 CORRELATION BETWEEN FAT INTAKE, BMI, WC AND WHR FOR AHMEDABAD AND BARODA (N=399)

Variables	Correlation	p value
Fat intake and BMI	0.05	0.39
Fat intake and WC	0.01	0.84
Fat intake and WHR	0.12	0.06
Fat intake and WSR	0.08	0.20
Fat intake and SBP	0.02	0.68
Fat intake and DBP	0.005	0.93
Fat intake and FBS	0.12	0.06
Fat intake and TC	0.04	0.52
Fat intake and LDL	0.01	0.81
Fat intake and TAG	0.11	0.08
Fat intake and TG/H	0.11	0.77
Fat intake and AIP	0.09	0.18

INTERLINKS BETWEEN CLINICAL CONDITIONS AND RISK FACTORS

In this study the effect of presence of one type of metabolic condition on alterations in other risk factors was analyzed. Therefore deviations in anthropometric/body composition, biophysical and biochemical parameters like WC, WSR, BMI, FBS, HOMA 2 IR, TC, TAG, LDL, HDL, TAG/H, TSH and AIP were studied in relation to presence and absence of clinical conditions like obesity, hypertension, metabolic syndrome and diabetes.

LINKS OF OBESITY WITH RISK FACTORS

The mean biochemical parameters were studied in relation to presence of obesity as defined by increased BMI; and in relation to abdominal obesity, as defined by increased WC and in relation to increased WSR. Data for Ahmedabad and Baroda has been described separately and analysis on pooled data has been shown as well in tables 4.15 to 4.23. In the pooled data, it is evident that in all the anthropometric indices, WC was the strongest predictor of aberrations in the biochemical parameters, because 7 biochemical parameters (PG2BS, Insulin, HOMA IR, TAG, HDL, LDL/HDL and TC/HDL) across categories of normal and increased WC differed significantly. In case of BMI, 4 parameters (Insulin, HOMA IR, TAG, and TAG/HDL) showed significant difference in the obese and overweight group. Regarding WSR, again 4 parameters (PG2BS, TAG, HDL and TAG/HDL) were significantly different in individuals with normal WSR than those with high WSR. The data from Baroda did not reflect any clear association between WSR (Table 4.22) and the risk factors, as did data from Ahmedabad (Table 4.21) though, where elevated WSR was associated with significantly high FBS, PG2BS, TAG, HDL, TC/HDL and TSH. Elevated WC was associated with significantly higher PG2BS, TAG, TC/HDL and lower HDL in subjects from Ahmedabad (Table 4.18). In Baroda, higher WC (Table 4.19) was found to be associated with significantly higher hemoglobin levels, TAG, LDL/H, TC/H and TAG/H. High BMI was found to be associated with significantly higher TAG levels and higher TC/H ratios in Ahmedabad (Table 4.15). In Baroda however high BMI did not appear to significantly influence any of the lipid parameters or glucose control parameters (Table 4.16).

TABLE 4.15 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH BMI (AHMEDABAD N=213)

	Normal N=31	Overweight N=30	Obese N=152	ANOVA p value
Hb(g/dl)	11.5±1.42	11.88±2.0	12.04±1.23	0.24
FBS(mg/dl)	101.3±24.9	107.47±37.1	103.16±21.5	0.60
PG2BS(mg/dl)	108.7±42.7	114.1±49.6	120.9±40.9	0.35
TC(mg/dl)	184.7±40.8	187.6±39.3	194.6±36.5	0.34
TAG(mg/dl)	87.1±49.9	94.3±40.5	110.8±53.4	0.04*
LDL(mg/dl)	108.7±34.0	115.4±33.1	119.5±30.9	0.23
HDL(mg/dl)	60.0±15.1	53.9±10.9	53.9±13.4	0.08
LDL/HDL	1.9±0.6	2.1±0.7	2.9±6.3	0.61
TC/HDL	3.2±0.8	3.5±0.8	3.7±1.1	0.03*
TSH(µIU/ml)	7.1±19.4	3.3±2.7	3.8±7.8	0.23
Insulin(mg/dl)	0.7±0.5	0.6±0.1	0.6±0.1	0.32

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.15a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS BMI CATEGORIES (AHMEDABAD N=213)

	p VALUE	
VARIABLES	**TAG**	**TC/HDL**
Normal Vs OW	0.29	0.05
Normal Vs Ob	0.01*	0.0008**
OwVs Ob	0.02	0.08

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.16 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH BMI (BARODA N=186)

Parameter	Normal N=30	Overweight N=39	Obese N=117	ANOVA p value
Hb (g/dl)	11.3 ± 1.3	12.0 ± 1.3	12.1 ± 1.3	0.02*
FBS (mg/dl)	84.6 ± 13.1	87.3 ± 25.7	94.8 ± 27.3	0.07
Insulin ()	8.4 ± 3.3	8.4 ± 3.8	9.3 ± 4.1	0.31
HOMA IR	1.1 0.4	1.1 ± 0.5	1.2 ± 0.5	0.20
TC (mg/dl)	189 ± 45.9	175 ± 29.4	182 ± 41.6	0.37
TAG (mg/dl)	110 ± 57.7	104 ± 48.3	122 ± 61.6	0.20
LDL (mg/dl)	115 ± 35.7	105 ± 24.5	109 ± 34.1	0.43
HDL (mg/dl)	51.7 ± 10.5	49.2 ± 10.2	48.4 ± 10.5	0.31
LDL/HDL	2.3 ±0.6	2.2 ± 0.7	2.3 ± 0.7	0.75
TC/HDL	3.7 ± 0.8	3.7 ± 0.9	3.9 ± 0.9	0.47
TAG/HDL	2.3 ± 1.5	2.3 ± 1.4	2.8 ± 0.9	0.21
TSH (µIU/ml)	3.0 ± 1.8	3.8 ± 2.4	6.8 ± 17.8	0.29

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.16a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS BMI CATEGORIES (BARODA N=186)

Hb	
	p value
Normal Vs OW	0.03
Normal Vs Ob	0.007*
OWVs Ob	0.9

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.17 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH BMI (TOTAL N=399)

	Normal N=60	Overweight N=70	Obese N=269	ANOVA p value
Hb (g/dl)	13.1 ± 1.3	12.4 ± 1.6	12.0 ± 1.3	0.34
FBS (mg/dl)	92.9 ± 21.7	106.6 ± 32.3	100.0 ± 26.0	0.13
PG2BS (mg/dl)	108.7±42.7	114.1±49.6	120.9±40.9	0.35
Insulin (µIU/ml)	7.63 ± 4.2	11.1 ± 4.8	10.5 ± 7.9	0.005**
HOMA IR	1.0± 0.6	1.5 ± 0.7	1.4 ± 0.9	0.001***
TC (mg/dl)	187.6 ± 41.4	198.3 ± 35.2	189.4 ±39.1	0.19
TAG (mg/dl)	98.9 ± 53.8	163.3 ± 45.5	115.9 ± 57.2	0.01**
LDL (mg/dl)	112.7 ± 33	119 ± 30.3	115.1 ± 32.6	0.28
HDL (mg/dl)	55.6 ± 13.9	45.2 ±10.7	51.4 ± 12.6	0.06
LDL/HDL	2.13 ± 0.7	2.6 ± 0.7	2.6 ± 4.7	0.50
TAG/HDL	1.97 ± 1.3	3.8 ± 1.3	2.5 ± 1.9	0.01**
TSH (µIU/ml)	5.0 ± 13	4.2 ± 2.6	5.1 ±13.2	0.62
Creatinine (mg/dl)	0.7±0.5	0.6±0.1	0.6±0.1	0.32

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.17a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS BMI CATEGORIES (TOTAL N= 399)

VARIABLES	p VALUE			
	Insulin	HOMA IR	TAG	TAG/H
Normal Vs OW	0.58	0.61	0.99	0.62
Normal Vs Ob	0.009*	0.005*	0.03	0.02
OwVs Ob	0.02	0.01*	0.02	0.05

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.18 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WC (AHMEDABAD N=184)

N=185	Normal N=82	High WC N=102	Students' 't' p value
Hb(g/dl)	11.8±1.6	12.1±1.1	0.08
FBS (mg/dl)	102.4±26.9	108.2±30.4	0.34
PG2BS (mg/dl)	107.8±28.5	122.8±39.8	0.003**
TC (mg/dl)	191.0±38.1	193.7±39.0	0.32
TAG (mg/dl)	94.3±38.2	111.8±55.3	0.007**
LDL (mg/dl)	116.1±33.2	119.1±32.6	0.27
HDL (mg/dl)	56.9±13.7	53.4±13.4	0.04*
LDL/HDL	3.1±8.5	2.3±0.8	0.19
TC/HDL	3.5±0.9	3.7±1.1	0.04*
TSH (µIU/L)	4.4±11.2	4.2±9.4	0.44
Creatinine (mg/dl)	0.6±0.3	0.6±0.1	0.09

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.19 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WC (BARODA N=186)

Parameter	Normal N=12	High WC N=174	Students' 't' p value
Hb (g/dl)	11.0 ± 1.5	12.0 ± 1.3	0.01*
FBS (mg/dl)	80 ± 7.2	92 ± 26.1	0.11
Insulin	9.0 ± 3.6	9.0 ± 3.9	0.99
HOMA IR	1.1 ± 0.4	1.2 ± 0.5	0.78
TC (mg/dl)	169 ± 34.0	183 ± 40.0	0.26
TAG (mg/dl)	75 ± 19.0	119 ± 59.4	0.01**
LDL (mg/dl)	100 ± 28.7	110 ± 32.8	0.34
HDL (mg/dl)	53.1 ± 9.7	48.8 ± 10.5	0.17
LDL/HDL	1.9 ± 0.5	2.3 ± 0.7	0.05*
TC/HDL	3.2 ± 0.6	3.8 ± 0.9	0.02*
TAG/HDL	1.5 ± 0.5	2.7 ± 1.8	0.02*
TSH (µIU/L)	3.1 ±2.5	5.7 ± 14.7	0.54

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.20 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WC (TOTAL N=370)

N=348	Normal N=38	High WC N=332	Students' 't' p value
Hb(g/dl)	11.5 ± 1.4	12.3 ± 5.7	0.38
FBS(mg/dl)	90.3 ± 10.4	98.7 ± 28.0	0.06
PG2BS(mg/dl)	107.8±28.5	122.8±39.8	0.003**
Insulin	6.9 ± 3.9	10.0 ± 7.4	0.01**
HOMA IR	0.89 ± 0.5	1.3 ± 0.9	0.007*
TC(mg/dl)	179 ± 35.7	188.3 ± 39.9	0.18
TAG(mg/dl)	76.2 ± 30.2	114.2 ± 55.2	0.000***
LDL(mg/dl)	107.6 ± 30.0	114.2 ± 33.2	0.23
HDL(mg/dl)	56.9 ± 13.1	51.5 ± 12.3	0.01**
LDL/HDL	1.95 ± 0.6	2.32 ± 0.8	0.004**
TC/HDL	3.23 ± 0.6	3.8 ± 1.0	0.0009***
TSH(µIU/ml)	6.5 ± 16.2	4.8 ± 11.9	0.43
Creatinine(mg/dl)	0.6±0.3	0.6±0.1	0.09

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.21 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WSR (AHMEDABAD N=184)

N=184	Normal N=15	High WSR N=169	Students' 't' p value
Hb (g/dl)	11.8 ± 1.5	12.0 ± 1.3	0.36
FBS (mg/dl)	92.6 ± 5.7	104.5 ± 25.5	0.04*
PG2BS (mg/dl)	95.4 ± 27.0	118.1 ± 36.0	0.009**
TC (mg/dl)	180.9 ± 34.6	193.5 ± 38.8	0.11
TAG (mg/dl)	73.0 ± 40.4	106.7 ± 48.9	0.005**
LDL (mg/dl)	105.6 ± 31.6	118.8 ± 32.8	0.06
HDL (mg/dl)	61.8 ± 15.2	54.4 ± 13.3	0.02*
LDL/HDL	1.8 ± 0.6	2.8 ± 6.0	0.26
TC/HDL	3.0 ± 0.7	3.7 ± 1.0	0.01**
TSH (µIU/L)	10.5 ± 26.1	3.8 ± 7.4	0.009**
Creatinine(mg/dl)	0.6 ± 0.1	0..6 ± 0.2	0.19

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.22 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WSR (BARODA N=186)

Parameter	Normal N=12	High WSR N=152	Students' 't' p value
Hb (g/dl)	10.6 ± 1.5	12.0 ± 1.3	0.005**
FBS (mg/dl)	79 ± 7.1	92 ± 25.9	0.15
Insulin	10.1 ± 3.9	8.9 ± 3.9	0.41
HOMA IR	1.24 ± 0.5	1.2 ± 0.5	0.63
TC (mg/dl)	165 ± 31.1	183 ± 40.4	0.21
TAG (mg/dl)	80 ± 20.2	118 ± 59.2	0.06
LDL (mg/dl)	97 ± 22.4	110 ± 32.9	0.26
HDL (mg/dl)	50.7 ± 10.8	49 ± 10.5	0.66
LDL/HDL	2.00.4	2.3 ± 0.7	0.16
TC/HDL	3.3 ± 0.5	3.8 ± 0.9	0.10
TAG/H	1.6 ± 0.5	2.6 ± 1.8	0.10
TSH (µIU/L)	2.6 ± 1.0	5.7 ± 14.5	0.55

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.23 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HIGH WSR (TOTAL N=370)

N=348	Normal N=24	High WSR N=346	Students' 't' p value
Hb(g/dl)	11.5 ± 1.6	12.3 ± 5.6	0.5
FBS (mg/dl)	88.0 ± 8.8	98.6 ± 27.6	0.06
PG2BS (mg/dl)	95.4 ± 27.0	118.1 ± 36.0	0.009**
Insulin	7.4 ± 4.1	9.8 ± 7.3	0.13
HOMA IR	0.95 ± 0.5	1.3 ± 0.9	0.08
TC (mg/dl)	174 ± 33.7	188 ± 39.8	0.08
TAG (mg/dl)	74.6 ± 33.6	12.8 ± 24.7	0.0008***
LDL (mg/dl)	101.8 ± 28.2	114.8 ± 33.1	0.07
HDL (mg/dl)	57.5 ± 14.5	51.7 ± 12.2	0.02*
LDL/HDL	1.9 ± 0.6	2.54 ± 4.2	0.43
TAG/H	1.4 ± 0.8	2.41 ± 1.6	0.002**
TSH (µIU/L)	7.5 ± 20.5	4.8 ± 11.7	0.31
Creatinine(mg/dl)	0.6 ± 0.1	0..6 ± 0.2	0.19

**** Significant at p<0.05, **p<0.01, ***p<0.001***

TABLE 4.24 SUMMARY TABLE FOR BIOCHEMICAL INDICATORS STRATIFIED BY ANTHROPOMETRIC INDICES (TOTAL)

VARIABLES	BMI	WC	WSR
FBS (mg/dl)	**NS**	**NS**	*
PG2BS (mg/dl)	**NS**	**	**
TAG (mg/dl)	*	**	**
HDL (mg/dl)	**NS**	*	*
TC/HDL	*	*	*
TSH (µIU/L)	**NS**	**NS**	**

**** Significant at p<0.05, **p<0.01, ***p<0.001***

BIOCHEMICAL PARAMETERS IN RELATION TO DIETARY INTAKE AND PHYSICAL ACTIVITY

The most important modifiable risk factors for development of adverse cardiovascular and metabolic conditions are dietary and lifestyle behaviors, including physical activity and intake of excess energy-dense foods. in this regard, the biochemical parameters were crosstabulated with by intake of fried/packaged snacks and physical activity (Table 4.25 and 4.26 respectively).
It was observed that frequent consumption of fried snacks did not appear to have any significant association with the biochemical parameters; however the frequent consumption group did have higher mean levels of FBS, PG2BS, Insulin and TSH in analysis of pooled data of Ahmedabad and Baroda (Table 4.25). Similarly, between individuals performing regular physical activity and those who did not, no significant difference was seen in the biochemical parameters, however, physical activity group had lower mean FBS, Insulin, TC, TAG and TSH values (Table 4.26).

ASSOCIATION OF CLINICAL CONDITIONS AND BIOCHEMICAL PARAMETERS

A comparative analysis of biochemical parameters in those without any clinical conditions like MS, hypertension or diabetes versus those who do, are depicted in Tables 4.27 to 4.37.

Hypertension

It was observed that the mean values of FBS, TAG and HDL were significantly affected by presence of hypertension and pre-hypertension (Table 4.27). Further post hoc testing (Table 4.27a) revealed that difference of FBS was highest in

TABLE 4.25 BIOCHEMICAL PARAMETERS STRATIFIED BY FREQUENT CONSUMPTION OF FRIED SNACKS (TOTAL)

N=422	<Weekly N=165	≥Weekly N=234	Students' 't' 2 tailed p value
Hb(g/dl)	11.9 ± 1.3	12.4 ± 6.8	0.29
FBS (mg/dl)	96.4 ± 21.9	99.7 ± 29.6	0.22
PG2BS (mg/dl)	117.7±48.1	119.3±55.1	0.38
Insulin	9.3 ± 5.8	9.9 ± 7.8	0.48
HOMA IR	1.22 ± 0.8	1.3 ± 0.9	0.54
TC (mg/dl)	189 ± 38.3	186 ± 39.4	0.48
TAG (mg/dl)	110 ± 52.5	110 ± 57.3	0.94
LDL (mg/dl)	115.6 ± 31.1	112 ± 33.1	0.28
HDL (mg/dl)	51.6 ± 11.9	52.5 ± 13.0	0.49
LDL/HDL	2.32 ± 0.7	2.6 ± 5.0	0.52
TC/HDL	3.77 ± 1.0	3.7 ± 1.0	0.54
TAG/H	2.9 ± 1.8	2.4 ± 1.7	0.90
TSH (µIU/L)	4.7 ± 10.3	4.9 ± 13.1	0.89
Creatinine (mg/dl)	0.8±0.4	0.8±0.2	0.10

TABLE 4.26 BIOCHEMICAL PARAMETERS STRATIFIED BY PHYSICAL ACTIVITY LEVEL (TOTAL)

N=348	No Physical Activity N=174	Regular Physical Activity N=225	Students' 't' 2 tailed p value
Hb(g/dl)	12.6 ±7.8	11.9 ± 1.4	0.19
FBS (mg/dl)	99.4 ± 29.8	97.4 ± 24.2	0.47
PG2BS (mg/dl)	118.3±48.7	118.9±55.2	0.45
Insulin	9.8 ± 7.5	9.6 ± 6.7	0.78
HOMA IR	1.3 ± 0.9	1.24 ± 0.8	0.57
TC (mg/dl)	188.3 ± 41.1	186.8 ± 37.2	0.70
TAG (mg/dl)	114.2 ± 65.5	107.5 ± 45.7	0.22
LDL (mg/dl)	112.9 ± 32.6	114.1 ± 32.1	0.72
HDL (mg/dl)	52.9 ±13.4	51.5 ± 11.8	0.24
LDL/HDL	2.2 ± 0.7	2.6 ± 5.1	0.26
TC/HDL	3.7 ± 1.0	3.8 ± 1.0	0.56
TAG/H	2.4 ± 1.9	2.3 ± 1.6	0.57
TSH (µIU/L)	5.2 ± 15.0	4.6 ± 9.1	0.60
Creatinine(mg/dl)	0.8±0.3	0.8±0.3	0.36

TABLE 4.27 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HYPERTENSION (TOTAL N=398)

N=420	Normal N=106	Pre-HTN N=114	HTN N=179	ANOVA p value
Hb	11.7 ± 1.4	12.8 ± 9.6	12.0 ± 1.4	0.28
FBS	93.8 ± 21.0	95.7 ± 23.4	103 ± 30.9	0.01**
PG2BS	113.0±43.7	111.0±40.5	125.3±42.4	0.08
Insulin	10.6 ± 10.5	8.5 ± 4.6	9.9 ± 5.9	0.13
HOMA IR	1.3 ± 1.1	1.1 ± 0.6	1.3 ± 0.8	0.15
TC	183.9 ± 35.8	188.8 ± 42.3	188 ± 38.5	0.54
TAG	107.1 ± 59	100 ± 52.2	118 ± 53.9	0.01**
LDL	112 ± 28.7	114 ± 35.7	113 ± 32.2	0.82
HDL	50.7 ± 11.8	54.8 ± 14.2	51.2 ± 11.6	0.02*
LDL/HDL	2.3 ± 0.7	2.9 ± 7.1	2.3 ± 0.8	0.42
TC/HDL	3.7 ± 1.1	3.6 ± 1.0	3.8 ± 1.0	0.20
TAG/H	2.4 ± 2.2	2.1 ± 1.7	2.5 ± 1.4	0.12
TSH	5.5 ± 13.4	4.3 ± 7.9	4.8 ± 13.3	0.76
Creatinine	0.62±0.1	0.65±0.1	0.69±0.3	0.34

HTN = Hypertension, * Significant at p<0.05, **p<0.01, ***p<0.001

TABLE 4.27a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS HYPERTENSION CATEGORIES (TOTAL N =398)

VARIABLES	p VALUE		
	FBS	TAG	HDL
Normal VsPreHTN	0.53	0.35	0.02
Normal VsHTN	0.009*	0.08	0.76
Pre HTN Vs HTN	0.04	0.003*	0.01*

Significant at p<0.01, ** p<0.001, p<0.0003 (Bonferroni adjusted p values)***

hypertension and normal groups followed by pre-hypertensive and hypertensive groups, in other words, pre hypertension and hypertension significantly raised the FBS levels compared to subjects with normal BP. In a more clear representation of this data in Figure 4.18, it is evident that an elevated SBP or DBP increases the risk of developing diabetes. This effect was markedly seen in Ahmedabad data (Table 4.29), as compared to Baroda (Table 4.28). An additional risk in Hypertensive females was observed that they had significantly higher TAG levels as compared to pre-hypertensive and normal women, thus indicating the role of hypertension in precipitating aberrations in lipid profile also.

Percent Prevalence of Clinical Conditions Among Hypertensive Subjects

The study subjects in both Ahmedabad and Baroda had a very high prevalence of high blood pressure, with the figure for pooled data coming up to 76%. Due to such disturbingly high prevalence, it was thought rational to study the presence of other clinical conditions in subjects with high blood pressure, to examine associations between conditions and hypertension. The pooled data revealed that less than half of the subjects (45.2%) were only hypertensives, rest all of them had either impaired glucose control or impaired thyroid metabolism or both along with high blood pressure (Table 4.30). The graphical representation of this data (Figure 4.19) gives a more vivid picture about this association.

This effect was not as pronounced in Baroda (Figure 4.20), where 60% of the hypertensives did not have any another concurrent metabolic condition (Table 4.31), whilst in Ahmedabad (Figure 4.21), a mere 30% of the hypertensives were devoid of any coexisting clinical conditions, rest all 70% of them had impaired glucose or thyroid metabolism (Table 4.32).

Another salient observation was that the prevalence of hypothyroidism was around 23% in hypertensives, which indicates predisposition to hypothyroidism in hypertensives; also the prevalence of IFG was close to 31%, which again is indicative of the propensity of hypertensives to have an impaired glucose control.

TABLE 4.28 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HYPERTENSION (BARODA N=186)

Parameter	Normal N=46	Pre HTN N=48	HTN N=92	ANOVA p value
Hb (g/dl)	11.5 ± 1.3	12.2 ± 1.0	12.0 ± 1.5	0.01**
FBS (mg/dl)	89 ± 22.4	89 ± 21.8	94.1 ± 28.5	0.41
Insulin	9.6 ± 3.9	8.5 ± 4.1	8.9 ± 3.8	0.37
HOMA IR	1.2 0.5	1.1 0.5	1.1 ±05	0.36
TC (mg/dl)	176 ± 36.1	188 ± 49.9	182 ± 36.0	0.30
TAG (mg/dl)	118 ± 67.8	115 ± 63.6	116 ± 51.2	0.97
LDL (mg/dl)	104 ± 27.5	115 ± 41.9	109 ± 29.1	0.28
HDL (mg/dl)	46.9 ± 9.5	50.8 ± 12.7	49.4 ± 9.5	0.18
LDL/HDL	2.3 ± 0.7	2.3 ± 0.6	2.3 ± 0.6	0.95
TC/HDL	3.9 ± 0.9	3.8 ± 0.8	3.8 ± 0.8	0.87
TAG/H	2.7 ± 1.8	2.6 ± 2.2	2.5 ± 1.4	0.85
TSH (µIU/L)	4.1 ± 3.9	5.3 ± 11.8	6.4 ± 18.2	0.67

HTN = Hypertension,* p<0.05, **P<0.01,P<0.001***

TABLE 4.28a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS HYPERTENSION CATEGORIES (BARODA N=186)

VARIABLES	p VALUE
	Hb
Normal Vs Pre-HTN	0.001***
Normal Vs HTN	0.04*
Pre HTN Vs HTN	0.34

Significant at p<0.01, ** p<0.001, p<0.0003 (Bonferroni adjusted p values)***

TABLE 4.29 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF HYPERTENSION (AHMEDABAD N=212)

Parameter	Normal N=47	Pre HTN N=64	HTN N=101	ANOVA p value
Hb (g/dl)	11.8±1.4	11.6±1.5	12.1±1.2	0.03*
FBS (mg/dl)	99.5±21.0	95.1±11.3	110.6±30.0	0.0001***
PG2BS (mg/dl)	113.0±43.7	111.0±40.5	125.3±42.4	0.08
TC (mg/dl)	190.6±35.6	186.4±34.9	196.4±39.6	0.23
TAG (mg/dl)	100.2±54.8	90.8±39.4	116.6±55.1	0.005**
LDL (mg/dl)	118.9±29.6	112.0±29.7	119.9±33.9	0.28
HDL (mg/dl)	53.0±12.9	57.4±15.1	53.9±12.6	0.15
LDL/HDL	2.34±0.7	3.37±9.6	2.33±0.8	0.43
TC/HDL	3.72±1.2	3.45±1.0	3.80±1.0	0.11
TSH (µIU/L)	7.68±19.7	3.33±2.1	3.21±2.5	0.07
Creatinine(mg/dl)	0.62±0.1	0.65±0.1	0.69±0.3	0.34

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.29a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY ACROSS HYPERTENSION CATEGORIES (AHMEDABAD N=212)

VARIABLES	p value		
	Hb	FBS	TAG
Normal VsPreHTN	0.13^{NS}	0.10^{NS}	0.14^{NS}
Normal VsHTN	0.14^{NS}	0.006**	0.05^{NS}
Pre HTN Vs HTN	0.007**	3.6^{-6}***	0.0003***

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

FIGURE 4.18 ROLE OF HYPERTENSION ON FBS LEVELS (TOTAL N=398)

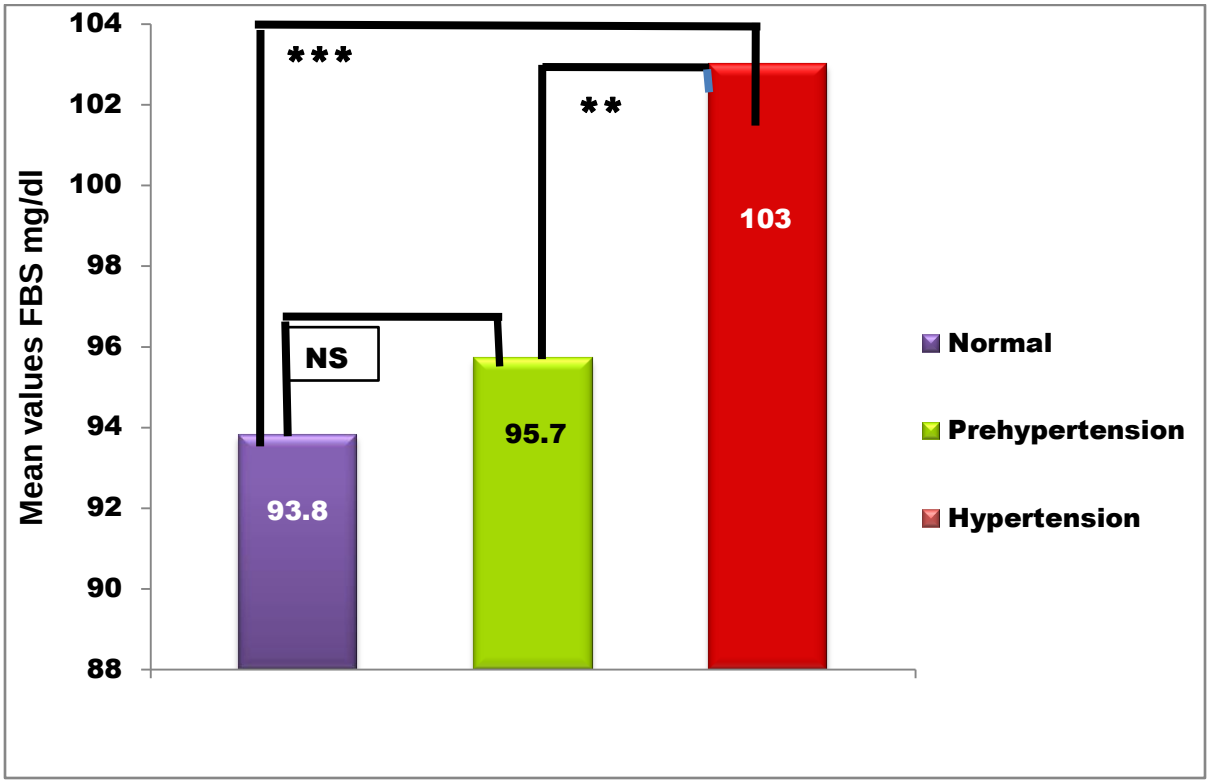


TABLE 4.30 PREVALENCE OF CLINICAL CONDITIONS AMONG HYPERTENSIVE SUBJECTS (TOTAL N=179)

CONDITIONS	N = 179	%
Only IFG	44	24.6
Only DM	12	6.7
Only HypoThyroidism	23	12.8
HypoThy + IFG	11	6.1
HypoThy + DM	8	4.5
Only HTN	81	45.2

FIGURE 4.19 PERCENT PREVALENCE OF CLINICAL CONDITIONS IN HYPERTENSIVES (TOTAL N=398)

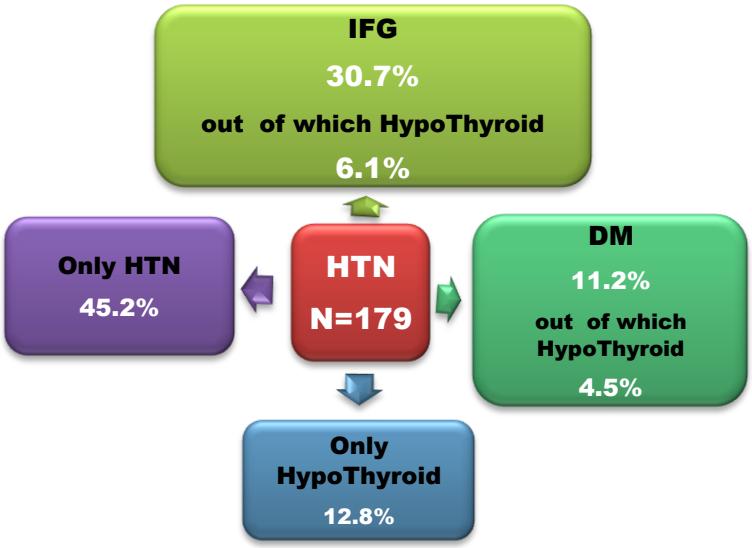


TABLE 4.31 PREVALENCE OF CLINICAL CONDITIONS AMONG HYPERTENSIVE SUBJECTS (BARODA N=90)

CONDITIONS	N = 90	%
IFG	12	13.4
DM	6	6.7
Only HypoThyroidism	15	16.7
HypoThyr + IFG	0	0
HypoThy + DM	3	3.4
Only HTN	54	60.0

FIGURE 4.20 PERCENT PREVALENCE OF CLINICAL CONDITIONS IN HYPERTENSIVES (BARODA N=186)

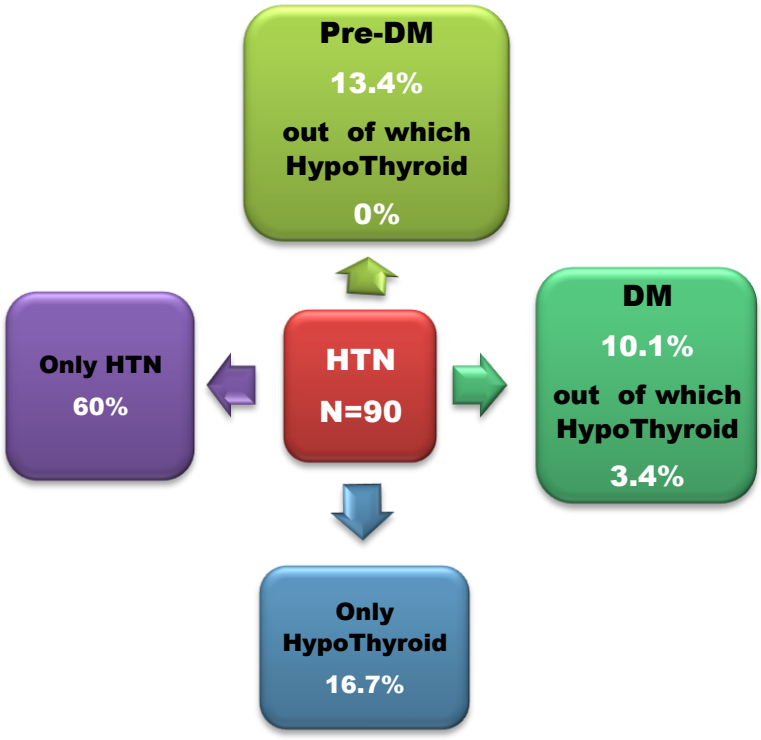
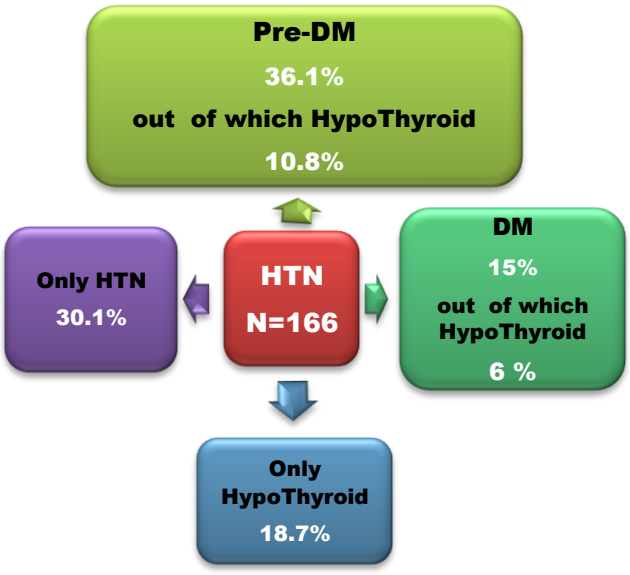


TABLE 4.32 PREVALENCE OF CLINICAL CONDITIONS AMONG HYPERTENSIVE SUBJECTS (AHMEDABAD N=212)

CONDITIONS	N = 166	%
IFG	28	16.9
IGT	4	2.4
Pre-DM	10	6.0
DM	15	9.0
Only HypoThyroidism	31	18.7
HypoThy + IFG	13	7.8
HypoThy + IGT	0	0
HypoThyr + Pre-DM	5	3.0
HypoThy + DM	10	6.0
Only HTN	50	30.1

FIGURE 4.21 PERCENT PREVALENCE OF CLINICAL CONDITIONS IN HYPERTENSIVES (AHMEDABAD N=212)



Metabolic Syndrome (MS)

Metabolic syndrome being clustering of risk factors, the parameters defining it are bound to be significantly different in the individuals with MS, than those who don't. The same is confirmed when risk factors were crosstablulated by presence of MS (Table 4.33). A significantly high mean value of FBS, TAG and HDL was observed in MS group, apart from these, significantly high mean values of PG2BS, Insulin. HOMA2 IR and TC/H were also observed. In Baroda (Table 4.34), apart from FBS, TAG and HDL, TC, LDL, LDL/H and TC/H were also significantly higher in the MS group compared to normals. This trend was consistent in Ahmedabad data as well (Table 4.35).

Diabetes

Presence of impaired glucose metabolism seemed to affect TAG, an effect seen consistently in both Ahmedabad and Baroda data (Tables 4.38 and 4.37 respectively)**.** In the pooled analysis (Table 4.36), the individuals with diabetes had significantly high SBP, DBP, Insulin, HOMA 2 IR, TAG, TC/H and TAG/H. The difference was more pronounced between normals & diabetics and normals and people with IFG, rather than between diabetics and individuals with IFG, as seen by post hoc statistics (Table 4.35a). This reinstates the importance of maintaining the glucose levels below 100mg/dl to avoid risk of atherogenicity due to increased TAG and TAG/H, as the levels are significantly higher in cases where FBS is higher than 100mg/dl.

Summary of Effect of Clinical Conditions on Biochemical Parameters

The overall summary of predictor variables getting altered by the presence of hypertension, diabetes and metabolic syndrome is given in Table 4.39 on page 65. Maximum number of variables (i.e.9) differed significantly when metabolic syndrome was present, as compared to diabetes (5 variables) and hypertension (3 variables).

TABLE 4.33 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF METABOLIC SYNDROME (TOTAL N=383)

N=348	Normal N=256	MS N=143	Student's 't' 2 tailed p value
Hb	11.8 ± 1.3	13.0 ± 8.5	0.02*
FBS	91.8 ± 15.2	109.9 ± 37.2	0.000***
PG2BS (mg/dl)	131.33 + 41.8	101.28 + 25.6	1.8^{-8}***
Insulin	8.9 ± 7.1	10.9 ± 6.9	0.01**
HOMA IR	1.13 ± 0.8	1.5 ± 0.9	0.000***
TC	185.7 ± 38.2	190.5 ± 65.1	0.24
TAG	89.4 ± 34.3	148.0 ± 40.1	0.000***
LDL	112.5 ± 31.7	115.6 ± 33.4	0.34
HDL	55.8 ± 12.5	45.4 ± 9.5	0.000***
LDL/HDL	2.4 ± 4.8	2.6 ± 0.8	0.57
TC/HDL	3.4 ± 0.8	4.3 ± 1.0	0.000***
TAG/H	1.8 ± 1.3	3.4 ± 1.9	0.000***
TSH	4.6 ± 10.2	5.3 ± 14.8	0.55
Creatinine	0.66±0.1	0.66±0.3	0.44^{NS}

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.34 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF METABOLIC SYNDROME (BARODA N=186)

Parameter	Present N =72	Absent N = 114	Student's 't' 2 tailed p value
Hb (mg/dl)	12.1 ± 1.4	11.6 ± 1.3	0.01**
FBS (mg/dl)	97 ± 30.7	82.5 ± 7.4	0.000***
Insulin	9.0 ± 4.0	8.9 ± 3.8	0.86
HOMA IR	1.2 ± 0.5	1.1 ± 0.5	0.57
TC (mg/dl)	192 ± 44.1	167 ± 26.5	0.000***
TAG (mg/dl)	133 ± 62.9	91 ± 39.7	0.000***
LDL (mg/dl)	117 ± 35.5	96 ± 9.6	0.000***
HDL (mg/dl)	47.3 ± 10.6	52 ± 9.6	0.002**
LDL/HDL	2.5 ± 0.6	1.9 ± 0.5	0.000***
TC/HDL	4.1 ± 0.8	3.3 ± 0.6	0.000***
TAG/H	3.0 ± 1.9	1.9 ± 1.2	0.000***
TSH (µIU/L)	6.5 ± 17.9	4.0 ±3.7	0.24

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.35 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF METABOLIC SYNDROME (AHMEDABAD N=197)

Parameter	Present N =94	Absent N = 103	t test p value
Hb (mg/dl)	12.12±1.3	11.82±1.4	0.06
FBS (mg/dl)	113.1±29.8	92.3±8.4	1.73^{-12}***
PG2BS (mg/dl)	131.33 + 41.8	101.28 + 25.6	1.8^{-8}***
TC (mg/dl)	194.42±40.8	188.92±37.3	0.15
TAG (mg/dl)	124.88±57.9	82.2±30.9	9.01^{-11}***
LDL (mg/dl)	119.06±35.6	115.92±28.0	0.24
HDL (mg/dl)	50.74±13.7	59.23±12.3	3.47^{-6}***
LDL/HDL	2.52±0.9	2.03±0.5	7.03^{-6}***
TC/HDL	4.07±1.2	3.30±0.6	4.9^{-8}***
TAG/H	2.87 ± 2.1	1.47 ± 0.7	1.9^{-9}***
TSH (µIU/L)	3.28±2.4	5.25±13.8	0.08
Creatinine(mg/dl)	0.66±0.1	0.66±0.3	0.44

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.36 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF IMPAIRED GLUCOSE CONTROL (TOTAL N=394)

N=348	Normal N=278	IFG N=90	DM N=31	Anova p value
Hb	11.9 ± 1.4	11.9 ± 1.2	12.1 ± 1.2	0.66
SBP	123 ± 17.4	132 ± 18.1	135 ± 19.9	0.000***
DBP	78 ± 10.3	82 ± 8.4	83 ± 11.30	0.02*
Insulin	8.8 ± 6.8	11.1 ± 7.1	12.4 ± 8.5	0.01**
HOMA IR	1.2± 0.7	1.5 ± 0.9	1.8 ± 1.2	0.000***
TC	186 ± 38.7	191 ± 40.9	187 ± 35.1	0.54
TAG	106.7 ± 54.4	113 ± 53.4	135 ± 62.6	0.01**
LDL	112 ± 31.3	116 ± 35.4	112.7 ± 32	0.56
HDL	52.5 ± 12.5	52.4 ± 13.0	112 ± 32.0	0.15
LDL/HDL	2.23 ± 0.7	3.22 ± 8.0	2.4 ± 0.8	0.10
TC/HDL	3.7 ± 0.9	3.9 ± 1.2	4.0 ± 0.9	0.04*
TAG/H	2.2 ± 1.6	2.5 ± 2.2	3.1 ± 1.8	0.03*
TSH	5.5 ± 14.2	3.3 ± 2.2	3.1 ± 2.5	0.22

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.36a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY IN RELATION TO GLUCOSE CONTROL

VARIABLES	p VALUE						
	SBP	DBP	Insulin	HOMA IR	TAG	TC/H	TAG/H
Normal Vs IFG	0.000***	0.02	0.02	0.001**	0.32	0.09	0.21
Normal Vs DM	0.000***	0.05	0.01*	0.000***	0.005*	0.02	0.006
IFG Vs DM	0.5	0.55	0.46	0.14	0.05	0.44	0.19

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.37 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF IMPAIRED GLUCOSE CONTROL (BARODA N=186)

Parameter	Normal N=157	IFG N=17	DM N = 12	ANOVA p value
SBP	126 ± 18.8	140 ± 17.2	144 ± 25.1	0.0002***
DBP	79.8 ± 11.6	86.2 ± 7.5	87 ± 15.5	0.01**
Hb (mg/dl)	11.9 ± 1.3	12.1 ± 1.5	12.4 ± 1.4	0.35
Insulin	9.0 ± 4.0	8.3 ± 3.3	9.7 ± 2.6	0.59
HOMA IR	1.1 ± 0.5	1.1 ± 0.4	1.5 ± 0.4	0.09
TC (mg/dl)	182 ± 39.5	172 ± 50.6	199 ± 26.8	0.18
TAG (mg/dl)	114 ± 57.4	100 ± 36.1	174 ± 71.9	0.001***
LDL (mg/dl)	109 ± 32.5	102 ± 39.9	116 ± 20.3	0.52
HDL (mg/dl)	49.1 ± 10.4	49.8 ± 11.7	48.9 ± 20.3	0.96
LDL/HDL	2.3 ± 0.7	2.1 ± 0.7	2.4 ± 0.4	0.29
TC/HDL	3.8 ± 0.9	3.5 ± 0.8	4.2 ± 0.8	0.10
TAG/H	2.5 ± 1.7	2.2 ± 1.0	3.8 ± 2.3	0.02*
TSH	5.9 ± 15.4	3.8 ± 3.3	3.7 ±3.0	0.76

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.37a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY IN RELATION TO GLUCOSE CONTROL (BARODA)

VARIABLES	p VALUE			
	SBP	DBP	TAG	TAG/H
Normal Vs IFG	0.003*	0.02	0.32	0.37
Normal Vs DM	0.002*	0.02	0.000***	0.01*
IFG Vs DM	0.64	0.73	0.001**	0.01*

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.38 BIOCHEMICAL PARAMETERS STRATIFIED BY PRESENCE OF IMPAIRED GLUCOSE CONTROL (AHMEDABAD N=208)

Parameter	Normal N=104	IFG N=43	IGT N=14	DM N = 47	ANOVA p value
Hb (mg/dl)	11.95±1.5	11.92±1.1	11.85±1.3	11.99±1.0	0.98
TC (mg/dl)	191.23 ± 37.0	199.91 ± 37.8	189.81 ± 38.5	179.64 ± 35.7	0.18
TAG (mg/dl)	95.55 ± 46.8	115.2 ± 59.3	124.43 ± 58.4	111.50 ± 42.3	0.02*
LDL (mg/dl)	116.90 ± 29.8	126.04 ± 38.8	117.65 ± 52.2	111.50 ± 42.3	0.31
HDL (mg/dl)	56.45 ± 13.7	53.38 ± 14.3	55.20 ± 11.5	50.18 ± 11.3	0.18
LDL/HDL	2.19 + 0.7	4.08 + 10.7	4.66 + 10.7	2.26 + 0.8	0.17
TC/HDL	3.50 + 0.9	4.01 + 1.3	3.64 + 1.0	3.73 + 0.9	0.05
TAG/H	1.86 + 1.8	2.64 + 2.6	2.44 + 1.2	2.43 + 1.2	0.03*
TSH	5.19 + 12.8	3.22 + 2.4	3.23 + 2.8	2.69 + 1.7	0.48
Creatinine	0.67 + 0.3	0.65 + 0.1	0.61 + 0.1	0.68 + 0.1	0.71

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.38a POST HOC TEST VALUES FOR VARIABLES DIFFERING SIGNIFICANTLY IN RELATION TO GLUCOSE CONTROL (AHMEDABAD)

VARIABLES	p VALUE	
	TAG	TAG/H
Normal Vs IFG	0.01*	0.02*
Normal Vs IGT	0.02 **	0.03*
Normal Vs DM	0.06^{NS}	0.03*
IFG Vs IGT	0.27^{NS}	0.32^{NS}
IGT Vs DM	0.20^{NS}	0.49
IFG Vs DM	0.37^{NS}	0.31

****Significant at p<0.01, ** p<0.001, p<0.0003*** (Bonferroni adjusted p values)***

TABLE 4.39 SUMMARY OF BIOCHEMICAL VARIABLES DIFFERING SIGNIFICANTLY BETWEEN CASES OF CLINICAL CONDITIONS AND NORMAL INDIVIDUALS

VARIABLES	MS	HTN	DM
Hb	*	NS	NS
FBS	***	***	NS
PG2BS	***	NS	NS
Insulin	*	NS	*
HOMA IR	***	NS	***
TC	NS	NS	NS
TAG	***	*	*
LDL	NS	NS	NS
HDL	***	NS	NS
LDL/HDL	NS	NS	NS
TC/HDL	***	NS	*
TAG/H	***	NS	*
TSH	NS	**	NS

Significantly different at P<0.05, ** P<0.01, *** P<0.001, NS – Non Significant

Aberrations In Risk Factors Across Quintiles Of Anthropometric Indices

To study the effect of anthropometric indices on trend of prevalence of aberrations in the parameters associated with glucose control, blood pressure and lipid profile , the data of all the above parameters was inspected across quintiles of the anthropometric indices of BMI, WC and WSR, as depicted in the Tables 4.40 to 4.48.

In the pooled data, a clear trend of steady increase in the parameters of SBP, DBP, HOMA2 IR, TAG and AIP across quintiles of WSR was seen (Table 4.40). Across BMI quintiles also, all these variables showed a gradual increase (Table 4.41). In case of WC quintiles, apart from SBP, HOMA2 IR and AIP, the TAG levels also increased steadily with each quintile (Table 4.42).

The data from Baroda also reflected the increase in the parameters across BMI as seen in the pooled data. Further, the elevations in TSH also appeared to gradually increase over the subsequent quintiles (Table 4.43). In case of WSR quintiles, it was seen that the prevalence of high TC and TAG peaked in the third quintiles, followed by a modest decline in the remaining quintiles (Table 4.44). Similar was the case across WC quintiles too (Table 4.45).

Data from Ahmedabad also saw similar trends in case of SBP, HOMA2 IR and AIP, however regarding the lipid fractions, it was seen that though an increasing trend was seen in progressive quintiles, most of the parameters dropped momentarily in the 3rd quintile, for all BMI, WSR and WC analyses (Tables 4.46 to 4.48). This might be indicative of the instance of health seeking for the increment in the blood pressure, blood sugar and lipid aberrations in the preceding quintiles. The graphical representation of the effect of analysis across quintiles has been depicted in Figures 4.22 to 4.29.

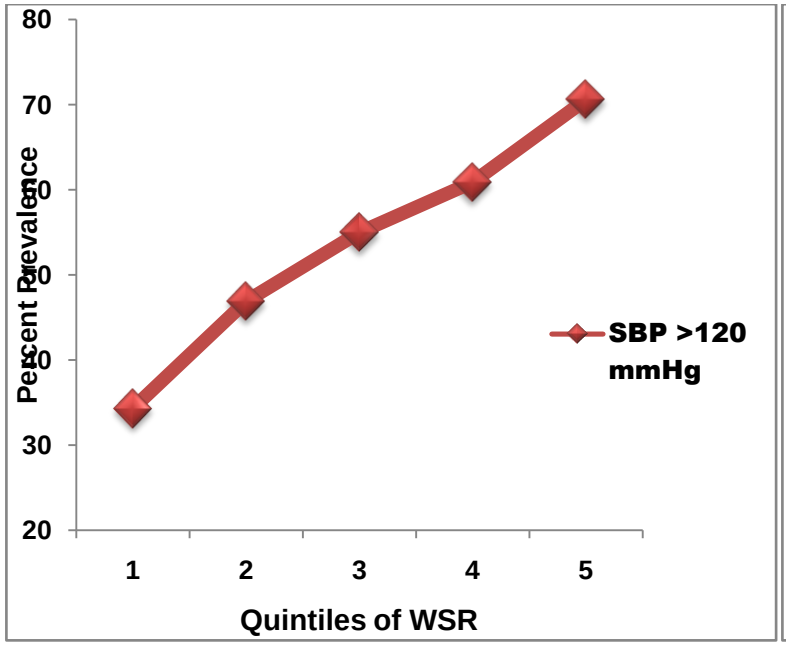
Hence, what can be inferred here is that anthropometric indices, especially WC, are very effective predictors of development of impairment in blood pressure regulation, glucose metabolism and lipid metabolism.

TABLE 4.40 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WSR (TOTAL N=370)ϕ

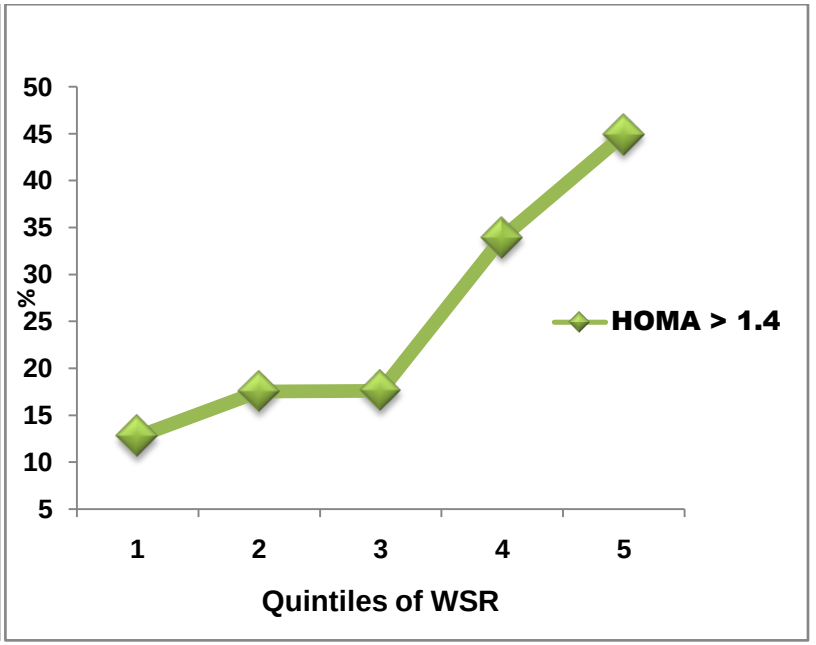
	N = 164	SBP >120	DBP >80	FBS ≥100	PG2/ PP2 ≥140	Insulin >12µl U/ml	HOMA 2 >1.4	TC >200	TAG >150	LDL >100	HDL <40	TAG/ H >3	AIP >0.21	TSH >4.5
1st Quintile(0.44-0.55)	70	24 (34.3)	13 (18.6)	15 (21.4)	3 (7.3)	8 (12.7)	8 (12.7)	17 (24.3)	4 (5.7)	43 (61.4)	21 (30.0)	4 (5.7)	27 (38.6)	19 (27.1)
2nd Quintile (0.56-0.58)	64	30 (46.9)	11 (17.2)	18 (28.1)	5 (20.8)	9 (15.8)	10 (17.5)	20 (31.2)	8 (12.5)	42 (65.6)	32 (50.0)	15 (23.4)	32 (50.0)	15 (23.4)
3rd Quintile (0.59-0.62)	80	44 (55.0)	27 (33.7)	19 (23.7)	3 (9.4)	10 (13.5)	13 (17.6)	32 (40.0)	18 (22.5)	52 (65.0)	35 (43.7)	15 (18.75)	49 (61.2)	13 (17.6)
4th Quintile (0.63-0.65)	64	39 (60.9)	20 (31.2)	25 (39.1)	5 (16.7)	15 (25.4)	20 (33.9)	20 (31.2)	9 (15.8)	45 (70.3)	40 (62.5)	18 (28.1)	51 (79.7)	18 (28.1)
5th Quintile (0.66-0.82)	92	65 (70.6)	41 (44.6)	32 (34.8)	10 (25.0)	24 (30.8)	35 (44.9)	33 (35.9)	24 (30.8)	59 (64.1)	46 (50.0)	32 (34.8)	64 (69.6)	23 (25.0)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

FIGURE 4.22 PREVALENCE OF HIGH SBP AND INSULIN RESISTANCE ACROSS QUINTILES OF WSR (TOTAL N=370)



4.22a SBP



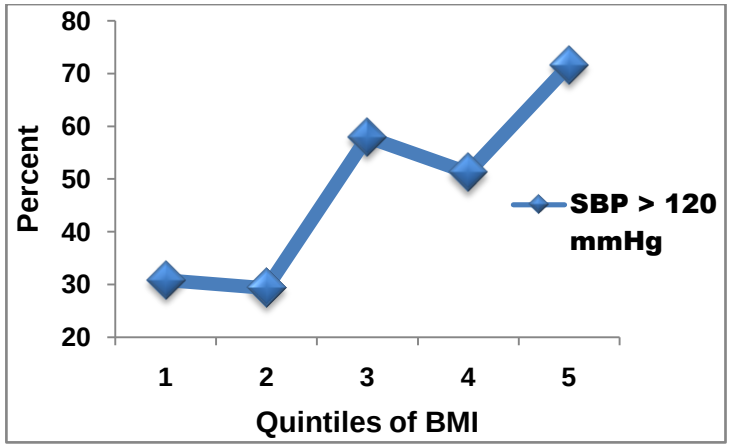
4.22b HOMA INSULIN RESISTANCE

TABLE 4.41 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF BMI (TOTAL N=399)ϕ

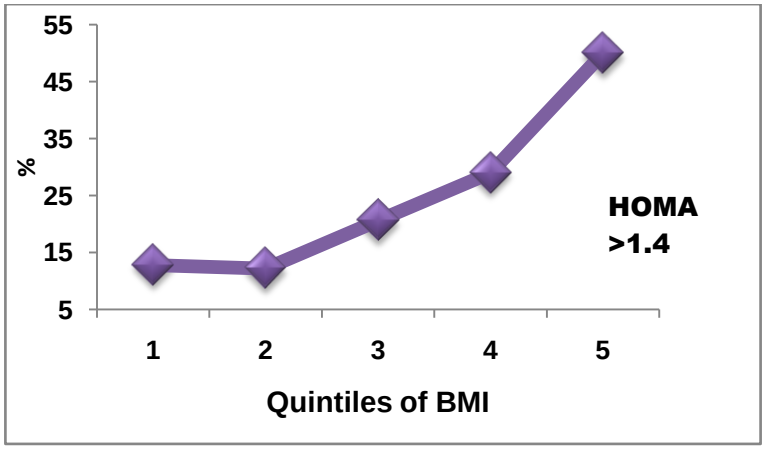
Quintiles	N= 209	WC> 80	SBP> 120	DBP> 80	FBS ≥100	Insulin > 12µIU/ml	HOMA 2 >1.4	TC >200	TAG >150	LDL >100	HDL <50	TAG/H >3	AIP >0.21	TSH >4.5
1st Quintile (15.3-23.6)	78	43 (60.6)	24 (30.8)	13 (16.7)	17 (21.8)	8 (12.1)	10 (12.8)	28 (35.9)	13 (16.7)	49 (62.8)	29 (37.2)	13 (16.7)	32 (41.0)	15 (19.2)
2nd Quintile (23.7-25.7)	82	69 (93.2)	39 (29.3)	24 (15.8)	18 (20.7)	8 (11.8)	10 (12.2)	27 (34.1)	13 (15.8)	54 (65.8)	42 (51.2)	15 (18.3)	47 (57.3)	20 (24.4)
3rd Quintile (25.8-27.9)	76	66 (86.8)	44 (57.9)	24 (31.6	25 (32.9)	9 (14.3)	13 (20.6)	27 (35.5)	17 (22.4)	51 (67.1)	35 (46.0)	19 (25.0)	48 (63.1)	13 (17.1)
4th Quintile (28-30.7)	82	75 (98.7)	42 (51.2)	21 (25.6)	21 (25.6)	17 (24.6)	20 (29.0)	20 (24.4)	10 (12.2)	49 (59.7)	42 (51.2)	20 (24.4)	50 (60.9)	22 (26.8)
5th Quintile (30.8-48)	81	79 (98.7)	58 (716)	31 (38.3)	40 (49.4)	25 (36.8)	34 (50.0)	30 (37.0)	14 (17.3)	55 (679)	39 (48.1)	22 (27.1)	61 (75.3)	23 (28.4)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

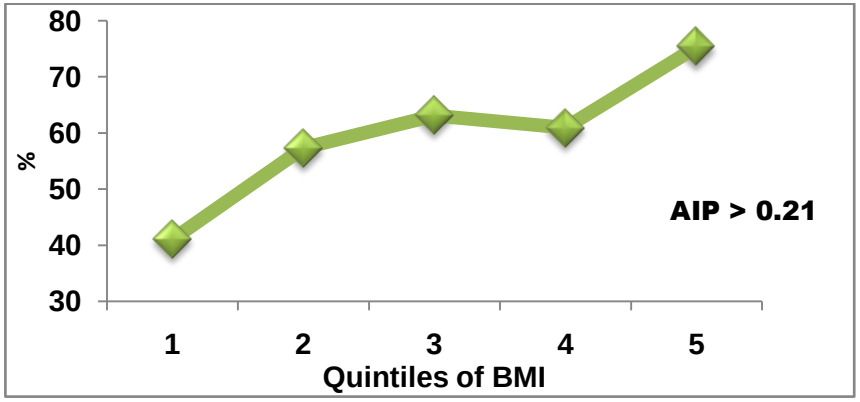
FIGURE 4.23 PREVALENCE OF ABERRATIONS IN SBP, INSULIN RESISTANCE AND AIP LEVELS ACROSS QUINTILES OF BMI (TOTAL)



4.23a SBP



4.23b HOMA INSULIN RESISTANCE



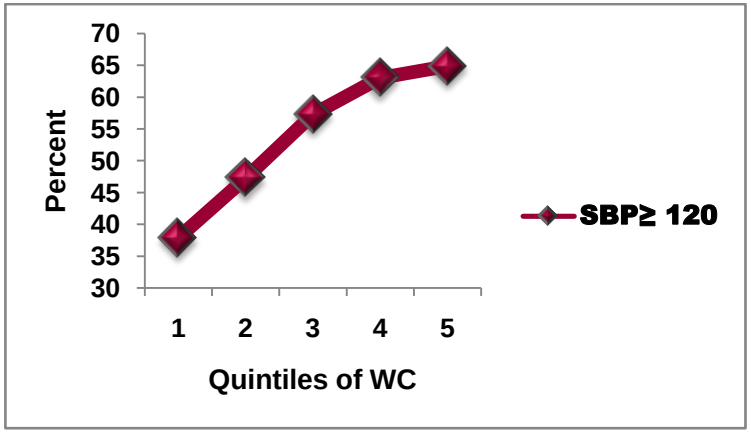
4.23c AIP

TABLE 4.42 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WC (TOTAL N=370) ϕ

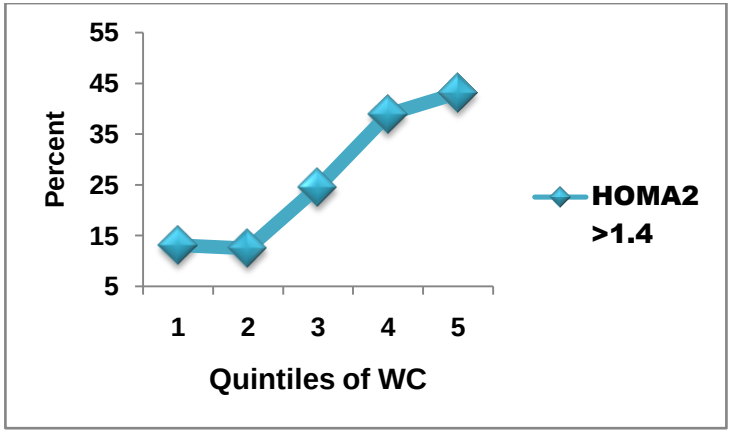
Quintiles	N = 164	SBP≥ 120	DBP≥ 80	FBS ≥100	PG2/ PP2 ≥140	Insulin >12	HOMA 2 > 1.4	TC ≥200	TAG ≥150	LDL ≥100	HDL ≤40	TG/H ≥3	AIP ≥0.21	TSH ≥4.5
1st Quintile (70-84.5)	66	25 (37.9)	11 (16.6)	16 (24.2)	4 (9.7)	8 (13.1)	8 (13.1)	18 (27.3)	5 (7.5)	37 (56.1)	19 (28.8)	5 (7.5)	23 (34.8)	17 (25.7)
2nd Quintile (85-89.5)	74	35 (47.3)	11 (14.9)	22 (29.7)	4 (11.8)	7 (10.9)	8 (12.5)	29 (39.2)	10 (13.5)	51 (68.9)	32 (43.2)	14 (18.9)	40 (54.0)	16 (21.6)
3rd Quintile (90-95.7)	75	43 (57.3)	31 (41.3)	16 (21.3)	4 (15.4)	11 (15.9)	17 (24.6)	21 (28.0)	15 (20.0)	46 (61.3)	42 (56.0)	17 (22.6)	49 (65.3)	14 (18.6)
4th Quintile (96-101)	81	51 (63.0)	27 (33.3)	30 (37.0)	5 (12.8)	22 (30.5)	28 (38.9)	30 (37.0)	16 (19.7)	56 (69.1)	39 (48.1)	27 (33.3)	57 (70.4)	18 (22.2)
5thQuintile (101.1-122.5)	74	48 (64.9)	33 (44.6)	25 (33.8)	9 (33.4)	18 (27.7)	28 (43.1)	24 (32.4)	17 (22.9)	46 (62.2)	42 (56.7)	21 (28.4)	54 (73.0)	23 (31.1)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

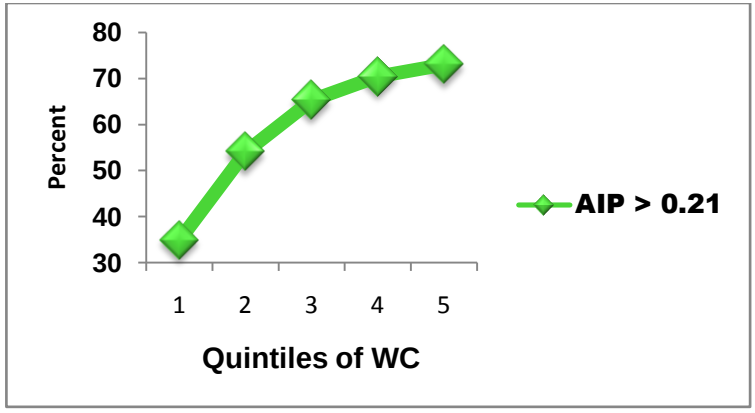
FIGURE 4.24 PREVALENCE OF HIGH BLOOD SUGAR, TAG AND AIP ACROSS QUINTILES OF WC (TOTAL)



4.24aBLOOD SUGAR



4.24b TAG



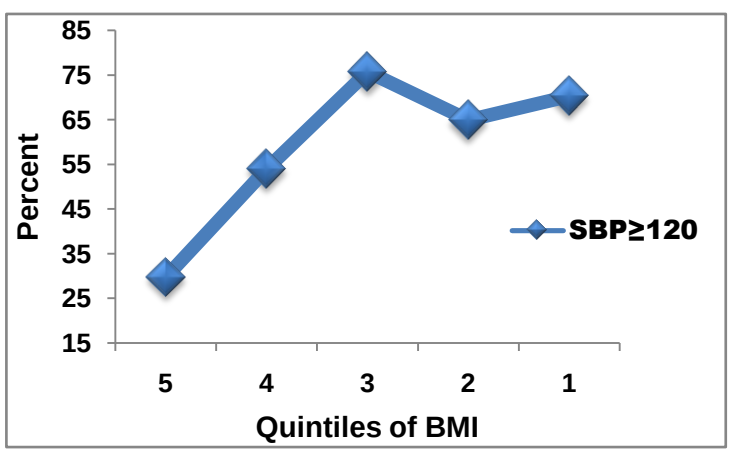
4.24c AIP

TABLE 4.43 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF BMI (BARODA N=186)

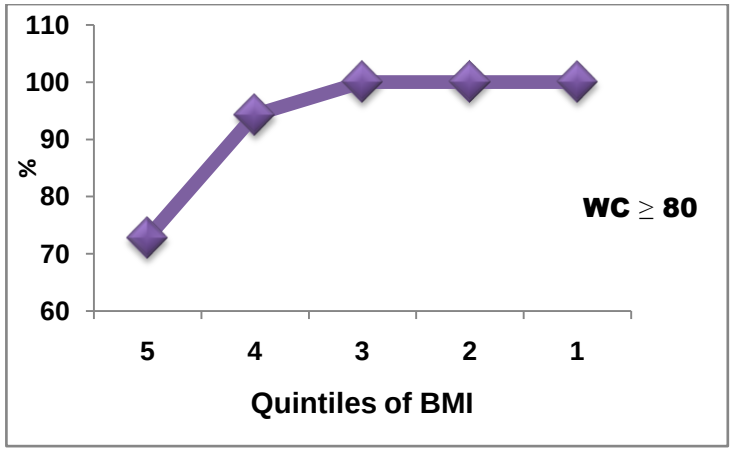
Quintiles	N= 209	WC> 90	SBP >120	DBP> 80	FBS ≥100	Insulin >12µlU /ml	HOMA >1.4	TC >200	TAG >150	LDL >100	HDL <40	TG/H >3	AIP >0.21	TSH >4.5
1st Quintile (15 .3-23.7)	37	27 (72.9)	11 (29.7)	9 (24.3)	2 (5.4)	5 (13.5)	6 (16.2)	12 (32.4)	9 (24.3)	24 (64.9)	16 (43.2)	9 (24.3)	19 (51.3)	5 (13.5)
2nd Quintile (23.7-25.2)	37	35 (94.6)	20 (54.0)	16 (43.2)	5 (13.5)	3 (8.1)	5 (13.5)	8 (21.6)	4 (10.8)	20 (54.0)	19 (51.3)	5 (13.5)	20 (54.0)	10 (27.0)
3rd Quintile (25.3-27.4)	38	38 (100)	28 (75.7)	20 (54.0)	5 (13.5)	3 (8.1)	6 (16.2)	14 (37.8)	10 (26.3)	23 (60.5)	21 (55.2)	10 (26.3)	26 (68.4)	11 (28.9)
4th Quintile (27.4-29.9)	37	37 (100)	24 (64.9)	19 (51.3)	6 (16.2)	10 (27.0)	13 (35.1)	6 (16.2)	8 (21.3)	19 (51.3)	28 (75.7)	15 (40.5)	29 (78.4)	6 (16.2)
5thQuintile (29.9-48)	37	37 (100)	26 (70.3)	20 (54.0)	11 (29.7)	5 (13.5)	9 (24.3)	11 (29.7)	7 (18.9)	20 (54.0)	18 (48.6)	7 (18.9)	28 (75.7)	15 (40.5)

Values in parenthesis indicate percentage

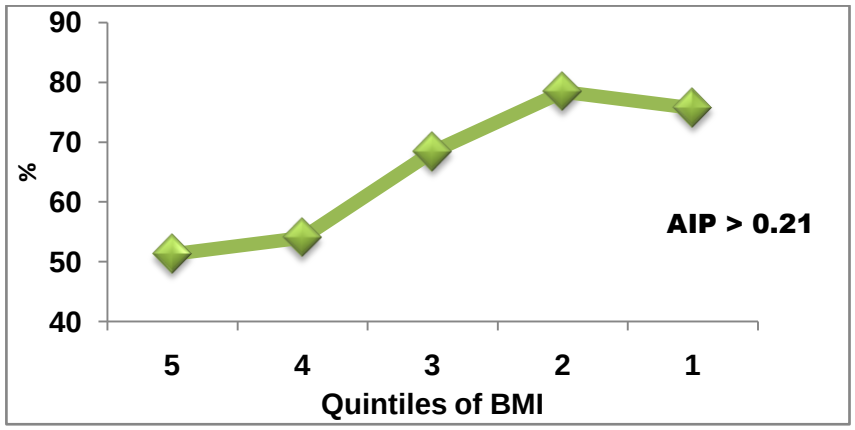
FIGURE 4.25 PREVALENCE OF ABERRATIONS IN SBP LEVELS, WC AND AIP ACROSS QUINTILES OF BMI (BARODA)



4.25a SBP



4.25b WC



4.25c AIP

TABLE 4.44 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WSR (BARODA N=186)

Quintiles	N = 164	SBP> 120	DBP> 80	FBS ≥100	Insulin >12µIU /ml	HOMA >1.4	TC >200	TAG >150	LDL >100	HDL <40	TG/H >3	AIP >0.21	TSH >4.5
1st Quintile (0.44-0.56)	36	14 (38.8)	12 (33.3)	3 (8.3)	5 (13.8)	5 (13.8)	6 (16.6)	4 (11.1)	21 (58.3)	15 (41.6)	4 (11.1)	15 (41.6)	8 (22.2)
2nd Quintile (0.57-0.59)	37	19 (51.3)	12 (32.4)	3 (8.1)	4 (10.8)	5 (13.8)	8 (21.6)	7 (19.4)	19 (51.3)	20 (54.0)	9 (24.3)	20 (54.0)	11 (29.7)
3rd Quintile (0.60-0.62)	36	19 (52.7)	15 (41.6)	7 (19.4)	3 (8.3)	5 (13.8)	15 (41.6)	9 (25.0)	25 (69.4)	18 (50.0)	8 (22.2)	23 (63.8)	7 (19.4)
4th Quintile (0.63-0.66)	35	25 (71.4)	19 (54.3)	6 (17.1)	6 (17.1)	10 (28.6)	10 (28.6)	7 (20.0)	21 (60.0)	27 (77.1)	12 (34.3)	32 (91.4)	10 (28.6)
5thQuintile (0.67-0.82)	42	32 (76.2)	26 (61.9)	10 (23.8)	8 (19.0)	14 (33.3)	12 (28.6)	11 (26.2)	21 (50.0)	22 (52.4)	13 (30.9)	32 (76.2)	12 (28.6)

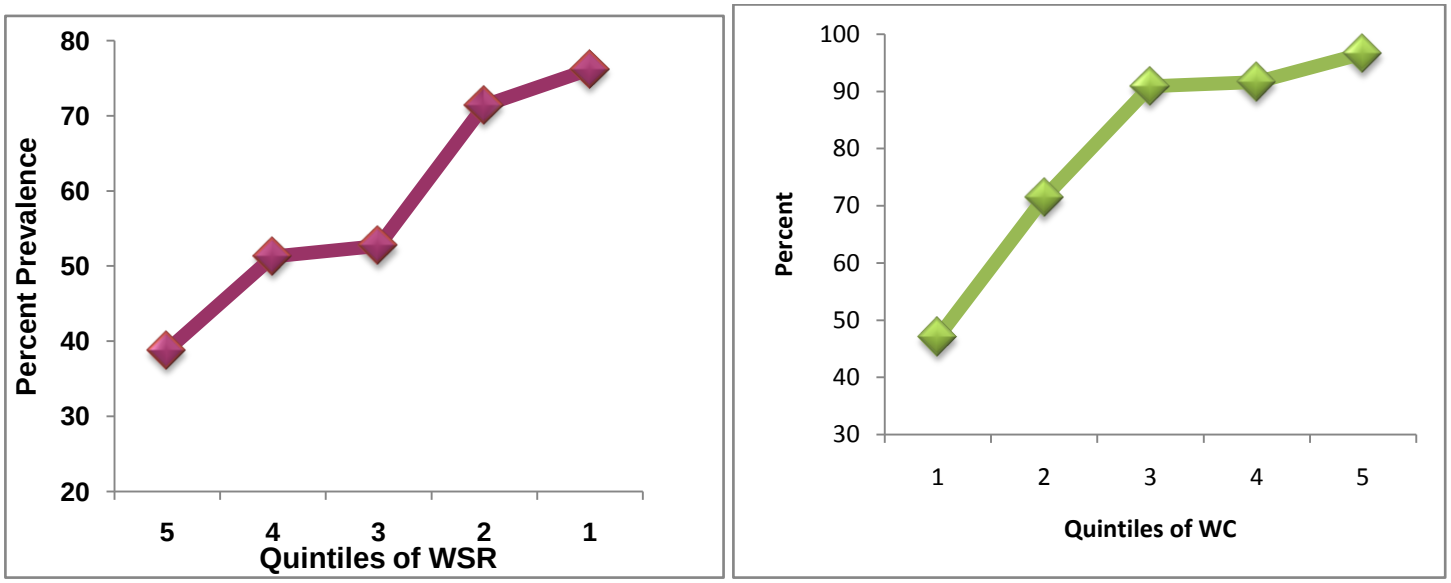
Values in parenthesis indicate percentage

TABLE 4.45 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WC (BARODA N=186)

Quintiles	N = 164	SBP ≥120	DBP ≥80	FBS ≥100	Insulin >12µlU /ml	HOMA >1.4	TC ≥200	TAG ≥150	LDL ≥100	HDL <40	TAG/ H ≥3	AIP ≥0.21	TSH ≥4.5
1st Quintile (68. 3-86.5)	36	14 (38.9)	9 (25.0)	2 (5.5)	5 (13.9)	5 (13.9)	8 (22.2)	5 (13.9)	19 (52.8)	14 (38.9)	5 (13.9)	13 (36.1)	9 (25.0)
2nd Quintile (87-91.4)	38	19 (50.0)	12 (31.6)	23 (60.5)	3 (7.9)	4 (10.5)	8 (21.0)	7 (18.4)	22 (57.9)	19 (50.0)	7 (18.4)	20 (52.6)	10 (26.3)
3rd Quintile (91.5-96.7)	36	21 (58.3)	20 (55.5)	6 (16.7)	3 (8.3)	4 (11.1)	10 (27.8)	9 (25.0)	21 (58.3)	22 (61.2)	9 (25.0)	29 (80.5)	8 (22.2)
4th Quintile (96-101.5)	34	25 (73.5)	18 (52.9)	6 (17.6)	7 (20.6)	10 (29.4)	11 (32.3)	8 (23.5)	19 (55.9)	25 (73.5)	14 (41.2)	28 (82.3)	8 (23.5)
5thQuintile (102-122.5)	42	30 (71.4)	25 (59.5)	32 (76.2)	8 (19.0)	16 (38.1)	12 (28.6)	9 (21.4)	23 (54.8)	22 (52.4)	11 (26.2)	32 (76.2)	13 (30.9)

Values in parenthesis indicate percentage

FIGURE 4.26 PREVALENCE OF ELEVATED AIP ACROSS QUINTILES OF WSR AND WC (BARODA)



4.26a AIP across WSR

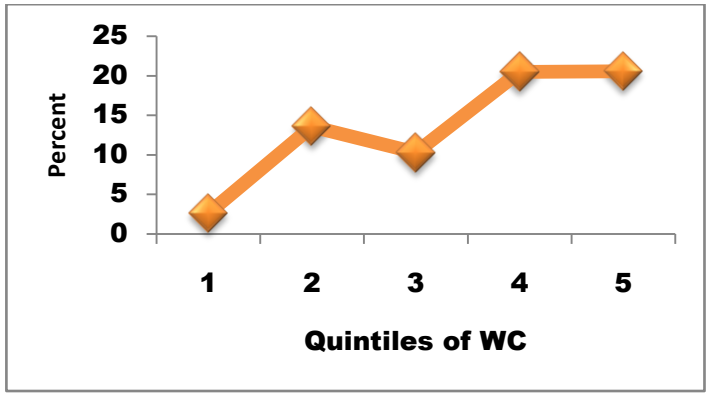
4.26b AIP ACROSS WC

TABLE 4.46 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WC (AHMEDABAD N=184)ϕ

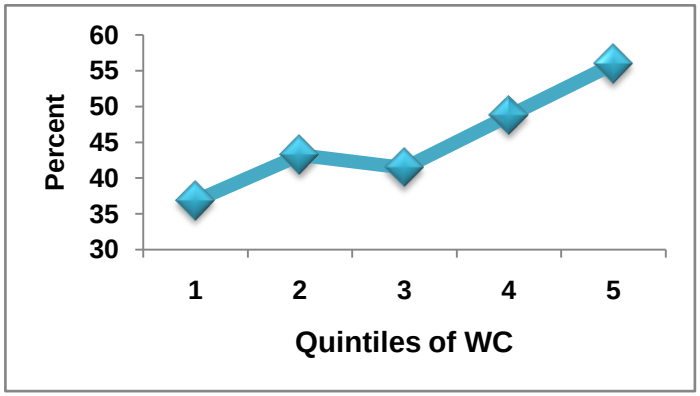
Quintiles	N = 164	WSR≥ 0.5	SBP≥ 120	DBP≥ 80	FBS ≥100	PG2/ PP2 ≥140	FBS≥ 100 &/or PPBS ≥140	TC ≥200	TAG ≥150	LDL ≥100	HDL ≤40	TG/H ≥3	AIP ≥0.21	TSH ≥4.5
1st Quintile (79-83	38	23 (60.5)	20 (52.6)	25 (65.8)	12 (31.6)	3 (7.9)	14 (36.8)	14 (36.8)	1 (2.6)	25 (65.8)	9 (23.7)	2 (5.3)	12 (31.6)	11 (28.9)
2nd Quintile(83.1 - 89.0)	44	44 (100)	32 (72.7)	34 (77.3)	18 (40.9)	10 (22.7)	19 (43.2)	22 (50.0)	6 (13.6)	32 (72.7)	17 (38.6)	10 (22.7)	27 (61.4)	9 (20.4)
3rd Quintile (89.1 - 95.0)	29	29 (100)	22 (75.9)	23 (79.3)	11 (37.9)	5 (17.2)	12 (41.4)	10 (34.5)	3 (10.3)	22 (75.9)	14 (48.3)	6 (20.7)	15 (51.7)	4 (13.8)
4th Quintile(95.1 - 100)	39	39 (100)	26 (66.7)	27 (69.2)	20 (51.3)	7 (17.9)	19 (48.7)	19 (48.7)	8 (20.5)	34 (87.2)	13 (33.3)	12 (30.8)	27 (69.2)	8 (20.5)
5th Quintile(100.1 - 118.5)	34	34 (100)	27 (79.4)	28 (82.3)	18 (52.9)	11 (32.3)	19 (55.9)	12 (35.3)	7 (20.6)	24 (70.6)	19 (55.9)	10 (29.4)	22 (64.7)	9 (26.5)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

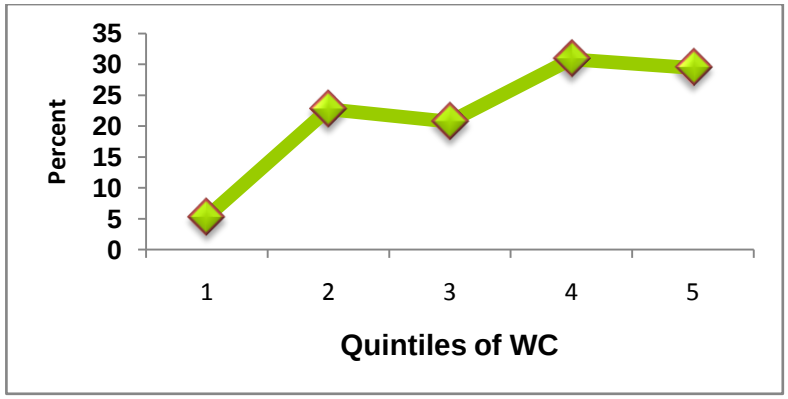
FIGURE 4.27 PREVALENCE OF TAG, FBS AND TAG/HDL ACROSS QUINTILES OF WC (AHMEDABAD)



4.27a TAG



4.27b FBS



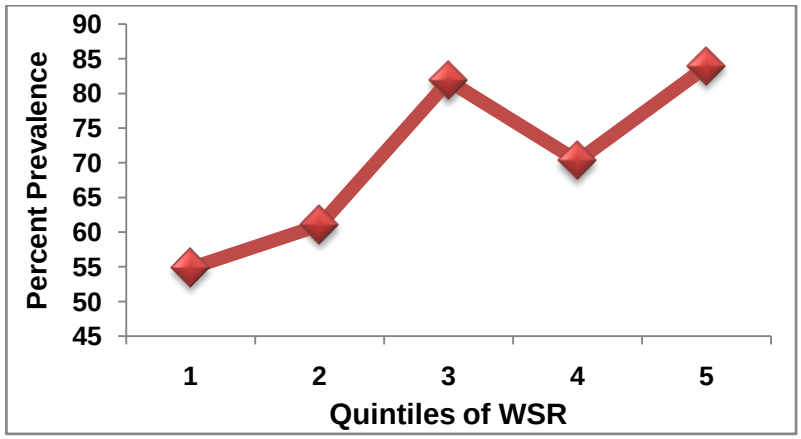
4.27c TAG/HDL

TABLE 4.47 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF WSR (AHMEDABAD N=184)ϕ

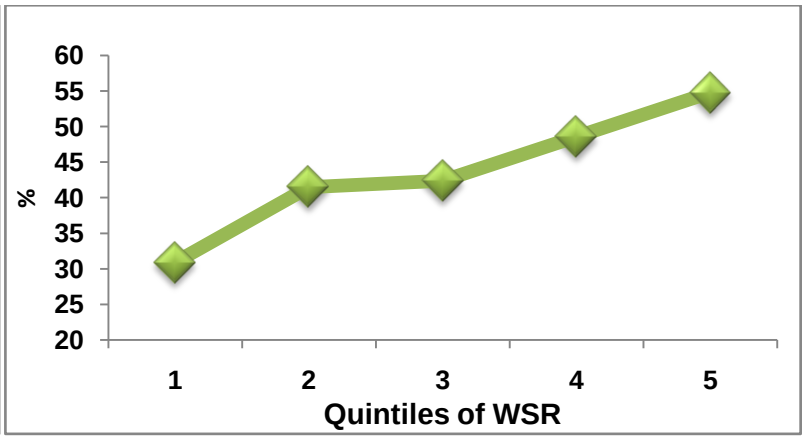
Quintiles	N = 164	WC >90	SBP >120	DBP >80	FBS ≥100	PG2/ PP2 ≥140	FBS, PPBS	TC >200	TAG >150	LDL >100	HDL <40	TG/H >3	AIP >0.21	TSH >4.5
1st 0.44-0.55)	42	19 (45.2)	23 (54.8)	29 (69.0)	13 (30.9)	3 (7.1)	15 (35.7)	14 (33.3)	1 (2.4)	27 (64.3)	11 (26.2)	3 (7.1)	16 (38.1)	13 (30.9)
2nd (0.56-0.59)	41	41 (100)	25 (61.0)	26 (63.4)	17 (41.5)	9 (21.9)	18 (43.9)	19 (46.3)	2 (4.9)	31 (75.6)	16 (39.0)	7 (17.1)	21 (51.2)	5 (12.2)
3rd (0.60-0.63)	33	33 (100)	27 (81.8)	27 (81.8)	14 (42.4)	7 (21.2)	14 (42.4)	14 (42.4)	9 (27.3)	23 (69.7)	13 (39.4)	9 (27.3)	21 (63.6)	5 (15.1)
4th(0.64-0.66)	37	37 (100)	26 (70.3)	27 (73.0)	18 (48.6)	6 (16.2)	18 (48.6)	17 (45.9)	5 (13.5)	30 (81.1)	17 (45.9)	11 (29.7)	24 (64.9)	11 (29.7)
5th(0.67 - 0.77)	31	31 (100)	26 (83.9)	28 (90.3)	17 (54.8)	11 (35.5)	18 (58.1)	13 (41.9)	8 (25.8)	24 (77.4)	15 (48.4)	10 (32.2)	21 (67.7)	6 (19.3)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

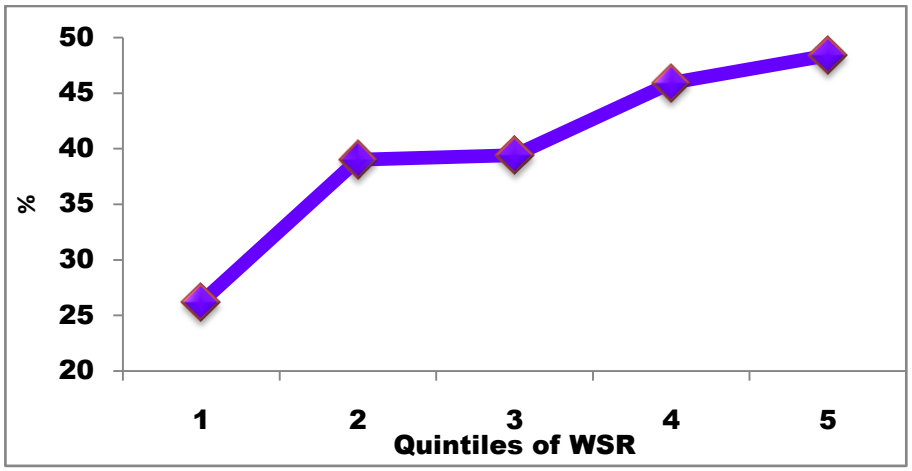
FIGURE 4.28 PREVALENCE OF HIGH SBP, FBS AND HDL ACROSS QUINTILES OF WSR (AHMEDABAD)



4.28a SBP



4.28b FBS



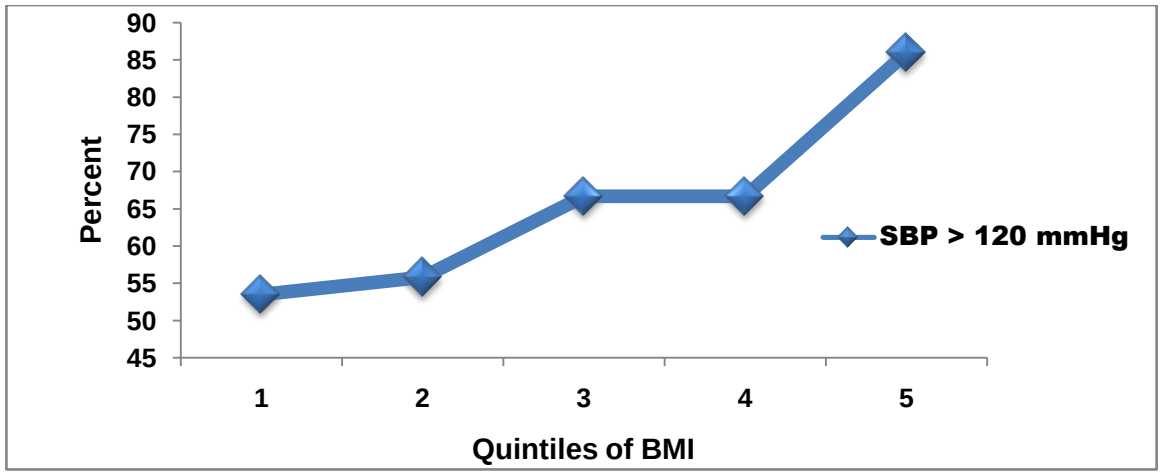
4.28c HDL

TABLE 4.48 PREVALENCE OF ABERRATIONS IN BIOCHEMICAL PARAMETERS ACROSS QUINTILES OF BMI (AHMEDABAD N=213)ϕ

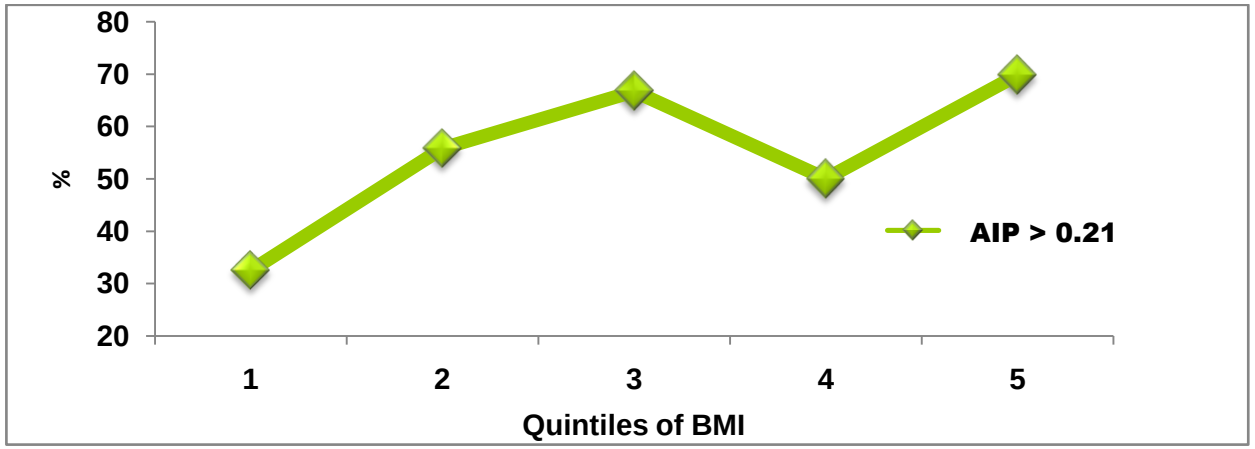
Quintiles	N= 209	WC> 90	SBP> 120	DBP> 80	FBS ≥100	PG2/PP2 ≥140	FBS≥ 100 &/or PPBS ≥140	TC >200	TAG >150	LDL >100	HDL <40	TG/H >3	AIP >0.21	TSH >4.5
1st (16.3-24.0)	43	20 (55.5)	23 (53.5)	26 (60.5)	15 (34.9)	8 (18.6)	17 (39.5)	17 (39.5)	4 (9.3)	25 (58.1)	15 (34.9)	5 (11.6)	14 (32.5)	12 (27.9)
2nd (24.1-26.0)	43	30 (90.9)	24 (55.8)	29 (67.4)	20 (55.5)	8 (18.6)	21 (48.8)	22 (51.2)	9 (20.9)	35 (81.4)	17 (39.5)	10 (23.2)	24 (55.8)	7 (16.3)
3rd (26.1-28.5)	42	33 (91.6)	28 (66.7)	30 (71.4)	16 (38.1)	3 (7.1)	17 (40.5)	15 (35.7)	6 (14.3)	31 (73.8)	18 (42.8)	11 (26.2)	28 (66.7)	8 (19.0)
4th(28.6-31.3)	42	37 (100)	28 (66.7)	29 (69.0)	19 (45.2)	10 (23.8)	20 (47.6)	13 (30.9)	3 (7.1)	31 (73.8)	16 (38.1)	7 (16.6)	21 (50.0)	9 (21.4)
5th(31.4 - 41.9)	43	41 (97.6)	37 (86.0)	38 ()88.4	21 (48.8)	12 (27.9)	21 (48.8)	20 (55.5)	7 (16.3)	33 (76.7)	18 (41.9)	11 (25.6)	30 (69.8)	10 (23.2)

Values in parenthesis indicate percentage, ϕ N for PG2BS, Insulin and HOMA is different from the N reported for other parameters in each quintile

FIGURE 4.29 PREVALENCE OF ABERRATIONS IN SBP AND AIP LEVELS ACROSS QUINTILES OF BMI (AHMEDABAD)



4.29a SBP



4.29b AIP

UNIVARIATE PREDICTORS OF CLINICAL CONDITIONS

HYPERTENSION

All the cardio-metabolic risk parameters were analyzed for their ability to predict risk of developing hypertension, by estimating the odds ratios for all these parameters (Tables 4.49 to 4.51). In pooled data, it was observed that an age of ≥ 40years, no college education, being post menopausal, high WC, WSR, BMI, FBS ≥100mg/dl and AIP > 0.21 were significantly associated with increased risk of developing hypertension in the subjects (Table 4.49).

The strongest predictor among all these was being post menopausal, which increased the chances of developing hypertension more than 4 times (OR 4.6, 95% CI: 2.4-8.8, p< 0.001). Thus, menopausal women have the double jeopardy of increased hypertension risk because of menopausal transition as well as advancing age, neither of which are modifiable. However, many researchers have argued about the individual contribution of menopausal status, free from that of age, because the observed effects of menopausal status can also be because of age. To ascertain the role of menopausal status here, the odds ratios for menopause, adjusted for age were also calculated (Figure 4.30) which clearly showed that the effects of menopausal status independent of age were still significant as compared to age, whose significance declined when adjusted for menopause. Thus women who have a menopause certainly have higher odds of developing hypertension than women who are not menopausal, irrespective of age.

The above trend was consistent in both Ahmedabad (Table 4.50) and Baroda (Table 4.51), where highest OR value was for amongst all the significant predictors.

TABLE 4.49 UNIVARIATE PREDICTORS OF HYPERTENSION (TOTAL)

Risk Factor	Odds Ratio (OR)	95% CI limits	'p' Value
Age ≥ 40yr	**4.4**	**2.2 – 8.7**	**0.000****
No college Education	**2.6**	**1.3 – 5.1**	**0.001*****
Post Menopausal	**4.6**	**2.4 – 8.8**	**0.000*****
No PA	0.77	0.4 – 1.2	0.27
High WC	**5.1**	**1.9 – 14.3**	**0.000*****
High WSR	**3.9**	**1.1 – 13.6**	**0.009***
BMI ≥23	**2.6**	**1.2 – 5.2**	**0.003****
Frequent Fried Food consumption	1.1	0.7 – 1.9	0.53
GLV consumption < weekly	1.2	0.7 – 2.3	0.35
FBS ≥ 100mg/dl	**4.1**	**2.1 – 7.8**	**0.000*****
HOMA2 > 1.4	0.9	0.5 – 1.7	0.86
TSH > 4.5µIU/ml	0.7	0.4 – 1.3	0.31
TC≥200	1.3	0.7 – 2.3	0.25
TAG≥150	1.7	0.8 – 3.5	0.09
LDL≥100	**0.8**	**0.5 – 1.5**	**0.65**
Low HDL	0.9	0.5 – 1.5	0.84
AIP>0.21	**1.7**	**1.03 – 2.9**	**0.02***

**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.50 UNIVARIATE PREDICTORS OF HYPERTENSION (AHMEDABAD)

Variable	Odds Ratio (OR)	95% CI limits	'p' Value
Age	**5.1**	**2.2 – 9.2**	**0.000*** **
Education	**2.82**	**1.49 – 5.33**	**0.001** **
No PA	0.66	0.34 – 1.30	0.22
Post-menopausal	**6.3**	**2.5 – 16.2**	**0.000*** **
High WC	1.05	0.44 – 2.51	0.91
High WSR	1.64	0.46 – 5.82	0.39
BMI ≥23	1.62	0.57 – 4.60	0.32
High Fried Food	0.78	0.38 – 1.60	0.48
GLV≥weekly	1.02	0.49 – 2.14	0.95
FBS>100mg/dl	**5.1**	**2.3 – 11.5**	**0.000***
HOMA >1.4	0.8	0.3 – 2.3	0.7
Hypo-thy	0.75	0.33 – 1.71	0.47
TC≥200	**1.98**	**1.04 – 3.80**	**0.03***
TAG≥150	1.09	0.42 – 2.84	0.86
LDL≥100	1.77	0.62 – 5.09	0.24
Low HDL	0.65	0.31 – 1.35	0.22
AIP >0.21	**2.0**	**1.0 – 4.0**	**0.03***

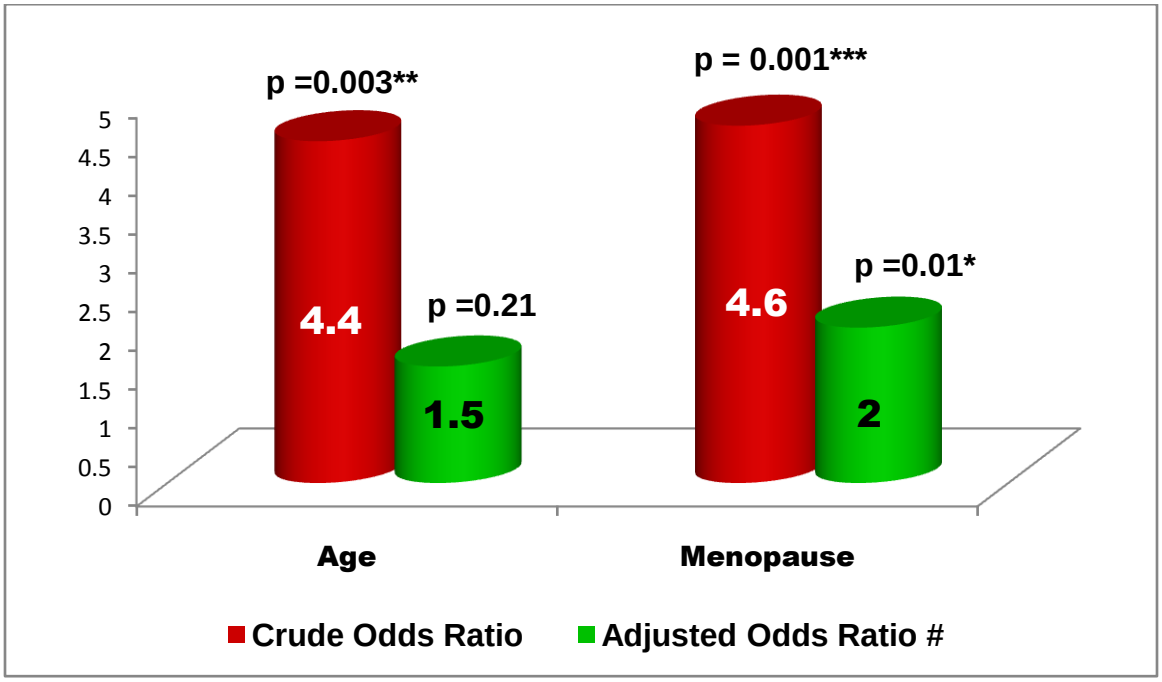
**** Significantly different at p<0.05,** P<0.01,***P<0.001***

TABLE 4.51 UNIVARIATE PREDICTORS OF HYPERTENSION (BARODA)

VARIABLE	ODDS RATIO (OR)	95% CI LIMITS	p VALUE
Age ≥ 40yr	**3.2**	**1.3 – 8.1**	**0.003****
No college Education	1.9	0.5 – 6.8	0.25
Post Menopausal	**4.1**	**1.5 – 11.1**	**0.001*****
No PA	0.8	0.3 – 1.1	0.62
High WC	1.6	0.3 – 7.6	0.46
High WSR	0.4	0.02 – 4.8	0.66
BMI ≥23	**2.6**	**1.0 – 7. 1**	**0.03***
Frequent Fried Food consumption	1.3	0.6 - 2.8	0.45
GLV consumption < weekly	1.1	0.4 – 2.5	0.89
FBS ≥ 100mg/dl	**4.1**	**1.1 – 19.0**	**0.01****
HOMA2 > 1.4	0.8	0.3 – 2.0	0.64
TSH > 4.5µIU/ml	0.8	0.3 – 2.0	0.67
TC≥200	1.1	0.4 – 2.9	0.68
TAG≥150	1.0	0.3 – 2.5	1.00
LDL≥100	0.9	0.4 – 1.9	0.8
Low HDL	0.6	0.3 – 1.4	0.27
AIP>0.21	1.0	0.4 – 2.2	1.00

Significantly different at P<0.05, ** p<0.01, *** P<0.001

FIGURE 4.30 ODDS RATIOS OF AGE AND MENOPAUSE FOR RISK OF DEVELOPING HYPERTENSION: MENOPAUSE PREDICTS HYPERTENSION INDEPENDENT OF AGE



 # Odds ratio for age adjusted for menopausal status and that for menopausal status adjusted for age

The non-modifiable significant predictor identified was FBS and AIP in Ahmedabad (FBS OR 5.1, p<0.001; AIP OR 2.0, p<0.05), and in Baroda it was FBS and BMI (FBS OR 4.1, p<0.01; BMI OR 2.6, p<0.05).

Thus, these results reflect the stronghold of non-modifiable risk factors in developing hypertension, and hence it is all the more important to keep the modifiable risk factors under strict control so as to keep the risk of developing hypertension in check.

DIABETES

The predictor variables identified for risk for developing diabetes have been described in Table 4.52, which depicts the combined data for Ahmedabad and Baroda. The main non-modifiable variables identified included age (OR 5.1, p<0.01), no college education (OR 7.6, p<0.001) and being post menopausal (OR 5.4, p<0.001). The main modifiable predictors included HOMA2 IR (OR 3.7, p<0.001) and AIP (OR 2.0, p<0.05).

City-specific data reflected greater influence of non-modifiable risk factors in predictability of diabetes. In case of Baroda (Table 4.53), except for being post menopausal, the major predictor variables were frequent fried food consumption (OR 5.3, p<0.05), HOMA2 IR (OR 3.3, p<0.05), TC (OR 4.1, p< 0.001) and TAG (OR 3.2, p< 0.05). With regard to data from Ahmedabad (Table 4.54), the main non-modifiable predictors were high WC (OR 2.37, p<0.05), WSR (OR 6.1, p<0.05) and BMI (OR 1.9, p<0.05).

Thus it can be observed is that a number of modifiable risk factors like anthropometric indices, lipid fractions and insulin resistance significantly influence development of diabetes, and should be made the focus of preventive strategies to deal with diabetes.

TABLE 4.52 UNIVARIATE PREDICTORS OF DIABETES (TOTAL)

Variables	OR	95% CI Limits	'p' value
Age ≥ 40yr	**5.1**	**1.1 – 31.6**	**0.01****
No college Education	**7.6**	**3.8 - 151**	**0.000*****
Post Menopausal	**5.4**	**1.7 – 19.1**	**0.000*****
No PA	0.9	0.4 – 1.95	0.89
High WC	4.1	0.5 – 84.7	0.13
High WSR	4.1	0.5 – 84.7	0.13
BMI ≥23	2.0	0.6 – 7.0	0.19
Frequent Fried Food consumption	1.3	0.6 – 2.7	0.44
GLV consumption < weekly	1.2	0.6 – 2.7	0.47
FBS ≥ 100mg/dl			
HOMA2 > 1.4	**3.7**	**1.6 – 8.5**	**0.000*****
TSH > 4.5µIU/ml	0.6	0.2 – 1.5	0.26
TC≥200	0.8	0.4 – 1.9	0.74
TAG≥150	1.8	0.7 – 4.2	0.12
LDL≥100	1.3	0.6 – 2.9	0.4
Low HDL	1.0	0.5 – 2.0	0.94
AIP>0.21	**2.0**	**0.98 – 4.6**	**0.04***

Significantly different at P<0.05, ** P<0.01, *** P<0.001

TABLE 4.53 UNIVARIATE PREDICTORS OF DIABETES (BARODA)

Variables	OR	95% CI Limits	'p' value
Age ≥ 40yr	2.1	0.4 – 14.3	0.32
No college Education	2.0	0.4 – 11.2	0.33
Post Menopausal	**4.3**	**0.89 – 30.9**	**0.05**
No PA	1.7	0.5 – 5.5	0.28
High WC	0.9	0.1 – 20.9	0.94
High WSR	0.9	0.1 – 20.9	0.94
BMI ≥23	0.4	0.04 – 11.9	0.51
Frequent Fried Food consumption	**5.3**	**1.1 – 35.6**	**0.01****
GLV consumption < weekly	1.2	0.3 – 4.0	0.73
HOMA2 > 1.4	**3.3**	**1.0 – 10.9**	**0.02***
TSH > 4.5µIU/ml	0.8	0.2 – 3.2	0.85
TC≥200	**4.1**	**1.6 – 10.7**	**0.000*****
TAG≥150	**3.2**	**0.99 - 104**	**0.04***
LDL≥100	2.2	0.6 – 8.7	0.16
Low HDL	0.7	0.2 – 2.4	0.65
AIP>0.21	2.55	0.6 – 11.7	0.14

Significantly different at P<0.05, ** P<0.01, *** P<0.001

TABLE 4.54 UNIVARIATE PREDICTORS OF DIABETES (AHMEDABAD)

Risk Factors	OR	95% CI	p value
Age	1.69	0.87 – 3.31	0.08
Education	**1.41**	**0.99 – 2.01**	**0.05**
Menopausal Status	**5.5**	**1.1 – 36.0**	**0.01****
No PA	0.71	0.49 – 1.03	0.06
High WC	**2.37**	**0.95 – 5.87**	**0.02***
High WSR	**6.15**	**0.92 – 41.20**	**0.008****
BMI ≥23	**1.92**	**0.92 – 4.00**	**0.04***
High Fried Food	0.93	0.64 – 1.36	0.7
GLV≥ weekly	1.02	0.69 – 1.52	0.9
HOMA2 >1.4	2.6	0.89 – 9.0	0.07
Hypo-thy	0.72	0.45 – 1.17	0.1
TC≥200	1.28	0.90 – 1.83	0.1
TAG≥150	1.50	1.00 – 2.26	0.08
LDL≥100	1.30	0.82 – 2.07	0.2
Low HDL	1.40	0.98 – 1.99	0.06
AIP >0.21	2.1	0.7 – 5.9	0.11

Significantly different at P<0.05, ** P<0.01, *** P<0.001

CORRELATION BETWEEN RISK FACTORS

It was well observed in the previous analyses that the aberrations in the anthropometric and biochemical parameters significantly predict the outcome of clinical conditions. However to test how linearly these parameters are associated with parameters defining the clinical conditions: namely SBP and DBP for hypertension and FBS, insulin and HOMA2 IR for diabetes, the correlations between each of these were computed (Table 4.55). It was observed that the all the anthropometric indices correlated very well with all the above mentioned dependent variables, except FBS, which was not significantly correlated with BMI. Apart from the anthropometric indices, SBP was significantly correlated with FBS (r=0.19), TC (r=0.10) and TAG (0.11). FBS was significantly correlated with TC (r=0.10), TAG (r=0.15), TAG/H (r=0.14) and AIP (r=0.14) and was negatively correlated with TSH (-0.10). It was observed that HOMA2 IR and Insulin were significantly correlated with most of the parameters including the anthropometric and lipid parameters.

In Baroda specifically (Table 4.56), FBS was correlated with SBP (r=0.17), DBP (r=0.13), TAG (r=0.24), TAG/H (r=0.28) and AIP (r=0.28). Insulin was also correlated significantly with most of these parameters, in addition to TSH (-0.18). Similarly HOMA2 IR was also significantly correlated with most the significant predictors of FBS.

With respect to Ahmedabad (Table 4.57), both SBP and DBP were correlated significantly with FBS, insulin, HOMA2 IR and lipid fractions. The glucose control parameters were well correlated with lipid fractions.

Thus it can be inferred that anthropometric and lipid parameters are correlated with parameters that define the metabolic conditions of hypertension and diabetes and hence should be the one of the target goals of therapy.

TABLE 4.55 CORRELATION OF RISK FACTORS WITH BLOOD PRESSURE, FBS, INSULIN AND HOMA2 IR (TOTAL)

Risk Factors	SBP	DBP	FBS mg/dl	Insulin µIU/ml	HOMA2 IR
BMI	0.265***	0.234***	0.219	0.316	0.329
WC	0.248***	0.240***	0.125*	0.359***	0.363***
WSR	0.30***	0.272***	0.141*	0.334***	0.341***
FBS mg/dl	0.193***	0.102*	1.000***	0.184***	0.268***
Insulin µIU/ml	0.043	0.024	0.184***	1.0***	0.991***
HOMA2 IR	0.062	0.041	0.268***	0.99***	1.0***
TC mg%	0.103*	0.062	0.101*	0.072	0.081
TAG mg%	0.116*	0.128*	0.150**	0.288***	0.301***
LDL C mg%	0.084	0.072	0.092	0.092	0.095
VLDL C mg%	0.121*	0*.136	0.141**	0.292***	0.305***
HDL C mg%	0.011	-0.062	-0.035	-0.238***	-0.239***
TAG/H	0.092	0.129*	0.141**	0.320***	0.332***
AIP	0.092	0.129*	0.141**	0.320***	0.332***
TSH µIU/ml	-0.052	-0.026	-0.106*	-0.135*	-0.137*

#Values are Spearman correlation coefficients

****significant at the .001 level (2-tailed)***

***** significant at the .01 level (2-tailed)***

****** significant at the .05 level (2-tailed)***

TABLE 4.56 CORRELATION OF RISK FACTORS WITH BLOOD PRESSURE, FBS, INSULIN AND HOMA IR (BARODA)

Risk Factors	SBP	DBP	FBS mg/dl	Insulin µIU/ml	HOMA_IR
BMI	0.295***	0.277***	0.219**	0.166*	0.188*
WC	0.28***	0.29***	0.24***	0.23***	0.25***
WSR	0.31***	.30	0.217**	0.180*	0.204**
SBP	1.0***	0.83***	0.173*	-0.03	-0.004
DBP	0.83***	1.0***	0.130	-0.015	0.012
FBS mg/dl	0.173	0.130	1.0***	0.23**	0.329***
Insulin µIU/ml	-0.032	-0.015	0.23**	1.0***	0.986***
HOMA2 IR	-0.004	0.012	0.329***	0.986***	1.0***
TC mg%	0.113	0.061	0.020	0.021	0.028
TAG mg%	0.081	0.096	0.245***	0.224**	0.259***
LDL C mg%	0.104	0.087	-0.007	-0.004	-0.007
HDL C mg%	0.059	-0.032	-0.20*	-0.168	-0.181*
TAG/H	0.033	0.086	0.282***	0.258***	0.291***
AIP	0.033	0.086	0.282***	0.258***	0.291***
TSH µIU/ml	-0.025	-0.041	-0.097	-0.185*	-0.180*

 # Values are Spearman correlation coefficients

***** Correlation is significant at the .01 level (2-tailed)***

**** Correlation is significant at the .05 level (2-tailed)***

TABLE 4.57 CORRELATION OF RISK FACTORS WITH BLOOD PRESSURE, FBS, INSULIN AND HOMA IR (AHMEDABAD)

	SBP	DBP	FBS mg/dl	PG2BS	Insulin µlU/ml	HOMA2 IR
BMI	0.268***	0.220***	0.144*	0.205**	0.505***	0.494***
WC	0.223**	0.153*	0.19**	0.292***	0.471***	0.465***
WSR	0.298***	0.231**	0.175*	0.326***	0.463***	0.457***
SBP	1.0***	0.765***	0.31***	0.239***	0.132	0.139
DBP	0.765***	1.0***	0.147**	0.189*	0.110	0.114
FBSl	0.31***	0.147*	1.0***	0.545***	0.304***	0.345***
PG2BS	0.239***	0.189*	0.545***	1.0***	0.34***	0.345***
Insulin	0.132	0.110	0.304***	0.34***	1.0***	0.996***
TC	0.136*	0.112	0.030	0.049	0.135	0.133
TAG	0.136*	0.151*	0.236***	0.312***	0.338***	0.339***
LDL	0.114	0.106	0.056	0.026	0.201*	0.201*
TAG/H	0.125	0.153*	0.248***	0.306***	0.358***	0.36***
AIP	0.125	0.153*	0.248***	0.306***	0.358***	0.36***
TSH	-0.094	-0.024	-0.118	-0.145*	-0.059	-0.062
HDL	0.006	-0.061	-0.187*	-0.139*	-0.277***	-0.281***
HOMA2 IR	0.139	0.114	0.345***	0.345***	0.996***	1.0***

 # Values are Spearman correlation coefficients

***** significant at the .01 level (2-tailed)***

**** significant at the .05 level (2-tailed)***

MULTIVARIATE PREDICTORS OF BLOOD PRESSURE AND BLOOD SUGAR

To investigate how multiple variables in this study synergistically predicted variations in the variables defining the outcomes of hypertension and diabetes (SBP, DBP and FBS that is), multivariate analysis was carried out on the dependent variables SBP, DBP and FBS (Table 4.58). It was observed the multivariate model that explained maximum amount of variation in SBP consisted of 6 significant variables: DBP, FBS, TC, LDL, TAG/H and AIP. This particular model directly explained 61% of variation in SBP. The semi partial correlation coefficients (adjusted partly for covariates DBP, BMI, WSR, WHR, WC, FBS, Insulin, HOMA2 IR, PG2BS, TC, TAG, LDL, HDL, TAG/H and AIP, but not for dependant variable SBP) of these variables indicates that even after adjusting, DBP had the strongest correlation (r=0.68), followed by FBS (r=0.12) and TC (r=0.13). AIP and LDL were found to have a negative coefficient (-0.10 and -0.15 respectively) indicating they had a suppressive effect compared to other variables in the model.

With regard to DBP, the best fit multivariate model was able to explain 58% of the variation in DBP (Table 4.58). The significant predictors included BMI (r=0.11, partly adjusted for all covariates mentioned above), WC (r=-0.10) and SBP (r=0.73), which is surprising that WC had a negative but weak correlation.

In case of FBS, it was seen that a number of variables affected variation in FBS, however, the most apt model that explained maximum possible variation (54%), included only PG2BS (partly adjusted r=0.50), Insulin (r=-0.24) and HOMA2 IR (r=0.25).

City-specific analyses depicted that in data from Ahmedabad the significant predictors were similar to those in the pooled analysis: anthropometric and lipid parameters for DBP, anthropometric for DBP and Insulin & HOMA2 IR for FBS (Table 4.59). However in case of Baroda (Table 4.60), it was seen that DBP and

TABLE 4.58 MULTIVARIATE PREDICTORS OF BLOOD PRESSURE AND BLOOD SUGAR (TOTAL, N=399)

SBP			DBP			FBS		
Predictor Variables	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**
DBP	0.61	0.689	BMI	0.58	0.119	PG2BS	0.54	0.505
FBS		0.128	WC		-0.106	Insulin		-0.245
TC		0.139	SBP		0.736	HOMA		0.252
LDL		-0.150						
TAG/H		0.130						
AIP		-0.103						

TABLE 4.59 MULTIVARIATE PREDICTORS OF BLOOD PRESSURE AND BLOOD SUGAR (AHMEDABAD, N=213)

SBP			DBP			FBS		
Predictor Variables	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**
DBP	0.63	0.688	BMI	0.57	0.109	PG2BS	0.54	0.492
FBS		0.121	WC		-0.095	Insulin		-0.226
TC		0.141	SBP		0.732	HOMA IR		0.234
LDL		-0.150				SBP		0.099
TAG/H		0.125						
AIP		-0.097						

TABLE 4.60 MULTIVARIATE PREDICTORS OF BLOOD PRESSURE AND BLOOD SUGAR (BARODA, N=186)

SBP			DBP			FBS		
Predictor Variables	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**	**Predictor Variables**	**Adjusted r^2**	**Adjusted Correlation**
WC	0.79	-0.175	WC	0.75	0.137	Insulin	0.60	-0.742
WSR		0.151	TC		-0.151	HOMA IR		0.720
DBP		0.769	LDL		0.165	TAG/H		0.152
TC		0.173	SBP		0.840			
LDL		-0.169						
TSH		0.129						

FBS also had lipid parameters as significant predictors, indicating the influence the lipid fractions in most of the dependant variables studied.

As a consequence, following inferences can be made from the multivariate analysis carried out:

- In case of blood pressure, the main predictors that collectively influence changes in SBP and DBP were observed to be BMI, WC, FBS, TC, LDL, TAG/H and AIP.
- In case of FBS, the major determinants identified were Insulin levels and HOMA2 IR and PG2BS, SBP and TAG/H.
- All of the above results point to the fact that both blood pressure and blood sugar are influenced to a great deal by simple, non-invasive, cost effective anthropometric indices and also by lipid parameters, this an implication to the fact that anthropometric indices and blood pressure should be an integral part of surveillance and diagnostic measures for early detection of hypertension and diabetes
- From the management point of view, it is evident that controlling the BMI (the weight, that is) especially the waist circumference, brings about a sea-change in the other prominent determinants like FBS levels and lipoprotein fractions

KEY DIFFERENCES IDENTIFIED BETWEEN THE CARDIO-METABOLIC RISK SITUATIONS OF AHMEDABAD AND BARODA

The subjects of Ahmedabad and Baroda were enrolled from different settings: in Ahmedabad, the subjects were chosen from a clinical setting where they were attending a health check up facility; whereas in Baroda, they were enrolled from the free-living population from all the major zones of the city. There were some key differences identified between the disease distribution and major determinants identified for these conditions between both the cities. Following are the major differences.

- The prevalence of waist circumference, low HDL and metabolic syndrome was higher in Baroda, while the prevalence of severe obesity, elevated TC and LDL was higher in Ahmedabad, suggesting that in a population presenting for a health check-up in an institution, suffers more from increased body and circulating fat (Table 4.61).
- The prevalence of all types of menopausal symptoms in subjects from Ahmedabad was higher than in those from Baroda (Table 4.61), indicating higher degree of estrogen withdrawal effects on physiologies of people presenting for health check-up, in addition to the problems mentioned in the above point.
- Pre-existing hypertension and diabetes were higher in Ahmedabad compared to Baroda (Table 4.61).
- Age-wise stratification of hypertension revealed that hypertension was mostly prevalent in the older age group of 51-60 years in case of Baroda, versus the maximum prevalence in case of Ahmedabad being in the younger age group of 31-40 years.
- The nutrient intakes were higher in Ahmedabad and so was consumption of fried snacks and bakery & confectionery items (Table 4.61).
- The main modifiable Univariate predictors of hypertension in Baroda were found to be BMI and FBS, versus TC, AIP and FBS in case of Ahmedabad.
- In case of Diabetes, the main Univariate predictors for Ahmedabad turned out to be all anthropometric: elevated WC, WSR and BMI. While the same for Baroda were found to be increased consumption of fried foods, in addition to elevated TC, TAG and insulin resistance.
- Multivariate risk factor analysis of systolic blood pressure revealed that subjects in Ahmedabad had more of lipid parameters that best predicted the variation in SBP, compared to those in Baroda, whose SBP varied more with changes in WC, WSR, TSH and lipid parameters like TC and LDL.
- Regarding FBS, it was found that it was mostly affected by insulin levels and HOMA IR, in addition to these, there was SBP which was included in the model that best predicted variation in FBS in case of Ahmedabad, versus TAG/HDL ratio in case of Baroda.

TABLE 4.61 KEY DIFFERENCES IN THE BEHAVIORAL AND CARDIO-METABOLIC RISK SITUATIONS BETWEEN AHMEDABAD (CLINICAL SETTING) AND BARODA

Parameter	Baroda	Ahmedabad
Consumption of Fried Snacks	38%	41% ↑
Consumption of Bakery/Confectionery items	33%	40% ↑
No Physical Activity	43%	45% ↑
Energy Intake	1562kcal	**1733kcal ↑ ***
Severe Obesity (Grade II & III)	19%	29 ↑
Pre existing Hypertension	32%	35% ↑
Pre-existing Diabetes	2.1%	9.1% ↑
Insulin Resistance	21%	**31% ↑****
TC	27%	**39% ↑ ****
LDL	57%	**73.% ↑*****
Vasomotor Symptoms	28%	30% ↑
Somatic Symptoms	21%	24% ↑
Psychological Symptoms	19%	22%↑
Urogenital Symptoms	16%	19%↑

DISCUSSION

Despite the widespread awareness about the alarming increases in mortality rates due to cardiovascular and metabolic diseases and the consequent reduction in the incidence of CVD mortality, it continues to be the leading cause of mortality worldwide (Shaw et al 2006). The figures have been worse for women: more coronary artery disease related deaths have occurred in women as compared to men in the US since the year 1984. The American Heart Association (2004) reported that almost 60,000 more CVD-related deaths were reported amongst females in the US compared to the males. From supplementary evidence it is evident that despite male admissions for CAD are more frequent; women have higher one-year death and reinfarction rates (Epstein et al 2003) as well as higher in-hospital mortality rates (Stramba-Badiale 2006).

All the underlying disarray in the metabolic parameters has been well documented to be affected by the endocrine disturbances in the hypothalamo-pituitary-ovarian axis, either directly, or in addition to their effect on body composition and body fat redistribution (Vitale et al 2010, Mercuro et al 2010, Davis et al 2012). These endocrine disturbances peak during the menopausal transition and increase the risk of menopausal women of becoming prey to shifts in cardio-metabolic equilibrium and in addition cause a range of disturbances known as the menopausal syndrome.

In the present study, the prevalence of menopausal symptoms was around 29% of vasomotor symptoms, 22% of somatic symptoms, 20% of psychological symptoms and 17% urogenital symptoms. It was observed that the women attending the health check up facility had higher prevalence of menopausal symptoms than the free-living population. Corroborating these findings, Singh (2012) also reported a higher prevalence of menopausal symptoms (75% vasomotor, 62% somatic, 32% psychological and 15.5% urogenital) in women attending a clinic for check up. Apart from this, several Indian authors have also reported that while comparing the relative prevalence of menopausal symptoms, it is the vasomotor symptoms that are more prevalent than somatic, psychological or urogenital symptoms (Kaur, Walia and Singh

2004; Sharma, Tandon and Mahajan 2007; Kakkar et al 2007; Bairy et al 2009; Kapur Sinha and Pereira 2009). Alternatively, Foo-Hoe (2007) reports that in Asian women the most prevalent symptom is joint pain, and amongst Asians, Indian women are more prone to experiencing symptoms than far eastern women, namely the Chinese, Japanese, Koreans and Philipino women.

Aside from the prevalence, vasomotor symptoms are the ones that form the main grounds for prescribing hormone therapy to menopausal women by physicians (Meherishi et al 2010).Vasomotor symptoms in Nepalese women were reported to be as high as 69.7% (Chunni and Sreeramareddy 2011), 35.8% in Bangladesh (Rahman, Salehin and Iqbal 2011), 41.6% in Malaysia (Rahman, Zainudin and Mun 2010) and the highest reported has been in 95.2% in Turkey (Ayranci et al 2010). The effects vasomotor symptoms exert on the biological systems have found to be highly deleterious. They are associated with significantly higher coronary artery calcification and development of atheromatous plaques (Allison et al 2010); greater subclinical cardiovascular disease including poorer endothelial function, that includes increased aortic calcification (Thurston et al 2008), as well as higher intima media thickness (Thurston et al 2011)and altered inflammatory processes (Yasui et al 2006, Bechlioulis et al 2010).

One particular metabolic disorder that aggravates around menopause is decline in the bone mineral density, especially around the hip bone and the hip-thigh bone joint (femoral neck). The present study found the prevalence of osteoporosis to be 11.9% and osteopenia to be 55%, with a prevalence of low bone mass of 67%, indicating that more than half of the women studied had suboptimal bone mass, which is a cause of grave concern. Aggarwal et al (2011) have also reported a low bone mass prevalence of 53% in peri and post menopausal women in North India, whereas Unni, Garg and Pawar (2010) reported a low bone mass prevalence of 45.7% in women above 40 years, irrespective of menopausal status. Studies on lower income women have reported the prevalence of osteoporosis to be as high as 28% (Shatrugna et al 2005), while some studies report a prevalence as high as 50% in post menopausal women in south India (Paul et al 2008).

In the present study, the highest prevalence of low bone mass was found in peri menopausal women (87%), than in pre and post menopausal women (65% and 62% respectively), however, the difference in the prevalence was not statistically significant, suggesting the fact that decline in bone mass, once assumed to be aggravated during the onset of menopause, is no longer confined to the post menopausal category, but affects all the women equally and needs universal strategies for managements across all menopausal stages.The main determinants of bone mass in that have been reported in Indian women are BMI (Paul et al 2008, Kumar et al 2010, Kadam et al 2010), physical activity (Kumar et al 2010, Aggarwal et al 2011), calcium intake (Keramat et al 2008, Kadam et al 2010, Kumar et al 2010, Aggarwal et al 2011). Except BMI, Indian women fare poorly on most of these parameters, especially calcium intake (Keramat et al 2008, Paul et al 2008), which has been identified as the major contributor of low bone mass across all menopausal stages.

It has been recently studied that menopause is associated with increased total body fat as well as increased abdominal fat, leading to increased incidence of obesity and abdominal obesity (Ghosh and Bhagat 2010, Davis et al 2012) . In the present study as well, the aggregate prevalence of obesity though very high (67.4%) the prevalence specific to post menopausal women (75%) was significantly higher ($p<.0.05$) than in pre menopausal women (53%). The Jaipur Heart Watch 5 (Gupta et al 2012), a large scale surveillance study of cardiovascular risk factors in India reported the prevalence of obesity in women to be 50%. Gupta et al (2012) in a large scale migration survey of 4608 individuals, reported a prevalence of 46.7% in urban women, while Ebrahim et al (2010) in a population based survey of 6510 participants, reported a prevalence of 53.5%. The analysis of results of the data on urban Indians of the large scale study Chennai urban rural epidemiology study/ CURES (Deepa et al 2009) revealed a prevalence of 47% in women as compared to 43% in men.

Abdominal obesity was found to be the highest prevalent problem in the subjects studied, with a prevalence of elevated WSR of 91.8% and high WC prevalence of 90.5%. The prevalence was found to be higher in the free-living population

(WC>80cm-93.5%, WSR>0.5 -95.7%) than in the population at the health check up clinic (WC>80cm – 87.5%, WSR>0.5 -91.8%). The analysis of the CURES urban arm (Deepa et al 2009) reported a prevalence of 56% in women (versus 35% in men, p<0.001). Khokhar, Kaur and Sidhu (2010) reported abdominal obesity (WC>80cm) to be 81% in middle aged women in Punjab, while Jyothi and Nayak (2010) report abdominal obesity to be 82.3% in working women in Karnataka.

Such high prevalence of obesity can be partly explained by the snacking patterns and lack of sufficient physical activity by the subjects. As high as 41% of the subjects consumed fried snacks more than once a week and almost 40% of then consumed sweets/bakery/confectionery items more than once a week. The fat intake of the subjects was way higher than the recommended daily intake (176% of the RDA), and for 78% of the subjects, more than 30% of the calories came from fat. Bhagat and Ghosh (2011), who studied the consumption of type of fats in rural Indian population, have reported that females had significantly higher (p<0.001) SFA: MUFA and SFA: PUFA consumption than their male counterparts and had higher mean BMI than the males studied (22.1kg/m^2 versus 21.9kg/m^2 respectively). Udipi et al (2006) reported the fat consumption to be higher than the recommended allowance in 72% in Kolkata, and 32% in Ghaziabad. Conversely, the dietary intake surveys led by National Nutrition Monitoring Bureau (NNMB) in the 10 states of India have reported the visible fat intake to be in a range of 9–13 g only.

To add to this anomaly in the dietary patterns of the subjects in this study, the proportion of those who engaged in more than 3 hours of regular physical activity was less than one-third. Singh and Pella (2007) have reported the prevalence of sedentary behavior as 59.3% among women, in a cross sectional population survey of 6940 individuals across 5 cities: Moradabad, Trivandrum, Kolkatta, Mumbai and Nagpur.Authors also reported a significant increasing trend in sedentary behavior in women after the age of 35-44 years. Sedentary behavior was significantly ($P < 0.05$) greater in Trivandrum, Calcutta, and Bombay compared to Nagpur and Moradabad.

In addition to the above discussed lifestyle factors, Indians are inherently predisposed to obesity on account of their body composition which is characteristically higher in body fat and lower in muscularity. For the same BMI, Asian Indian men have reported to have 7-8% points higher % body fat compared to Caucasians, while Pacific Islanders have 4% points % body fat compared to Caucasians (Rush et al 2004). Compared to European men for the same % body fat, BMI was 2-3 units higher for Pacific Islanders and 3-6 units lower for Asian Indian. In addition to percent body fat, it has also been found that Indians have larger stores of metabolically active central adipose fat depot than peripheral adipose tissue, as compared to Caucasians and other races (Chowdhury, Lantz and Sjostrom 1996, Sniderman et al 2007, Rush et al 2004), which is a predisposing factor and partially the explanation of higher prevalence of metabolic anomalies in south east Asians.

This trait of body composition in southeast Asians has links to various polymorphisms associated with multiple genes that are involved in lipid metabolism and storage. Bhatt and co-workers (2012) have reported polymorphisms in *Myostatin* gene to be associated with increased abdominal fat mass and less of lean body mass in Asian Indians. Radha et al (2007) have reported single gene polymorphisms in lipoprotein lipase gene which may be implicated in predisposing Indians to obesity. In addition, a Q223R SNP in the leptin receptor gene has also been found to be associated with obesity in Indians (Murugesan 2012). In the present study too, the prevalence of aberrations like hypertension, impaired glucose metabolism and lipid metabolism and clustering of all these aberrations was disturbingly high, as is discussed below.

Hypertension

The infamous silent killer that is responsible for 57% of all types of deaths related to stroke and almost one-fourth of all deaths related to cardiovascular events, stands at 48.2% in urban women in India, as reported in the most recent large scale survey involving 4,608 women in Delhi, Haryana, Jaipur, Pune, Kolkata, Kochi and Gandhigram (Gupta et al 2012), implicating that every other woman in urban India suffers from hypertension. The present study estimated the prevalence of

hypertension to be almost the same: 48.3% which included the existing cases and the ones that were diagnosed during the study. The Jaipur Heart Watch 5 study however reported a prevalence of 24.6%, which is almost half of found in this study. Another large sale survey of 88,653 individuals from Mumbai, reported a prevalence of 48.4% of hypertension in women, and the mean SBP was higher in women in all age groups compared to men (Gupta et al 2004).

Major determinants of hypertension that have been documented include age, height, weight, BMI, low education, female gender, smoking, diabetes and high fat diet (Gupta et al 2004; Devi et al 2012, Gupta et al 2012). Other predictors include gene polymorphisms namely mineralocorticoid receptor gene (Sia et al 2012), β 1 adrenoreceptor (Kong et al 2012), methylene tetrahydrofolate reductase (Alghasham et al 2012), inducible nitric oxide synthase (Oliveira-Paula et al 2012), serine-threonine kinase (Maatta et al 2012), angiotensin II receptor (Katsuya and Morishita 2012), PPAR α (Ding et al 2012) to name a few. The main determinants of hypertension in the present study were identified to be almost the same as above: age>40, no college education, overweight/obesity, abdominal obesity, high blood sugar and high levels of atherogenic index of plasma (AIP>0.21); nonetheless an additional determinant turned out to be post-menopausal status, which was the strongest predictor of hypertension in the study (crude OR 4.6, age adjusted OR 2.0). A number of studies disputing the role of menopause in precipitating hypertension have been reported (van Beresteyn, van t Hof, De Waard 1989; Casiglia et al 1996; Luoto et al 2000) which state that the effect of age cannot be ruled out while considering the strength of association of menopause and hypertension. However, even after adjusting for age, the odds ratio of menopause and hypertension was still significant ($p<0.01$) in the present study.

The major effects of menopause on hypertension have been attributed to vasomotor symptoms. Hot flashes have been associated with increased SBP even after controlling for age, race, ethnicity, BMI and even menopausal status (Gerber et al 2007, Gallicchio et al 2010, Sadeghi et al 2011). Part explanation of this association can be attributed to relation of both to central sympathetic activity. During the bouts of hot flashes, the peripheral vasoconstriction and increased cardiac output, both caused

by baroreflex dysfunction, might also have been responsible for accompanying increments in SBP. It has been reported (by Hart, Charkoudian and Miller 2011) that sex hormones interact to modulate several neuroeffector mechanisms which also includes regulation of the Sry gene effect on synthesis, release and re-uptake of monoamine neurotransmitters. Apart from this, estrogen receptors in brain areas are in themselves associated with autonomic control. Thus, timely management of vasomotor symptoms appears relevant to control the blood pressure so that the person is not rendered a hypertensive by the end of the menopausal transition.

Diabetes

The metabolic anomaly that has many a researchers and treatment providers still searching for answers is diabetes, which not only continues to soar in India but the secondary complications of which also prey on a considerable proportion of those affected. The 2012 update by International Diabetes Federation reveals that the burden of diabetes in India is 63 million diabetics. The latest analysis of CURES study where the researchers assessed the comparative prevalence of diabetes by FBS and by glycated Hb in 2188 individuals, reported the prevalence of diabetes to be 6.1%, excluding the existing diabetic cases (Nazir et al 2012). The same prevalence in the present study came out to be 5.5%. Another recent survey on 1178 individuals from the East Indian population (Prasad et al 2012) revealed a prevalence of 15.7%, which is three times the prevalence found in the present study. The Jaipur Heart Watch 5 has reported a prevalence of 10.8% diabetes in urban women, which is twice the prevalence found in the present study. The prevalence of insulin resistance in the study was found to be 25.7%, which was considerably higher in the population attending the health check up section (31.7%), compared to the free living population (21%). However, the prevalence of diabetes was found to be higher in the free living population (6.4%), compared to the subjects attending the health check-up (4.7%). Peterson et al (2006) reported insulin resistance across various ethnic groups using the Insulin sensitivity index (ISI); among them and it was found that Asian Indians had significantly higher insulin resistance (59%) compared to Blacks (33%), Eastern Asians (30%), Caucasians (20%) and Hispanic (18%). Kumar et al (2005) reported the

prevalence of insulin resistance in north Indian population using HOMA, to be 12.8%; the same prevalence according to ISI was 37.8%. The authors also reported that ISI was found to as promising an indicator as HOMA, on account of it poor specificity. The Chennai Urban Population Study (CUPS) conducted on 1262 adult subjects from two residential areas in Chennai, found a prevalence of 11.2% according to HOMA (Deepa et al 2002). MengKhoo et al (2011) reported a comparative account of insulin resistance across Asians, where they found that Asian Indians had highest mean HOMA levels (3.18 ± 3.18), compared to Chinese (1.58 ± 1.43) and the Malays (2.28 ± 1.84), the difference being highly significant (P<0.001).

Major determinants of diabetes identified in several studies are family history, age, male gender, BMI, WHR, blood pressure, serum cholesterol levels (Rahman, Rahim and Nahar 2007; Ramachandran et al 2001, Ajay et al 2008, Ravikumar et al 2011, Bharati et al 2011). The genetic factors that play a role in making south Asians susceptible to diabetes include body composition and central adiposity. Wannamethe et al (2010) in a 7 year prospective study, showed that adiposity measures including BMI, WC and WHR were significant predictors of diabetes and insulin resistance in women, with significant adjusted relative risks being 4.10 (95% CI 2.16–7.79), 12.18 (95% CI 4.83–30.74) and 5.61 (95% CI 2.84–11.09) for BMI, WC and WHR, respectively. In the present study itself, anthropometric parameters were found to have significant correlations with blood sugar values (WC-0.12*, WSR-0.14*), in addition to it, the percent prevalence of high FBS showed a significant trend of increase when analyzed across quintiles of WSR, WC and BMI. However, anthropometric parameters have greater significant association with insulin values and insulin resistance than FBS values itself (correlation between Insulin and WC - 0.35***, Insulin and WSR - 0.34***, HOMA2 and WC - 0.36***, HOMA2 and WSR – 0.34***). Visceral fat depot has been found to be associated with insulin resistance. Increased secretion of free fatty acids, inflammatory cytokines and decreased secretion of adiponectin are molecules mediating obesity and insulin resistance (Boden and Shulman 2002, Matsuzawa 2006). Studies on insulin resistance suggest a dearth of evidence directly addressing the relationship between waist circumference

and hyperinsulinemia or insulin resistance (Lemieux et al 2000, Wahrenberg et al 2005). However, epidemiological observations linking abdominal obesity and insulin resistance have been documented well. A cross-sectional analytical study by Wahrenberg et al (2005) reported a linear increase in hyperinsulinemia prevalence across deciles of waist circumference in Canadian 185 healthy men. Similarly, yet another cross-sectional study conducted on 2746 volunteers, including 1948women, reported that waist circumference was strongly correlated with HOMA-IR in the subjects.

Partial explanation for this association of abdominal obesity can be the characteristic elevated levels of circulating plasma free fatty acids/ FFAs (Koutsar and Jensen 2006). In normal situations,, insulin is able to suppress the intracellular adipocyte lipolysis through the hormone sensitive lipase, and at the same time stimulates intravascular adipose tissue lipoprotein lipase (LPL) for enhancement of circulating triglycerides hydrolysis and subsequent capture and storage of the fatty after a meal (Berger and Bernard 1999, Coppack, Jensen and Miles 1994, Lewis et al 2002). Thus, a diminished ability for re-esterification of fatty acids seems be an one of the important mechanisms of excess availability of FFAs in abdominal obesity. Peripheral insulin resistance is thus a consequence of elevated circulating FFAs (Groop et al 1991; Riemens, Sluiter and Dullaart 2000; Heilbronn, Smith and Ravussin 2004). Similarly, visceral adipose tissue is specifically responsive to lipolytic stimuli, which creates the potential for an elevated FFA delivery to the liver. As a result, there is minimal or no suppression of hepatic glucose production, while the hepatic insulin clearance is not inhibited either (Golay et al 1987, Boden et al 2002).

Another major predictor of diabetes in the present study came out to be post menopausal status (crude odds ratio 5.4, 95% CI: 1.7 – 19.1, $p<0.001$), which was observed consistently in both the free living population and the subjects attending health check up facility. Several animal model molecular studies have demonstrated the beneficial effect of estrogen in insulin sensitivity and euglycemia. Insulin-stimulated glucose uptake in skeletal muscle, mediated by the glucose transporter

isoform GLUT4, is suppressed in the absence of estrogen receptor/ ERα (Bryzgalova et al 2006). In addition, ERβ acts as an inhibitor of PPARγ activity, a major inhibitory regulator of glucose and lipid metabolism (Foryst-Ludwig et al., 2008). Estrogens have been found to increase hepatic insulin sensitivity by decreasing gluconeogenesis and glycogenolysis (Ahmed-Sorour and Bailey, 1981), and increasing insulin release in islets of Langerhans (Alonso-Magdalena et al., 2008).

Apart from the non-modifiable risk factors and body composition, the major modifiable determinants of diabetes identified in several studies include, blood pressure, serum cholesterol levels (Rahman, Rahim and Nahar 2007; Ramachandran et al 2001, Ajay et al 2008, Ravikumar et al 2011, Bharati et al 2011). A recent review by Riccardi, Giacco and Rivellese (2004) has pointed out quite a few dietary factors which were found to be associated with insulin resistance specifically among South Asians, such as higher intakes of refined carbohydrates, SAFA, trans-fatty acids (TFA), -6 PUFA coupled with lower n-3 PUFA intakes, in addition to a low intake of fiber. All these indicate that this characteristic Asian diet may be an important contributory feature for the high prevalence of impaired glucose tolerance.

Thus, given the significant ability of abdominal obesity to predict and precipitate insulin resistance and eventually diabetes, it is of utmost importance that Southeast Asians control the amount of buildup of belly fat as a preventive measure

Dyslipidemia

The most pressing problem identified in the present study came out to be dyslipidemia, with the prevalence of high TC being 33.8%, elevated TAG being 17%, decreased HDL being as high as 47.3% and the most prevalent problem being elevated LDL which was found in, more than 65% of the subjects. The difference in the free living subjects in the study and the subjects attending the health check up was that the prevalence of low HDL was higher in the free living (54.8% in free living versus 39% health check-up) and the prevalence of high LDL was higher in health check up subjects (72.9% versus 57.5%) Various studies have looked at dyslipidemia as one of the risk factors while studying metabolic syndrome, diabetes and cardio

vascular disease, however, the focus remains on the former conditions, even as lipid aberrations continues to be the most prevalent. A recent large scale survey on 6198 individuals across 11 cities in India reported the aggregate prevalence of high total cholesterol levels to be 25.3% among women (Gupta et al 2012), which was lower than that found in men (24.8%), even though marginally so. Yet another large scale comparative study on the rural and urban divide conducted on 4624 women reported the prevalence of high TC to be 27.7% in urban women, compared to a significantly lower 13.5% in the rural counterparts (Pandey et al 2011). The Jaipur Heart Watch 5 (Gupta et al 2012) reported the recent prevalence of high TC to be 32.7% in women and low HDL to be 25.1%.

The trend that the authors have observed over a 20 year period through a series of successive Jaipur Heart Watch studies (Gupta et al 2012) indicates that there was an increasing trend in prevalence of high TC, TAG and high non-HDL ($p_{trend}<0.001$). Global trends in serum cholesterol (Farzadfar et al 2011) indicated that in 2008, age-standardized mean total cholesterol worldwide was 4.64 mmol/L (95% uncertainty interval 4.51-4.76) for men and 4.76 mmol/L (4.62-4.91) for women. These values did not change much globally between 1980 and 2008, where the fall in the values was less than 0.1 mmol/L per decade in both the sexes. There was a fall in the total cholesterol levels in the high-income region, which consisted of North America,Australasia and western Europe while the regional declines in central and eastern Europe were about 0.2 mmol/L per decade. The mean total cholesterol increased in only in the south east and East Asia and Pacific by 0.08 mmol/L per decade (-0.06 to 0.22, posterior probability=0.86) in men and 0.09 mmol/L per decade (-0.07 to 0.26, posterior probability=0.86) in women. This steep increase in the dyslipidemia in the populations may be the one of the pivotal causes of rise in metabolic anomalies in the population.

The determinants of hyperlipidemia have been reported in population of South Western China, where age, alcohol consumption, a preference for meat and animal products, regular dining out, and BMI were found to be the main determinants of hyperlipidemia in women, while high prevalence of salt intake was associated with

hyperlipidemia in men (Deng et al 2012). A study on determinants of hyperlipidemia in Turkish populations (Erem et al 2008) reported that dyslipidemia was significantly associated with age, BMI, WC (except for TC and LDL-C), hypertension (only for LDL-C and TG), FBS (only for LDL-C and TG), education level, cigarette smoking (only for HDL-C and TC/HDL-C ratio), alcohol consumption (except for HDL-C and TC/HDL-C ratio), occupation (especially housewives), marital status (widows and widowers), and a family history (for only TC). Another study reported the effect of intake of SFA intake on the serum lipids in two APOE polymorphism genotypes- rs429358 and rs7412 (Petkeviciene et al 2012). The findings indicated that age, genotype APOE2 (rs7412), SFA intake, and body mass index (BMI) were significant determinants of TC and LDL-C level (with p values ranging from 0.043 to 0.001) as assessed by multivariate linear regression analysis.

Thus, it is evident that the rise in hyperlipidemia is quite steep and needs to be contained through whatever modifiable determinants that affect it.

Metabolic Syndrome

The condition representing utmost risk with regard to cardio-metabolic health, is the metabolic syndrome (MS), which is cause of concern worldwide on account of its rapidly acceleration among all populations. The findings in the present study indicated, much to the dismay that more than one third of the subjects suffered from metabolic syndrome (35.8%), with the prevalence being higher in the subjects from the free living population (41.9%), as compared to the subjects attending the health check up facility who had a prevalence of 30.5%. A city based survey by on 548 subjects Samant et al (2011) in Mumbai, revealed that the prevalence of metabolic syndrome in the women to be 12.5%, which is less than half of that found in this study. Another large scale survey by Prasad et al (2012) on East Indian urban population reported the prevalence of metabolic syndrome in females to be 42.3% in females, compared to 24.9% in males. A yet another survey from Mumbai (Pandey et al 2010) conducted on 498 women reported the prevalence of metabolic syndrome to be 55% in post menopausal women and 45% in premenopausal women, which higher than the

prevalence found in this study. Rampal et al (2012) studied the comparative prevalence of metabolic syndrome across Asian populations settled in Malaysia. The findings revealed an prevalence of 27.5% in the mixed group, with prevalence being least in the Chinese and highest among Indians, with the prevalence ratios compared to Malays being 1.25 for Indians and 0.86 for Chinese and 0.94 for the indigenous Sarawakians. Sinha et al (2012) reported the prevalence in 300 women from south Delhi to be 29.6%.

Several studies have reported older age, female gender, general obesity, BP, HbA1c, hypercholesterolemia, inadequate fruit intake and middle-to-high socioeconomic status to be significant predictors of increased risk of metabolic syndrome (Pandey et al 2010; Das, Pal and Ghosh et al 2011;Prasad et al 2012; Sinha et al 2012). The present study found the main determinants of metabolic syndrome to be FBS, elevated lipid levels. In addition, fasting insulin levels and insulin resistance were also found to be significant predictors ($p<0.05$ and $p<0.001$ respectively).

SALIENT OBSERVATIONS

1. The extent of menopausal symptoms in the subjects was 29% of vasomotor symptoms, 22% somatic symptoms, 20% psychological symptoms and 17% urogenital symptoms.
2. The prevalence of osteoporosis was found to be 11.9% and osteopenia was 55%, indicating aa prevalence of low bone mass to be as high as 67%.
3. Overall obesity was found to be 67.4%, with post menopausal women having a prevalence of 75% and premenopausal women 53%.
4. Abdominal obesity was the highest prevalent, with elevated WSR at 91.8% and elevated WC at 90.5%

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.
6. 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).

7. The prevalence of diabetes in this study was found to be 6.1%, 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.

8. The subjects in the clinical setting (Ahmedabad) were found to suffer from increased prevalence of severe obesity and serum lipid fractions and menopausal symptoms, compared to subjects from free-living population in Baroda.

OVERALL SUMMARY

- The middle aged women studied had a high prevalence of cardio-metabolic risk factors, especially central obesity
- All the metabolic aberrations showed a trend of being significantly higher in the menopausal category than other categories, barring the prevalence of low bone mass, anemia, high AIP and elevated TSH, which were high across all the categories, and hence need special focus.
- The major non-modifiable predictors of the clinical conditions were found to be age>40years, no college education and most importantly, post- menopausal status.
- The most significant modifiable predictors of the clinical conditions in the study came out to be the adiposity measures, especially abdominal obesity, that is WSR and WC, proved to be significant predictors of diabetes and insulin resistance, as indicated by univariate and multivariate analyses.
- Reduction of abdominal obesity and decreasing the waistlines should be an integral part of all management strategies targeted at control of dyslipidemia, diabetes, hypertension and metabolic syndrome.
- The subjects from the clinical setting seemed to have higher degree of estrogen withdrawal and circulating and body fat compared to subjects from free living population.
- 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.

PHASE II

THE IMMEDIATE AND LONGITUDINAL OUTCOMES OF HEALTH CHECK-UP ON WOMEN'S HEALTH CARE PRACTICES

Given that the health situation of menopausal women in India is highly compromised due to high prevalence of multiple risk factors and aberrations in metabolism, it is reasonable to plan intervention strategies for target the situation. However, certain other factors that come into play while administering health services to women in India, that are beyond the purview of health services, include reasons that are social in nature. A considerable proportion of women in India shy away from seeking health services for reasons ranging from economic constraints to lax attitude towards their health to sidelining their health to accommodate the family's wellbeing.

For this reason, it was felt essential to study the health seeking practices of the women to see whether women if despite being informed that they are suffering from certain risk factors proven to predispose them to adverse health events, do they seek medical help or not. For this purpose, the women studied in the first (exploratory research) phase were followed up after 2 years. During the exploratory research, the women who had elevated levels of risk factors studied in that phase, were informed of their high risk situation and were asked to see a doctor for further diagnosis and treatment, if any.

After a period of two years, of the 186 subjects studied in the exploratory research phase, 107 could be followed up, for studying the longitudinal outcomes of the health check-up conducted as a part of the formative research. Of the total 186, only 107 could be contacted, because 27 had permanently moved, 42 were temporarily unavailable because of either being out of station or having changed their contact details, 7 were not willing to share any details and 3 unfortunately, had expired.

The results indicated that the 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 2years (Figure 4.31). 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 (Figure 4.31). The mean blood pressure of the subjects had reduced from 130mmHg to 127mmHg SBP, which was still in the pre-hypertensive category. The DBP had reduced from 82mmHg to 79mmHg (Figure 4.31). 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.

Regarding the health seeking practices of the subjects, it was observed that indeed the women had shockingly low health seeking practices, as reflected by the fact that very few of them actually took some action when they discovered they need health consultation (Figure 4.32). Of the 107 subjects that were followed up, only a mere 3.04% had seen a doctor and more than half of them (57.7%) of the subjects had not taken any action after getting the results of the health check up. The remaining 39.9% reported that they did not consult a doctor because the results were normal. This takes attention to the fact that the health seeking practices of women in India is abysmally low, awareness needs to be created among them so that they realize that health consultation if sought early, will revert most of the adverse health conditions they are predisposed to.

A number of studies have reported that menopausal women in India do not avail the health services available and most of them remain untreated (Sengupta 2003). Despite availability of evidence based medicine, a large proportion of women goes untreated or relies on unproven alternative therapies.

SALIENT OBSERVATIONS

Following observations and conclusions can be made from this small but significant study:

FIGURE 4.31 LONGITUDINAL TRENDS IN BODY COMPOSITION AND BLOOD PRESSURE IN THE SUBJECTS (N=107)

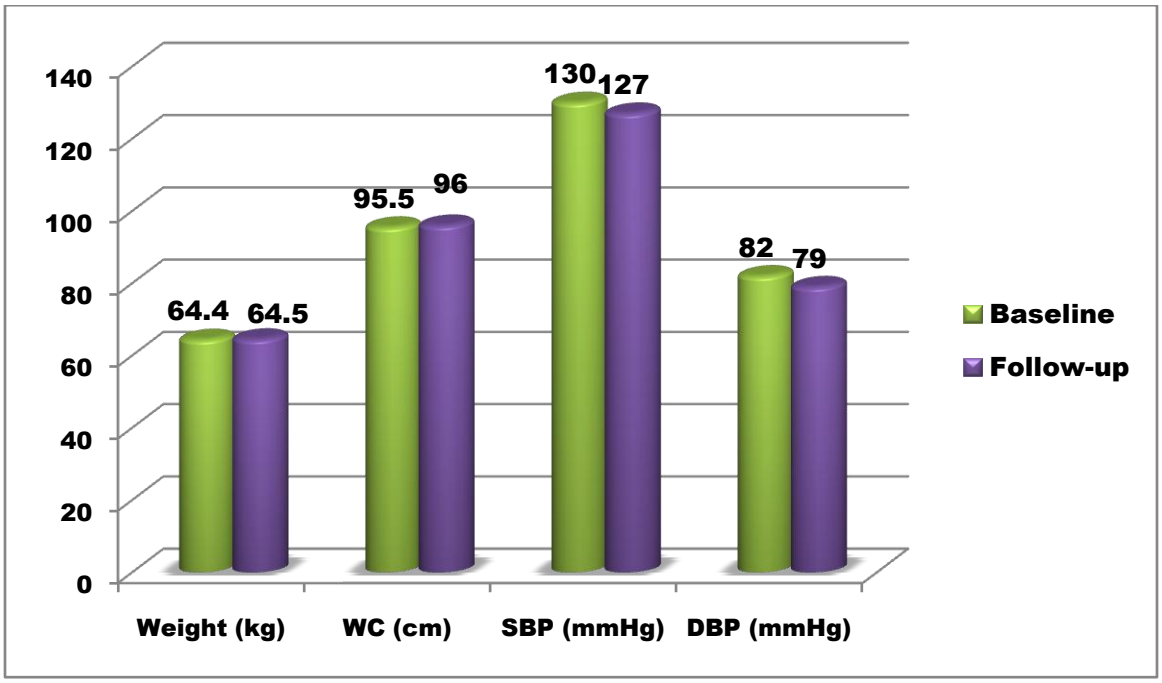
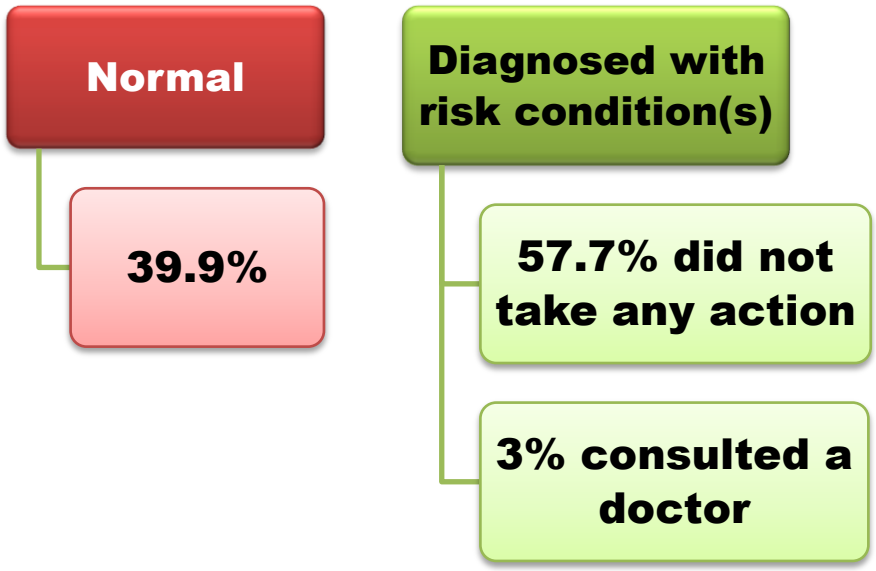


FIGURE 4.32 LONGITUDINAL OUTCOMES OF A HEALTH CHECK UP IN THE SUBJECTS (N=107)



1. The longitudinal data on body composition did not reflect any significant changes in the body composition measures, namely Weight and WC.
2. The longitudinal effect on blood pressure of the subjects was that there was a mean reduction in the blood pressure values over a period of 2 years, this could implicate initiation of hypertension therapy by some subjects, which indicates that women sought medical help.
3. Thus from this small but significant study, the main finding is that despite being informed that they need medical consultation and despite adequate health facilities being available, middle aged menopausal women refrain from seeking health consultation.

CONCLUSIONS

From the results summarized above, it can be concluded that women tend to refrain from seeking health care even in the face of presence innumerable risk factors. This is a cause of concern because this lax attitude can hinder and affect the outreach of health services.

PHASE III

ANALYSIS OF NUTRITIONAL QUALITY OF WHEATGRASS POWDER, ITS INCORPORATION IN DIFFERENT RECIPES AS A FUNCTIONAL FOOD AND ITS ACCEPTABILITY TRIALS

Research in nutrition is one arena which has immense applications in everyday life. However, despite much of remarkable discoveries being done in nutrition science, only a meager part of it percolates down to a common man's life. Innovative approaches to translate scientific insight into tangible results that can be directly applied to the clinical and population setting is the need of the hour. Thus, translational research that is geared towards translating discoveries made in the laboratory into community and patient practices cannot be overemphasized in this regard. Research involving detection, isolation and health benefits of a range of bioactive compounds found in various plant products has paved the way for the major area in translational nutrition, namely: Nutraceuticals. The term stands for any non-toxic food/plant extract that has sufficiently proven health benefits for both disease treatment and prevention. One such breakthrough that the field of nutraceuticals has witnessed is wheatgrass (to follow a botanical description, the shoot of *Triticum aestivum* Linn), which is proposed to have remarkable antioxidant capacity and can prove to be a functional food for the management of chronic diseases. Thus an attempt was made to look into the nutrient content of freeze-dried wheatgrass powder, incorporate it in day to day recipes and investigate the acceptability of these recipes, with the objective of sculpting wheatgrass as a nutraceutical.

NUTRIENT CONTENT ANALYSIS OF FREEZE-DRIED WHEATGRASS POWDER

For this purpose, Freeze-dried nitrogen packed wheatgrass powder was procured from an exporting firm in Vadodara. The nutrient component

analysis (conducted by Analytical & Environmental Sciences, Vadodara) included quantitative testing of energy, protein content, total fat, fibre, iron, moisture, ash, carbohydrate & sugar content, ascorbic acid, and β carotene. The analysis of wheatgrass powder revealed that wheatgrass is an excellent source of many nutrients, as is evident from Table 4.62. Wheatgrass was not a significant source of macronutrients, but had considerable amounts of β-carotene and iron and fibre.

ACCEPTABILITY TESTING OF WHEATGRASS INCORPORATED RECIPES

Freeze-dried wheatgrass powder was incorporated in selected Indian recipes for evaluation of the organoleptic properties. The recipes tested included *Khakhra, Thepla, Muthiya, Dal* and Buttermilk. All the recipes involved different method of cooking and buttermilk did not involve heat application at all. 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

Sensory evaluation of the wheatgrass incorporated recipes revealed that the recipes were equally acceptable at all levels of incorporation, and the mean scores of same recipes at different levels of incorporation did not differ significantly. The overall range of the mean scores of all the recipes was from 6.5 to 7.4, thus indicating moderate to good acceptability. The scores for individual recipes are as follows.

DAL

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 (Table 4.63), indicating moderate acceptability at all levels. The variability of the scores for the within the

TABLE 4.62 NUTRIENT COMPOSITION OF WHEATGRASS POWDER

Nutrients	Result of Nutrient Analysis per 100g
Energy (Kcal)	354
Protein (gm)	28.7g
Carbohydrate (gm)	49.9
Fat (gm)	4.43g
B carotene (µg)	1,08,100
Ascorbic acid (mg)	32.3
Iron (mg)	57.9
Fibre (gm)	25.5
Moisture (%)	6.6

TABLE 4.63 SCORES FOR SENSORY ATTRIBUTES OF WHEATGRASS INCORPORATED *DAL* (MEAN + SD)

Attributes	Levels of Incorporation of Wheatgrass			ANOVA p value
	1.0g	1.5g	2.0g	
Appearance	6.4±1.6	6.5±1.5	6.5±1.2	0.988
Colour	6.9±1.4	7.0±1.7	6.6±1.6	0.795
Flavour	6.9±1.8	6.9±1.7	6.9±1.7	0.992
Consistency	7.2±1.5	7.2±1.6	7.0±1.4	0.910
Aroma	6.4±1.8	6.6±2.0	6.9±1.8	0.832
After taste	6.5±1.5	6.2±1.6	6.5±1.3	0.820
Overall Score	7.1±1.5	6.8±1.3	7.1±1.3	0.766

various levels of incorporation of wheatgrass was not significantly different, as depicted in Table 4.61 yet, there was a trend of decline in the scores towards higher concentration of wheatgrass in the recipes.

BUTTERMILK

In case of buttermilk (Table 4.64), the mean overall scores for the level 1.5g was 7.5 which was significantly higher ($p<0.01$) than for the level 1g (5.7) and level 2g (6.4). This difference in the overall scores was contributed by the difference in the sensory attributes of flavor and aftertaste, which were significantly higher for the level 1.5g ($p<0.05$ in both cases). Thus the best accepted level of incorporation of wheatgrass was at the concentration of 1.5g per serving in case of buttermilk.

MUTHIYA

For *Muthiya* again, the mean overall scores did not differ significantly across the varying concentrations of wheatgrass (Table 4.65), though the mean scores for any of the attributes or the mean score did not vary significantly across the three levels of wheatgrass concentrations. 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

Khakhra was well accepted at both 1g and 2g level (Table 4.66) indicated by similar mean overall scores (7.1 and 7 respectively); while mean scores for the 1.5g level stood at 6.7, indicating comparatively less acceptability. However, the mean scores for all the three concentrations of wheatgrass did not vary significantly among each other as depicted by the analysis of variance.

TABLE 4.64 SCORES FOR SENSORY ATTRIBUTES OF WHEATGRASS INCORPORATED BUTTERMILK (MEAN ± SD)

Attributes	Levels of Incorporation of Wheatgrass			ANOVA p value
	1.0g	1.5g	2.0g	
Appearance	6.2±1.8	7.4±1.4	6.6±1.4	0.120
Colour	6.1±1.8	7.4±1.1	6.5±1.5	0.061
Flavour	5.1±1.5	6.9±1.4	5.7±1.5	0.009*
Consistency	6.7±1.6	7.2±1.5	6.9±1.4	0.696
Aroma	6.0±2.1	7.1±4.1	6.4±1.8	0.251
After taste	5.1±1.3	6.8±1.3	5.8±1.5	0.006*
Overall Score	5.7±1.5	7.5±1.0	6.4±1.3	0.002**

** Significantly different at p<0.05, **p<0.01*

TABLE 4.65 SCORES FOR SENSORY ATTRIBUTES OF WHEATGRASS INCORPORATED *MUTHIYA* (MEAN + SD)

Attributes	Levels of Incorporation of Wheatgrass			ANOVA p value
	1.0g	1.5g	2.0g	
Appearance	6.6±1.4	6.4±1.7	6.4±1.2	0.948
Colour	6.6±1.4	6.6±1.3	6.7±1.2	0.983
Flavour	6.3±1.3	6.1±2.2	6.1±1.3	0.911
Consistency	7.2±1.4	7.0±1.3	7.1±1.2	0.954
Aroma	6.3±1.5	5.8±1.5	6.1±1.3	0.777
After taste	6.7±1.7	6.3±2.1	6.8±1.5	0.784
Overall Score	6.6±1.2	6.5±1.4	6.4±1.1	0.920

TABLE 4.66 SCORES FOR SENSORY ATTRIBUTES OF WHEATGRASS INCORPORATED *KHAKHRA* (MEAN ± SD)

Attributes	Levels of Incorporation of Wheatgrass			ANOVA p value
	1.0g	1.5g	2.0g	
Appearance	7.1±1.3	6.9±1.2	7.1±1.3	0.824
Colour	6.6±1.3	6.7±1.1	6.8±1.4	0.958
Flavour	6.5±1.7	6.6±1.1	7.1±1.4	0.450
Consistency	6.4±1.6	6.5±1.1	6.5±1.8	0.990
Aroma	7.0±1.7	7.2±1.5	7.4±1.3	0.753
After taste	6.1±1.8	6.0±1.2	6.7±2.0	0.482
Overall Score	7.0±1.6	6.7±1.0	7.1±1.6	0.720

THEPLA

The best accepted recipe of the lot was *Thepla*, with mean overall score of 7.9 for the level 1g, followed by 7.4 at 1.5g level and 6.8 at 2g level (Table 4.67). The inter-concentration variation revealed significantly low scores for appearance and aftertaste at 2g level. The difference in the mean overall scores nonetheless, was not significantly different.

Comparative ranking of the recipes based on the total mean scores of all the recipes indicated that the recipes were best accepted in the following sequence: *Thepla, Dal, Khakhra*, Buttermilk and *Muthiya*

DISCUSSION

Herbal drugs constitute a major share of all the officially recognized alternative medicine systems in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. With more than 70% of India's 1.1 billion population using alternative systems of medicine (Vaidya and Devasagayam 2007), natural foods and plant products have immense utility in this regard. The major difference between these herbal products and nutraceuticals is that the field of nutraceuticals is evidence-based. It is therefore all the more rational to have evidence against herbal products and supplements, and promote nutraceuticals.

The nutrient content analysis of freeze-dried wheatgrass reflected that dosage as small as 2g can meet significant levels of the daily requirements of micronutrients, especially β-carotene (360%) and iron (6.8%), while increasing the macronutrient intake only by a marginal percentage of the RDA (energy: 0.3%, protein: 0.95%, fat: 0.4%). Also, being the leaf part of the wheat plant, its chlorophyll content would be quite high. The antioxidant capacity of wheatgrass has already been estimated to be fairly good with FRAP values of 0.573 mmol of Trolox equivalents being reported for ethanol

TABLE 4.67 SCORES FOR SENSORY ATTRIBUTES OF WHEATGRASS INCORPORATED *THEPLA* (MEAN + SD)

Attributes	Levels of Incorporation of Wheatgrass			ANOVA p value
	1.0g	1.5g	2.0g	
Appearance	7.5±0.7	7.2±10.7	6.6±0.8	0.014*
Colour	7.3±1.0	7.0±1.0	6.4±0.8	0.070
Flavour	8.1±1.2	7.3±1.6	6.8±1.4	0.099
Consistency	7.9±1.0	7.5±1.2	7.3±1.1	0.338
Aroma	7.5±1.2	7.3±1.2	6.9±1.3	0.528
After taste	8.1±1.4	7.3±1.5	6.6±1.3	0.042*
Overall Score	7.9±1.1	7.4±1.4	6.8±1.2	0.081

** Significantly different at p<0.05, **p<0.01*

extracts from 100g fresh wheatgrass at 15days (Kulkarni et al 2006). Apart from the nutrients, wheatgrass is also documented to contain a variety of non-nutrient phytochemicals: polysaccharides-glucans, fatty oil (2%); phospholipids (1%); glycolipids (0.5%): particularly acyldigalactosylglycerols; steroids (0.3%): sterol esters and Lignin & Alkyl resorcinols in the range of 0.1 – 0.2% (Kumar et al 2011). Apart from this, wheat grass contains significant amount of Polycosanols (approximately 137-274 mg/kg) and Phytosterols (approximately 834-1206 mg/kg); with octacosanol, tetracosanol, docosanol, hexacosanol, and tricontanol being the main polycosanol components. Approximately 60-76% of the total Phytosterol content of wheatgrass is beta-sitosterol (Dunford and Edwards 2009).

This indicates the competency of wheatgrass to be a functional food targeted at improving the micronutrient and anti-oxidant status in a broad spectrum of clinical conditions and to maintain general health as well.

Wheatgrass in its natural form has innumerable nutraceutical compounds as mentioned above; however, dehydration employed for manufacture of wheatgrass powder reduces the nutrient content of wheatgrass (Das et al 2012). Freeze drying on the other hand has been found to preserve all the nutrients and phytonutrients: phytosterols, flavonoids and phenolic compounds as well as the antioxidant capacity as compared to conventional heat dehydration (Das et al 2012)

The incorporation of wheatgrass was thus a significant stride in translating the encouraging results of nutrient composition analysis of wheatgrass. The acceptance of recipes was moderately good in case of some recipes namely; buttermilk and *Muthiya*, while other recipes like *Khakhra, Thepla* and *Dal* were highly acceptable. Acceptability trials of wheatgrass incorporated recipes have been conducted in the department as well. One such trial by Iyer et al in 2001 (unpublished M.Sc. dissertation) studied the acceptability of five common Indian recipes (*Paratha, Dhebra, Cutlet, Samosa* and *Muthia*)

incorporated with fresh wheatgrass at the level of 10g, 15g and 20g. The results revealed maximum scores till 15g level of incorporation using fresh wheatgrass.

Freeze-dried wheatgrass has also been successfully incorporated in other formulations and has shown good degree of acceptability: Iyer and co-workers (Iyer et al 2011) formulated a cereal-pulse enriched health drink by incorporating freeze-dried wheatgrass powder and investigated its tolerance in adult population. The formulation was well accepted and has pleasant organoleptic qualities. Not only did the formulation not give rise to any allergic reactions, but also caused suppression of satiety in the subjects

An observation that can be made here is that the recipes with low water-content like *Khakhra and Thepla* were better accepted and increase in the water content of the recipe reduced the acceptability of the recipe as indicated by the scores, for example buttermilk and *Muthiya*. Thus it can be stated that extent to which wheatgrass powder adversely affects the sensory qualities of a recipe is proportional to the water content in the recipe, however, this aspect has to be studied further.

IMPLICATIONS AND USAGE

Nutraceuticals with the nutrient and phytonutrient content equal to that of wheatgrass, has the potential of being used as a general health supplement, if not for specific conditions. Wheatgrass is very easy to grow at a household level and can be incorporated into various common recipes, to enable ingestion of biologically viable concentrations. Alternatively, freeze-dried powder can also be procured from health supplement marketing agencies, which then can be used to be consumed as such or by adding to already prepared foods, without affecting the acceptability as found by the sensory evaluation in this study.

SALIENT OBSERVATIONS FROM THE STUDY

1. Freeze dried wheatgrass was found to contain good quantities of nutrients which can be used to supplement the daily diet, which more often than not in this age of convenience foods, lacks most of the nutrients
2. Because of the vast variety of phytonutrients in wheatgrass, which have been purported to exert specific health benefits, it can be considered as adjunct therapy in various conditions like hypertension, diabetes, dyslipidemia, cancer, CHD, etc
3. The ease of incorporation of wheatgrass into commonly made recipes as well as the considerably good acceptability of these recipes, in light of the nutrient quality of wheatgrass, suggests that it should be propagated as a supplement, which has far reaching applications.

PHASE IV

IMPACT OF WHEATGRASS POWDER SUPPLEMENTATION ON ATHEROGENICITY, INFLAMMATION AND MENOPAUSAL SYMPTOMS IN PRIMARY HYPERLIPIDEMIC WOMEN

Cardiovascular disease (CVD) physiology, etiology and burden differs in women as compared to men and it has compound causative associations to regulation of the sex steroid metabolism and the endocrine effects it exerts on other organ systems, the main perpetrator of this transgression being estrogen withdrawal resulting during onset of menopause (Vlassoff 2007, Cagnacci et al 2011, Cagnacci et al 2012). For the same reason the manifestation, stage of diagnosis and treatment of CVD in women tends to differ and has been purported to have underlying links to the menopausal stage the woman is in. The body of evidence that exists for possible benefits of therapy for menopausal symptoms on CVD health is not conclusive, at the same time; the line of therapy for CVD is not of any assistance for menopausal symptoms (Guay et al 2006, Dessapt and Gourdy 2012).

On the other hand, nutraceutical compounds in many natural functional foods have been proposed to exert holistic health benefits, particularly with regard to cardiovascular, metabolic and oncogenic events. One such substance which is hypothesized to contain a plethora of nutraceutical polyphenols, antioxidant vitamins and pigments, is wheatgrass. Wheatgrass has been shown to decrease oxidative stress and atherogenic indices in animal model studies and *in vitro* molecular and genetic studies (Kulkarni et al 2006; Kothari et al 2011; Das, Hakim and Mittal 2012). It was therefore felt worthwhile to evaluate the multifaceted effect of wheatgrass powder on the atherogenicity, inflammation, glucose control and menopausal symptoms as well, in hyperlipidemic women. Consequently this randomized controlled study was planned with the objective of evaluating the impact of freeze-dried wheatgrass powder supplementation on the above mentioned aspects in primary

hyperlipidemic pre, peri and post menopausal women. As described in more detail in the methods chapter, 60 primary hyperlipidemic women were enrolled for the study from the free-living population, and randomized into two study groups: one being the experimental group (N=29), receiving the supplementation in the form of freeze-dried wheatgrass powder capsules containing 1.25g wheatgrass per day for 10 weeks; and a control group (N=30), which was observed for 10 weeks without giving any intervention. The information pertaining to background information, menopausal status, lifestyle and dietary intake was collected at baseline. Information on menopausal symptoms, anthropometry and biochemical indicators, was collected twice: before and after the intervention period for both the groups. The results of the study are as follows

Background Characteristics of the Subjects

The age profile of the subjects is given in Figure 4.33. It can be seen that majority of the subjects were in the 40-60year age-group, both in the control and the experimental group (76.7% and 82.7% respectively). The distribution of the subjects based on their menopausal status (Figure 4.34) revealed that women from all 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). The proportion of hysterectomized women was less in both control and experimental groups (13.3% and 10.3% respectively). The prevalence of menopausal symptoms (Figure 4.35) revealed that in the controls, somatic symptoms were the highest prevalent symptom (31%) while in the experimental subjects vasomotor symptoms were the highest prevalent group of symptoms (24.1%). Urogenital symptoms were the least prevalent in both the groups (Control: 17.2%, experimental 13.8%), though in experimental group the prevalence of urogenital and psychological symptoms were the same.

FIGURE 4.33 AGE DISTRIBUTION OF THE SUBJECTS

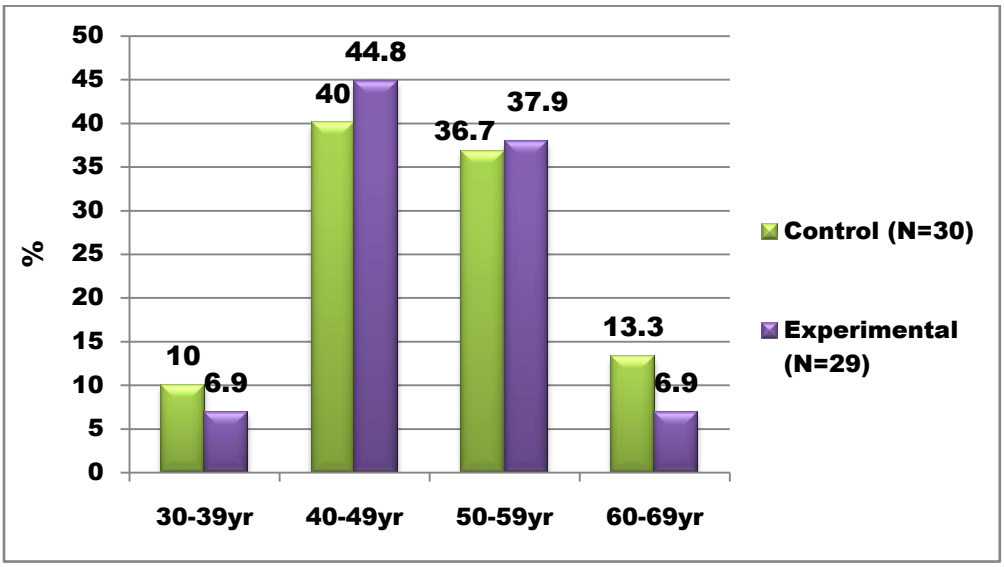


FIGURE 4.34 DISTRIBUTION OF THE MENOPAUSAL STATUS OF THE SUBJECTS

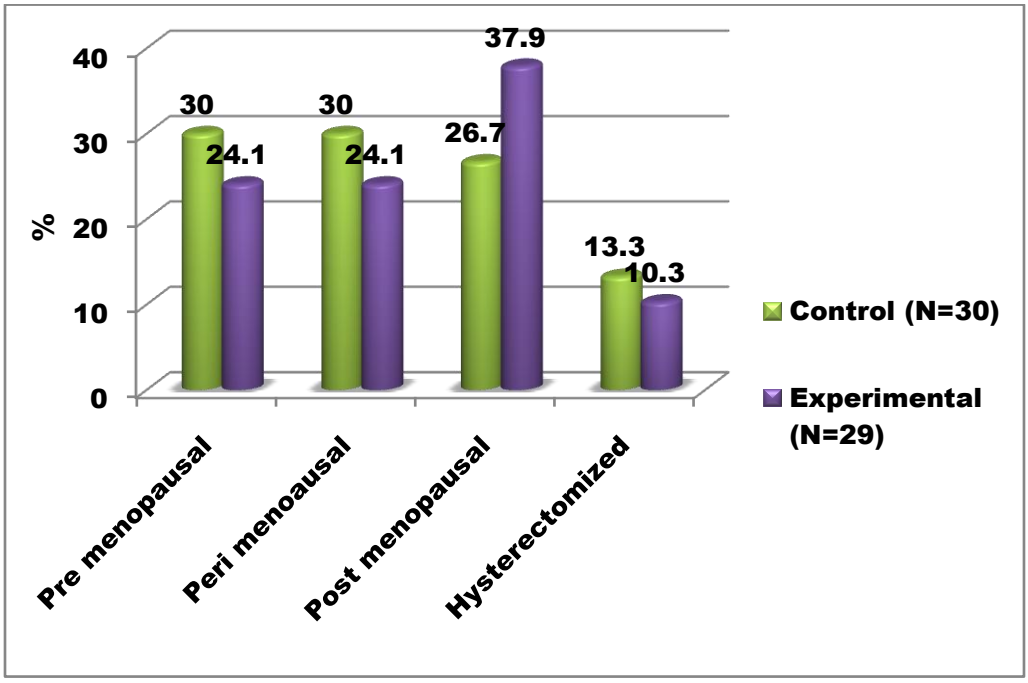
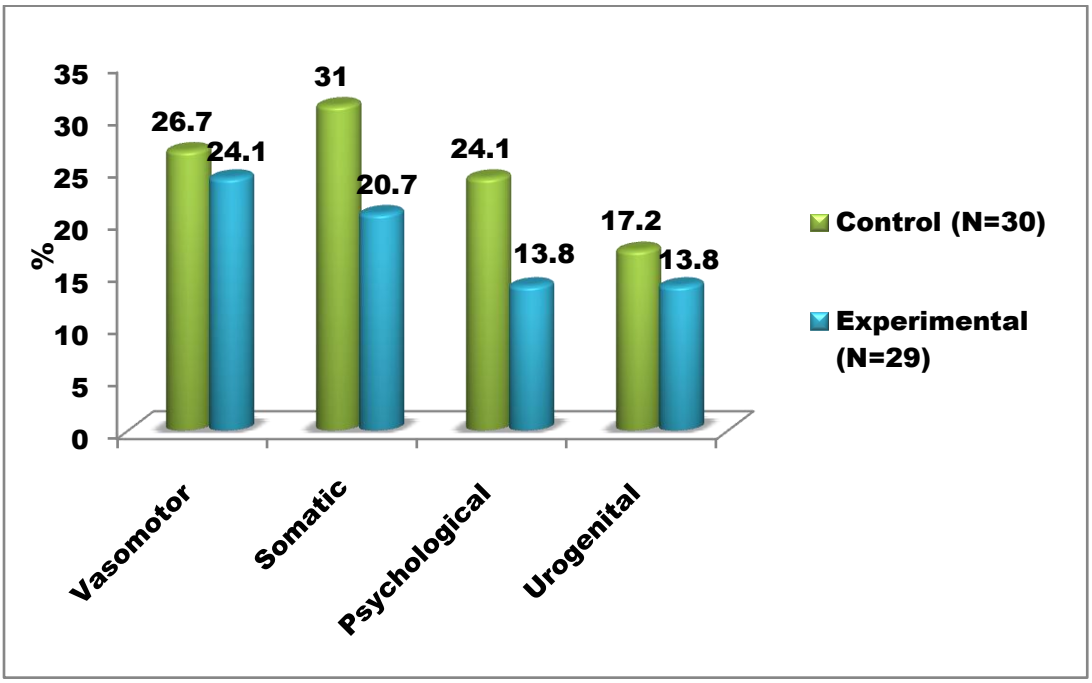


FIGURE 4.35 PREVALENCE OF MENOPAUSAL SYMPTOMS IN THE SUBJECTS*



**menopausal symptoms categories are not mutually exclusive*

CLINICAL PROFILE OF THE SUBJECTS

The information on anthropometric variables and blood pressure (Table 4.68) revealed that the clinically the subjects in both the control and experimental groups were comparable at the beginning of the study and there was no significant difference between the two groups. The mean BMI in both the groups was above the normal category (controls – 27.2kg/m^2, experimental – 25.8 kg/m^2). The waist circumference and hip circumferences were also comparable among both the groups; however, the waist stature ratio (WSR) was marginally higher in the control group subjects (0.62 $\pm$ 0.04) versus the experimental group subjects (0.60 $\pm$ 0.10). The mean blood pressure in both the groups was in the pre-hypertension category and was comparable between the two groups.

LIFESTYLE HABITS OF THE SUBJECTS

In either group, no prevalence of substance abuse (smoking, tobacco usage or alcohol consumption) was reported (Table 4.69). The frequency of any physical activity was similar and very low in both the control and experimental groups (26.7% and 27.6% respectively).

NUTRIENT INTAKE OF THE SUBJECTS

The nutrient intakes of the subjects (Table 4.70) was more or less similar across the control and experimental groups, with the exception of the iron intake, which was higher in the experimental group subjects (11.2g/dl) as compared to control group subjects (8.9g/dl), with the difference being statistically significant ($p<0.05$).

TABLE 4.68 CLINICAL PROFILE OF THE SUBJECTS (MEAN ± SD)

Parameter	Control (N=30)	Experimental (N=29)	Students' 't' p value
Age (years)	50 ± 8.9	48 ± 7.6	0.47
Weight (kg)	63.4 ± 10.7	62.5 ±11.5	0.75
BMI (kg/m2)	27.2 ± 3.9	25.8 ± 4.0	0.18
WC (cm)	95.9 ± 7.3	93.7 ± 9.8	0.32
WSR	0.62 ± 0.04	0.60 ± 0.1	0.05*
HC (cm)	102.4 ± 7.6	102.8 ± 8.4	0.84
SBP (mmHg)	135 ± 15.6	133.4 ± 52.8	0.76
DBP (mmHg)	82 ± 7.4	81.8 ± 12.8	0.86

TABLE 4.69 LIFESTYLE HABITS OF THE SUBJECTS*

Parameter	Control (N=30)	Experimental (N=29)	Chi square p value
Physical Activity	8 (26.7)	8 (27.6)	0.93
Tobacco Usage	0	0	-
Smoking	0	0	-
Alcohol Consumption	0	0	-

** Figures in parenthesis indicate percentages*

TABLE 4.70 NUTRIENT INTAKES OF THE SUBJECTS (MEAN ± S.D.)

Nutrient	Control (N=30)	Experimental (N=29)	p value
Energy (kcal)	1523 ± 164	1592 ± 287	0.32
CHO (g)	186 ± 22.6	192 ± 36	0.44
Fat (g)	59 ± 12.6	64 ± 16.8	0.16
Protein (g)	43 ± 10.8	45 ± 13.2	0.34
Iron (mg)	8.9 ± 3.7	11.2 ± 4.6	0.03*
Vitamin C (mg)	70.4 ± 24.1	77.8 ± 18	0.18
β-Carotene (μg)	1398 ± 754	1498 ± 833	0.63
TDF (g)	23.8 ± 3.5	23.8 ± 5.7	0.97

TDF: Total Dietary Fiber (only for values reported by NIN),

**significantly different from control at p<0.05*

IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE BIOPHYSICAL AND BIOCHEMICAL PARAMETERS OF THE SUBJECTS

IMPACT ON THE BLOOD PRESSURE OF THE SUBJECTS

The mean systolic and diastolic blood pressure in the experimental group subjects showed significant reductions after the intervention as indicated by the paired t test 2 tailed p values in Table 4.71. The mean systolic BP went down by 0.75% ($p<0.05$); and the mean diastolic BP went down 1.2% ($p<0.05$). However, the comparison between the post-intervention values between the control and the experimental group did not reflect any statistically significant difference. Though significant reductions were seen in the systolic and diastolic blood pressure levels in the experimental group, it may not carry any significant physiological impact as the reduction was of only 1mmHg.

IMPACT ON THE LIPOPROTEIN FRACTIONS IN THE SUBJECTS

The supplementation with wheatgrass powder showed significant reductions in serum total cholesterol (TC); highly significant reductions in apo B; near-significant reductions in triacylglycerols (TAG) and very low density lipoprotein (VLDL); and significant decrease in high density lipoprotein (HDL) and apo A as shown in Table 4.72.

The mean TC in the experimental group reduced by 5.3% ($p<0.01$), while the mean TC increased 0.74% in the control group. The mean TAG levels in the experimental group showed a near significant reduction 9.7% ($p=0.07$), while in the control group there was an increase of 3.5%. The LDL levels came down by 4.5% in the experimental group at the end of the intervention; while in the control group, it increased by 0.9%. The HDL levels also declined in the experimental group at the end of the intervention by 6.2% ($p=0.05$), whereas in the control group, there was a marginal reduction of 1.3%. The VLDL levels

TABLE 4.71 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE BLOOD PRESSURE OF THE SUBJECTS (MEAN ± S.D.)

Blood Pressure		Control (N=30)	Experimental (N=29)	Students' t test 2 tailed p value (post data)
SBP	Pre	135 ± 15	133 ± 26	
	Post	135 ± 14	132 ± 24	0.57
	Paired t 2 tailed p value	0.92	**0.04***	
DBP	Pre	82 ± 7	82 ± 13	
	Post	82 ± 7	81 ± 12	0.55
	Paired t 2 tailed p value	0.36	**0.02***	

**Significantly different from baseline at p<0.05*

TABLE 4.72 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE SERUM LIPID FRACTIONS OF THE SUBJECTS (MEAN ± S.D.)

Lipid Fraction		Control (N=30)	Experimental (N=29)	Students' t test 2 tailed p value (post data)
TC	Pre	215.6 ± 26.9	223.4 ± 28.8	
	Post	217.2 ± 34.2	211.5 ± 39.5	0.54
	Paired t 2 tailed p value	0.71	**0.01****	
TAG	Pre	138.0 ± 53.9	146.4 ± 53.8	
	Post	142.8 ± 54.7	132.2 ± 44.7	0.41
	Paired t 2 tailed p value	0.29	**0.07**	
LDL	Pre	134.8 ± 27.9	138.8 ± 22.9	
	Post	136.0 ± 30.8	132.6 ± 31.3	0.77
	Paired t 2 tailed p value	0.79	0.1	
HDL	Pre	52.9 ± 8.3	56.6 ± 11.4	
	Post	52.2 ± 8.8	53.1 ± 10.4	0.70
	Paired t 2 tailed p value	0.31	**0.05***	
VLDL	Pre	28.9 ± 15.9	28.6 ± 11.1	
	Post	29.0 ± 14.1	25.7 ± 9.3	0.3
	Paired t 2 tailed p value	0.96	**0.07**	
Apo A	Pre	139.1 ± 19.8	134.4 ± 21.1	
	Post	126.1 ± 17.7	109.0 ± 18.4	0.1
	Paired t 2 tailed p value	**0.001*****	**0.000*****	
Apo B	Pre	111.9 ± 16.6	111.1 ± 17.2	
	Post	106.3 ± 17.1	96.5 ± 20.5	**0.05**$^{\#}$
	Paired t 2 tailed p value	**0.07**	**8.08E-05*****	

** Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001,*

 # Significantly different from control at p<0.05

in the experimental group reduced by 10.1% (p=0.07); on the other hand, control group saw a marginal increase of 0.3% at the same time.

Impact on the Apolipoprotein Fractions in the Subjects

The serum apo A fraction saw highly significant reductions in both the groups, with the reduction being higher in the experimental group of 18.6% (Table 4.72). Similarly, even the apo B levels reduced by 13% (p<0.000) in the experimental group and 5% in the control group (p=0.07). In case of post-intervention apo B values, there was a significant difference between the control and experimental groups (p<0.05).

Impact on the Atherogenic Indices of the Subjects

Regarding the atherogenic indices, it was seen that the TAG/HDL ratio had decreased marginally in the experimental subjects by 3.6%, while the same in the control group had increased at the end of the intervention period by 3.6% (Table 4.73). The mean AIP levels in the experimental group also decreased by 5%, however the decline was not statistically significant; and on the other hand, the mean AIP levels in the controls increased by 7.7% at the end of the intervention period. The apo B / apo A ratio had decreased significantly in the experimental group subjects (6.4%, p<0.05); whereas in the control group subjects the ratio increased by 4.9% after the intervention period. Thus, though apo A and HDL levels decline, overall, there was a decline in the apo B/A ratio indicating a net positive response.

Impact on FBS Levels of the Subjects

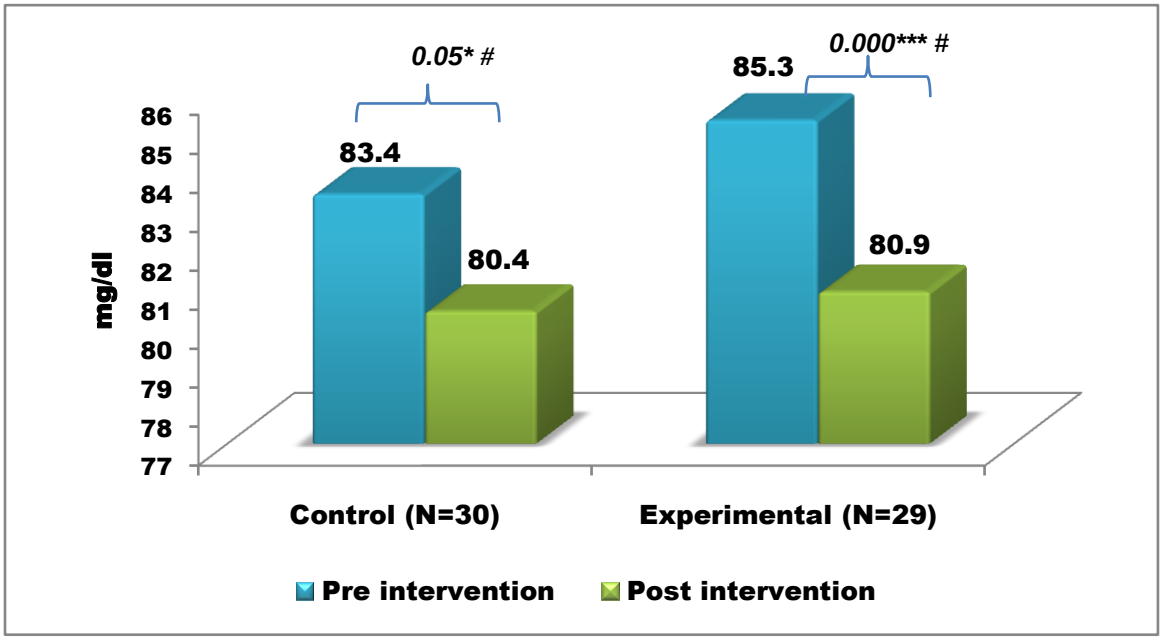
The mean FBS levels in the experimental group (Figure 4.36) saw a significant reduction of 5.1% after the intervention (paired t test 2 tailed p<0.001). However, the mean FBS levels in the control groups subjects also came down towards the end if the intervention period by 3.6% (p=0.05). Thus only a transient significant reduction in the normal range was seen among the subjects of both the groups.

TABLE 4.73 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE SUBJECTS (MEAN ± S.D.)

Atherogenic Index		Control (N=30)	Experimental (N=29)	Students' t test 2 tailed p value (post data)
TAG/HDL	Pre	2.8 ± 1.4	2.8 ± 1.5	
	Post	2.9 ± 1.4	2.7 ± 1.3	0.52
	Paired t 2 tailed p value	0.21	0.49	
AIP	Pre	0.39 ± 0.2	0.40 ± 0.2	
	Post	0.42 ± 0.2	0.38 ± 0.2	0.49
	Paired t 2 tailed p value	0.17	0.40	
Apo B/ Apo A	Pre	0.81 ± 0.16	0.78 ± 0.17	
	Post	0.85 ± 0.16	0.73 ± 0.18	**0.04**#
	Paired t 2 tailed p value	0.11	**0.05***	

**Significantly different from baseline at p=0.05, # significantly different from control at p<0.05*

FIGURE 4.36 IMPACT OF WHEATGRASS SUPPLEMENTATION ON FBS LEVELS OF THE SUBJECTS



 # 2 tailed significance between baseline and post intervention values

Impact of Wheatgrass Supplementation on Inflammation in the Subjects

The impact of the intervention was also seen on the inflammation in the subjects, as assessed by the serum hs-CRP levels (Figure 4.37). It was seen that the mean hs-CRP levels went down by 10% in the experimental group subjects; the difference however was not statistically significant. On the contrary, the mean levels of hs-CRP went up by 11% in the controls at the end of the intervention period.

Impact on Hemoglobin Levels of the Subjects

The hemoglobin levels in the experimental group subjects increased non-significantly by 0.98% (Figure 4.38). The control group subjects saw a decline in the mean hemoglobin levels at the end of the intervention period 1.2% (p=0.05).

Impact of Wheatgrass Supplementation on the Prevalence of Dyslipidemia in the Experimental Group Subjects

It was seen that the prevalence of dyslipidemia in the experimental group subjects went down at the end of the intervention period (Figure 4.39). 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. In all the cases, the p values for Mantel-Haenszel chisquare test for the difference between the initial and latter prevalence was not statistically significant.

FIGURE 4.37 IMPACT OF WHEATGRASS SUPPLEMENTATION ON INFLAMMATION IN THE SUBJECTS AS REFLECTED BY hs-CRP LEVELS

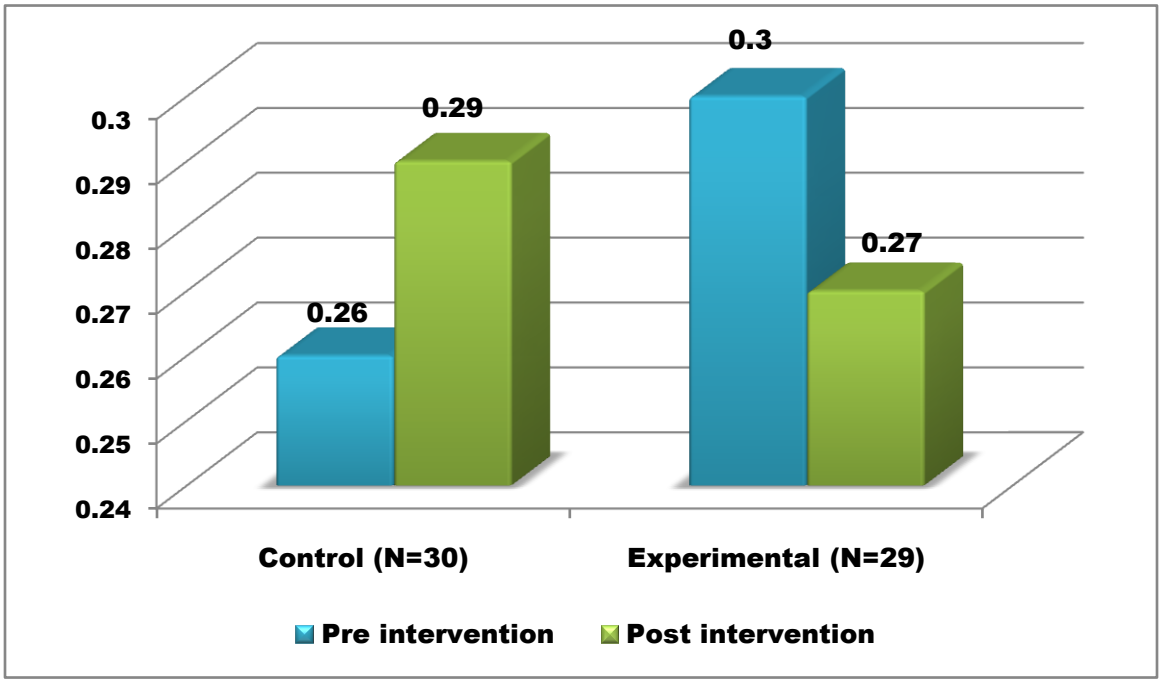
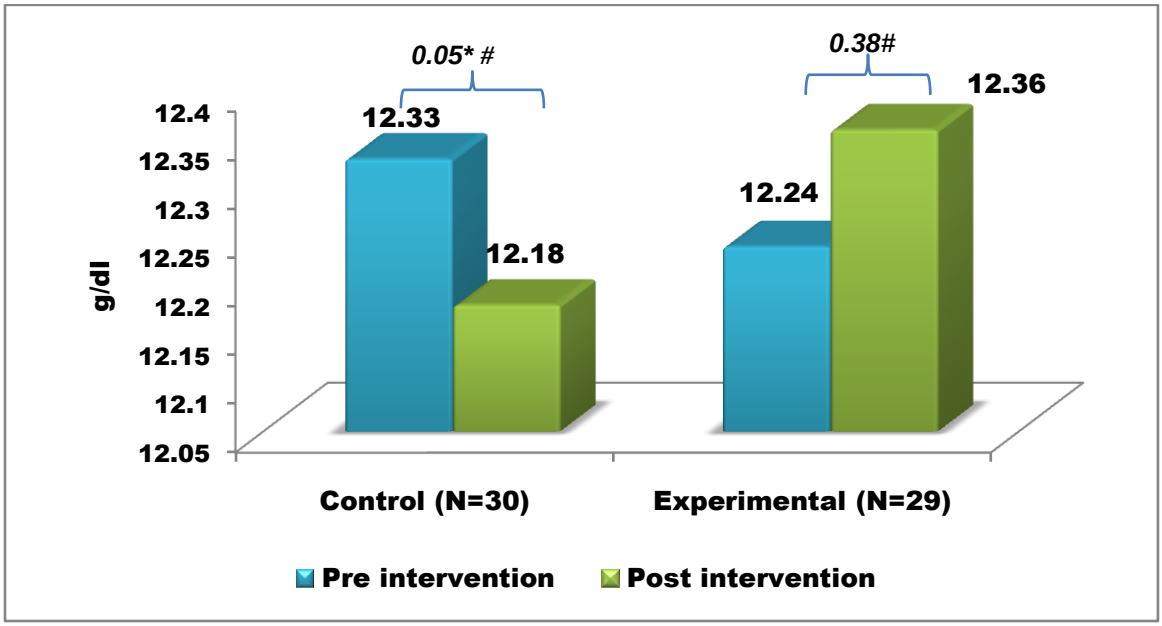
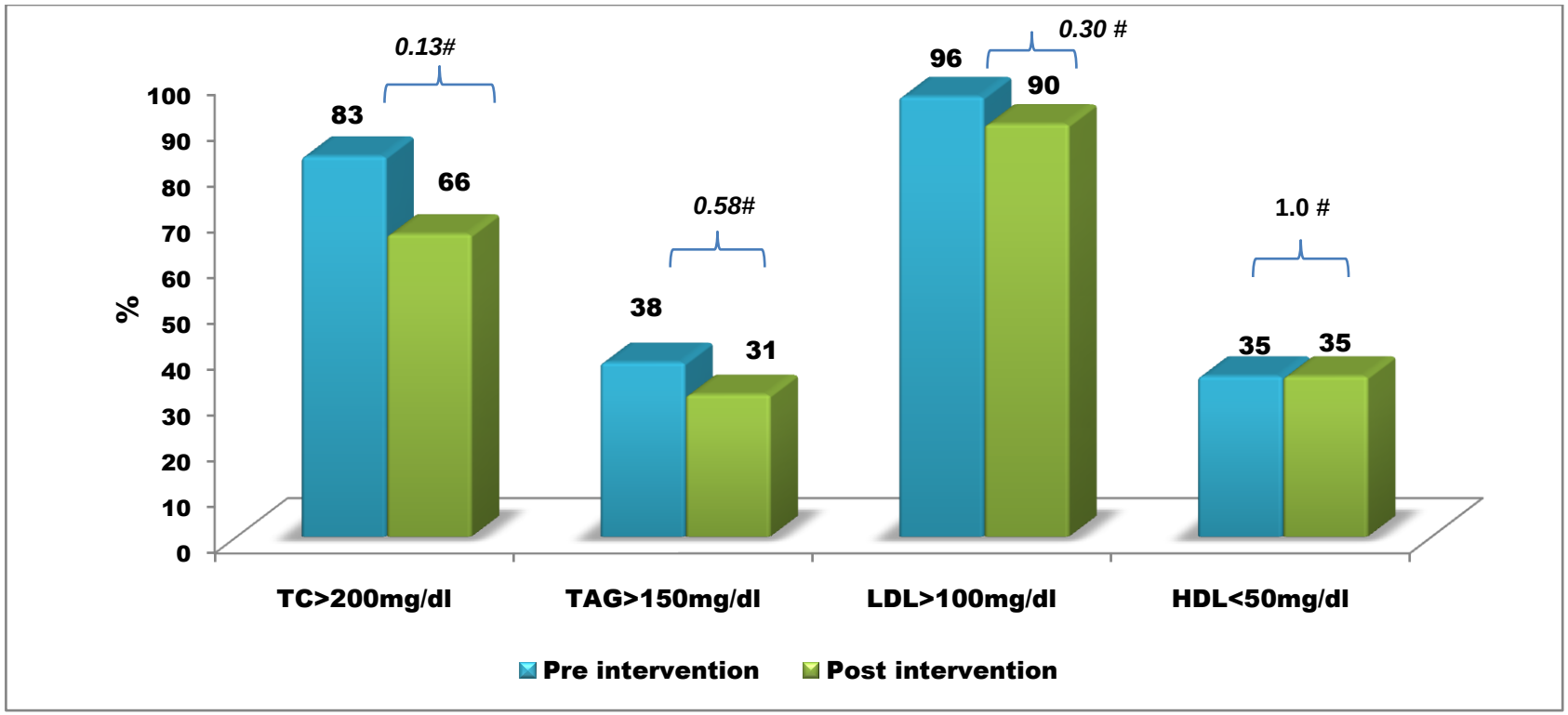


FIGURE 4.38 IMPACT OF WHEATGRASS SUPPLEMENTATION ON HEMOGLOBIN LEVELS OF THE SUBJECTS



 # 2 tailed significance between baseline and post intervention values

FIGURE 4.39 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE PREVALENCE OF DYSLIPIDEMIA IN THE EXPERIMENTAL GROUP SUBJECTS



 # 2 tailed significance for Mantel-Haenszel Chisquare test between baseline and post intervention values

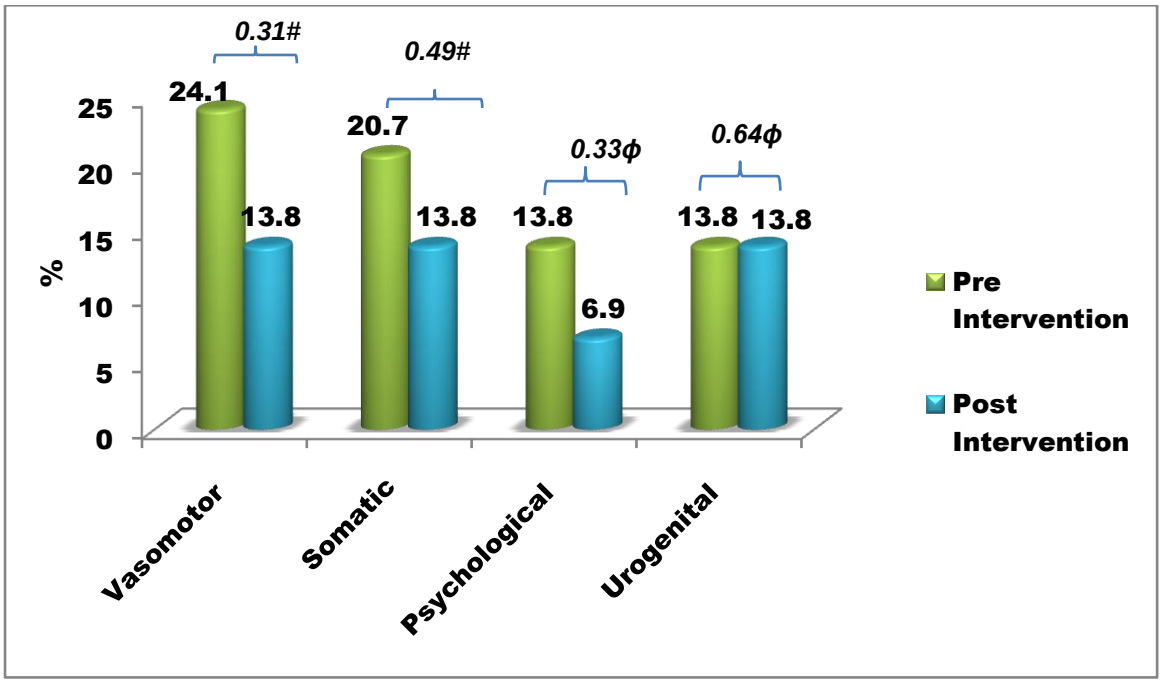
Impact of Wheatgrass Supplementation on Menopausal Symptoms in the Subjects

One of the important the objectives of this supplementation trial, was to study the effect of wheatgrass on the menopausal symptoms in the subjects. Interestingly, the results (Figure 4.40) revealed that in the experimental group, the prevalence of vasomotor symptoms saw a decline of 42% from the initial prevalence of 24.1% to 13.8% after the intervention period however, the Mantel-Haenszel chisquare p values did not indicate any statistical significance (2 tailed p=0.31). Similarly the prevalence of somatic symptoms declined by 33% from the initial 20.7% prevalence during the beginning of the trial to 13.8% at the end (p=0.49). In case of psychological symptoms, the prevalence decreased from initial by 50% after the supplementation, however the difference was not statistically significant (p=0.33). Only in case of urogenital symptoms, it was observed that the prevalence remained unchanged at 13.8% from the baseline till the end of the supplementation. The prevalence of none of the menopausal symptoms changed in the control group after the intervention.

Impact of Wheatgrass Supplementation on the Lipid and Lipoprotein Fractions of the Subjects Based on The Initial TC Levels

An attempt was made to analyze the pre and post intervention cholesterol values depending upon the initial TC levels, i.e. subjects who had <200mg/dl TC levels and subjects who had $\geq$200mg/dl TC levels at the beginning of the supplementation (Table 4.74). The separate analysis revealed that among the experimental group subjects, people with higher TC levels showed significant reductions in TC (p=0.02), HDL (p=0.02), apo A (p=0.001) and apo B (p=0.000) values as compared to experimental subjects with low initial TC levels. The experimental subjects with low initial TC however showed significant decline in TAG levels (0.005) and VLDL levels (p=0.02) as compared to subjects who had high initial TC level, who saw only a marginal reduction in these values. These trends were not seen in the control group.

FIGURE 4.40 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE MENOPAUSAL SYMPTOMS IN THE SUBJECTS*



**menopausal symptoms categories are not mutually exclusive*

 # p values for Mantel Haenszel Chisquare test between baseline and post intervention values

ϕ p values for Fisher's Exact test between baseline and post intervention values

TABLE 4.74 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE LIPID AND LIPOPROTEIN FRACTIONS OF THE SUBJECTS BASED ON THE INITIAL TC LEVELS (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	Post	Paired t 2 tailed p value	Pre	Post	Paired t 2-tailed p value
TC< 200mg/dl	**N=8**			**N=5**		
TC	184 ± 11.0	177 ± 14.9	0.35	181± 25.5	174 ± 23.8	0.19
TAG	152 ± 56.3	145 ± 59.2	0.50	123 ± 25.7	99.8 27.2	**0.005****
LDL	103 ± 24.8	102 ± 17.0	0.86	114 ± 19.2	109 ± 19.9	0.28
HDL	49 ± 7.2	47 ± 8.6	0.33	46.6 ± 5.9	48.5 ± 4.9	0.29
VLDL	35 ± 25.3	33 ± 19.9	0.71	20.7 ± 4.2	16.8 ± 3.7	**0.02***
Apo A	138 ± 23.3	118 ± 9.1	**0.02***	142 ± 28.8	128.8 ± 19	0.22
Apo B	106 ± 11.0	90 ± 12.7	**0.01****	96.3 ± 16	86.1 ± 26.7	0.32
TC≥ 200mg/dl	**N=22**			**N=24**		
TC	227 ± 21.2	231 ± 26.6	0.37	232 ± 20.8	219 ± 37.9	**0.02***
TAG	132 ± 53.3	142 ± 54.5	0.07	151 ± 57.2	138 ± 45.0	0.2
LDL	146 ± 18.9	148 ± 24.9	0.70	144 ± 20.3	137 ± 31.2	0.15
HDL	54.3 8.4	54 ± 8.3	0.69	57.9 ± 11.3	54.0 ± 11.0	**0.02***
VLDL	26.5 ± 10.7	27.2 ± 11.4	0.50	30.2 v 11.4	27.9 ± 9.0	0.16
Apo A	139 ± 18.9	129 ± 19.3	**0.01***	147 ± 25.8	135 ± 21.7	**0.001*****
Apo B	114 ± 18.0	112 ± 14.7	0.6	114 ± 16.1	98 ± 19.0	**0.000*****

**Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001*

Impact of the Supplementation on the Atherogenic Indices of the Subjects Based on The Initial TC Levels

The atherogenic indices of the subjects were also analyzed separately based on the initial TC levels (Table 4.75). It was found that the experimental subjects who had lower TC to begin with (<200mg/dl) showed a significant decline in the TAG/HDL ratio (p=0.01) and the AIP (p=0.02), as compared to the subjects who had higher initial TC levels (≥200mg/dl). The apo B/ apo A ratio however showed a significant decline (p=0.05) in the experimental subjects who had higher initial TC levels as compared to subjects with lower TC, who showed only a marginal decline (p=0.7). The data emphasizes that wheatgrass powder helped to reduce the atherogenic small dense lipoprotein indicator TAG/H and AIP in subjects who had normal TC levels as compared to hypercholesterolemic subjects.

Impact of the Supplementation on the FBS Levels of the Subjects Based on The Initial TC Levels

The separate analysis of FBS levels depending upon higher TC levels (≥200mg/dl) and lower initial TC levels (<200mg/dl) as shown in Table 4.76; showed that experimental subjects with higher initial TC levels showed a higher decline of 4.6% (p=0.009) in their FBS levels as compared to subjects with lower initial TC levels, whose levels declined non significantly by 7.3% (p=0.07). However the subjects remained in the normal physiologic levels of blood glucose.

Impact on Inflammation in the Subjects Based on The Initial TC Levels

Following the supplementation, the inflammatory marker hs-CRP showed only a marginal decline in the experimental subjects. Analyzing the results separately for subjects with initial high and low TC levels, did not reveal any significant difference (Table 4.77). Subjects with low initial TC levels saw an 18% decline (p=0.59), and subjects with higher initial TC levels saw a decrease of 6.4% (p=0.60).

TABLE 4.75 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE SUBJECTS BASED ON THE INITIAL TC LEVELS (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	**Post**	**Paired t 2 tailed p value**	**Pre**	**Post**	**Paired t 2-tailed p value**
TC< 200mg/dl	**N=8**			**N=5**		
TAG/HDL	3.13 ± 1.24	3.19 ± 1.54	0.82	2.7 ± 0.7	2.1 ± 0.7	**0.01****
AIP	0.47 ± 0.18	0.46 ± 0.22	0.87	0.42 ± 0.11	0.35 ± 0.15	**0.02***
Apo B/ Apo A	0.79 ± 0.15	0.77 ± 0.13	0.57	0.70 ± 0.18	0.67 ± 0.18	0.70
TC≥ 200mg/dl	**N=22**			**N=24**		
TAG/HDL	2.6 ± 1.4	2.8 ± 1.4	0.15	2.84 ± 1.7	2.78 ± 1.4	0.83
AIP	0.36 ± 0.21	0.40 ± 0.19	**0.05***	0.40 ± 0.2	0.40 ± 0.2	0.93
Apo B/ Apo A	0.83 ± 0.16	0.89 ± 0.16	**0.04***	0.79 ± 0.2	0.74 ± 0.2	**0.05***

**Significantly different from baseline at p<0.05, **p<0.01*

TABLE 4.76 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE FBS LEVELS OF THE SUBJECTS BASED ON THE INITIAL TC LEVELS (MEAN ± S.D., mg/dl)

	FBS (mg/dl)	
	Control	**Experimental**
TC<200mg/dl	**N=8**	**N=5**
Pre	85 ± 17.3	82.0 ± 6.82
Post	80 ± 15.5	76.0 ± 5.36
Paired t 2 tailed p value	0.24	0.07
TC≥200mg/dl	**N=22**	**N=24**
Pre	82 ± 9.4	86 ± 19.5
Post	80 ± 8.9	82 ± 10.4
Paired t 2 tailed p value	0.12	**0.009****

***Significantly different from baseline at p<0.01*

TABLE 4.77 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE INFLAMMATION IN THE SUBJECTS BASED ON THE INITIAL TC LEVELS (MEAN ± S.D., mg/dl)

	hs-CRP (mg/dl)	
	Control	**Experimental**
TC<200mg/dl	**N=8**	**N=5**
Pre	0.30 ± 0.30	0.27 ± 0.20
Post	0.37 ± 0.39	0.22 ± 0.22
Paired t 2 tailed p value	0.47	0.59
TC≥200mg/dl	**N=22**	**N=24**
Pre	0.25 ± 0.23	0.31 ± 0.3
Post	0.27 ± 0.24	0.29 ± 0.3
Paired t 2 tailed p value	0.44	0.6

Impact of the Supplementation on the Hemoglobin Levels of The Subjects Based on The Initial TC Levels

The hemoglobin levels of the experimental subjects showed marginal improvement following the supplementation. When analyzed separately for subjects with normal TC levels and those with elevated TC (Table 4.78), it was seen that the values remained unaltered.

Influence of Initial TC Levels on Change in Percent Prevalence of Dyslipidemia in Supplemented Group

The influence of the initial TC levels was studied on the change in percent prevalence of dyslipidemia in the experimental subjects (Figure 4.41). It was seen that subjects with elevated TC in the beginning of the study, saw reductions in prevalence of elevated TC and LDL, but a parallel increase in the prevalence of low HDL levels. In case of subjects with normal initial TC levels in the beginning, there was a 100% reduction of prevalence of elevated TAG and 50% reduction in the prevalence of low HDL levels.

SUMMARY OF IMPACT BASED ON INITIAL TC LEVELS ON OUTCOME PARAMETERS

- 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 (Figure 4.42). However, reduction in prevalence of dyslipidemia showed no discernable patterns when stratified according to initial TC levels.
- 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 4.39) at baseline compared those who were hypercholesterolemic at baseline (18.8%, $p<0.01$ for TAG, and 18.8%, $p<0.05$ for VLDL).
- Surprisingly, apo A and HDL levels declined significantly in experimental subjects who were hypercholesterolemic at baseline compared to those who had normal TC levels.

TABLE 4.78 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE HEMOGLOBIN LEVELS OF THE SUBJECTS BASED ON THE INITIAL TC LEVELS (MEAN ± S.D., g/dl)

	Hb (g/dl)	
	Control	Experimental
TC<200mg/dl	**N=8**	**N=5**
Pre	12.4 ± 1.6	11.98 ± 1.38
Post	12.1 ± 1.5	12.26 ± 1.74
Paired t 2 tailed p value	0.25	0.51
TC≥200mg/dl	**N=22**	**N=24**
Pre	12.3 ± 0.2	12.3 ± 1.0
Post	12.2 ± 1.1	12.4 ± 1.2
Paired t 2 tailed p value	0.14	0.56

FIGURE 4.41 CHANGE IN PERCENT PREVALENCE OF DYSLIPIDEMIA IN SUPPLEMENTED GROUP STRATIFIED BY INITIAL TC LEVELS

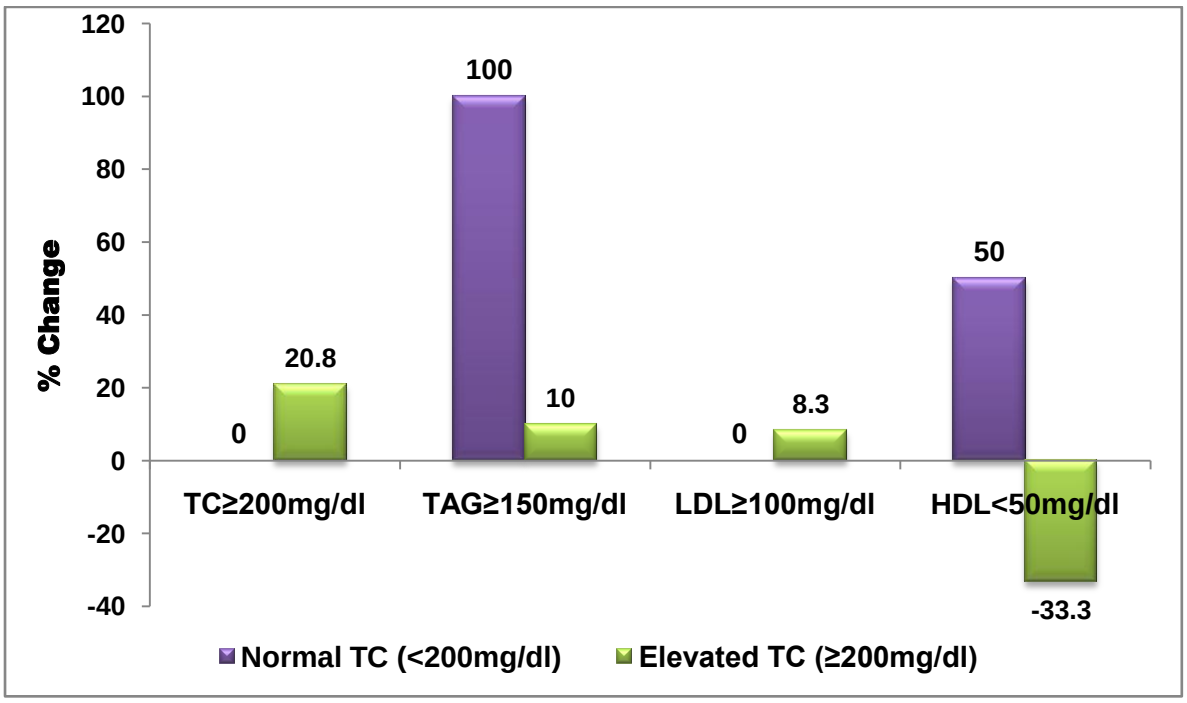
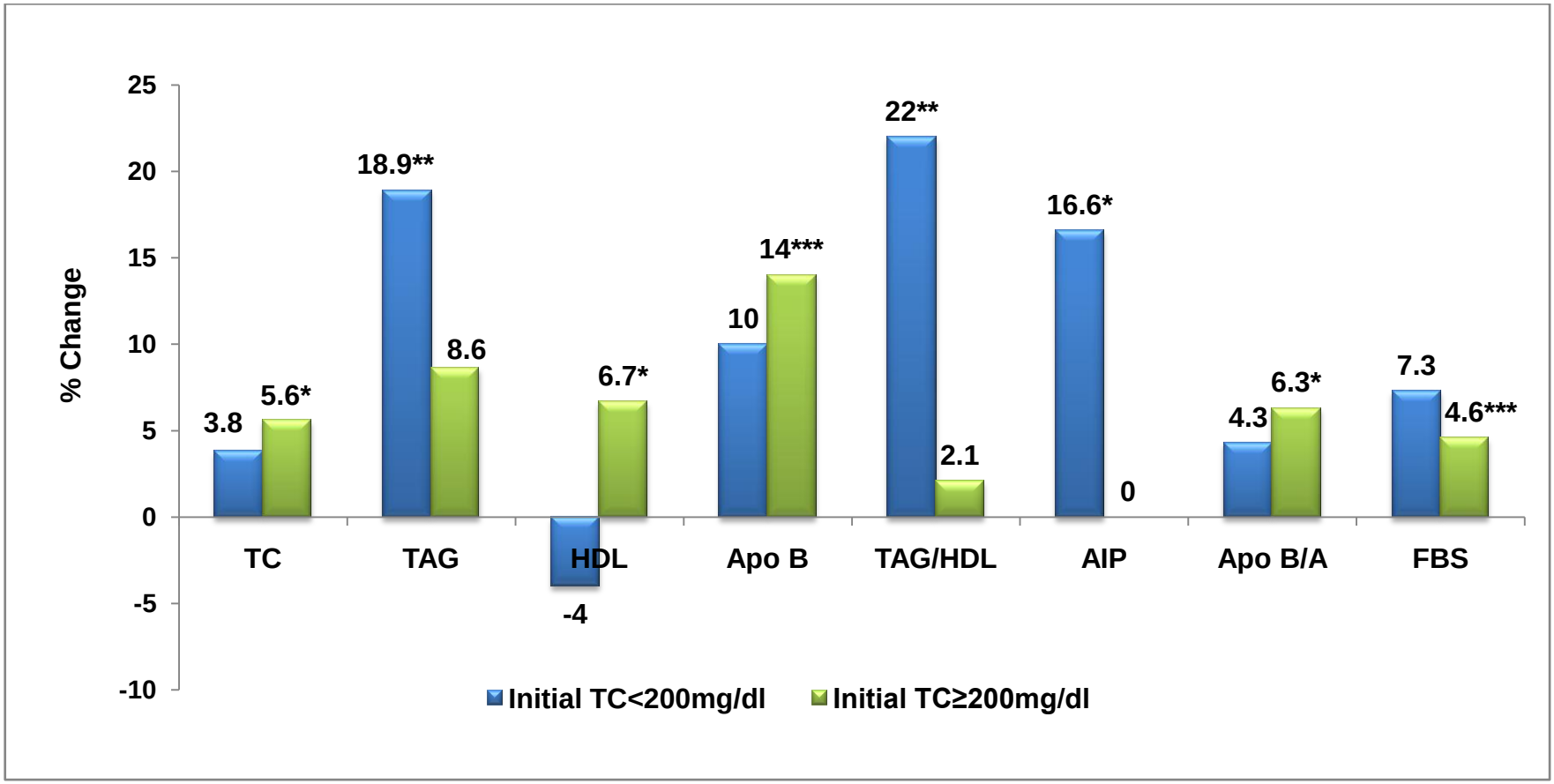


FIGURE 4.42 INFLUENCE OF INITIAL TC LEVELS ON THE % CHANGE IN OUTCOME PARAMETERS IN SUPPLEMENTED SUBJECTS



Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001

- The hs-CRP and hemoglobin levels showed no conceivable difference of baseline TC levels on the impact of the intervention.

Impact of Wheatgrass Supplementation on the Lipid and Lipoprotein Fractions of the Subjects Based on The Initial TAG Levels

The results of supplementation on the lipoprotein and apolipoprotein levels was also analyzed separately based on elevated and normal initial TAG levels, i.e. TAG<150mg/dl and TAG≥150mg/dl (Table 4.79). Here a clear trend of significant decreases in all of the lipoprotein fractions and the apolipoproteins was seen in case of experimental subjects with elevated TAG levels. In case of subjects with normal TAG levels, none of the cholesterol fractions showed a statistically significant decline, however, the apo A and apo B levels declined significantly (apo A by 8.2%, p=0.02; and apo B by 12.7%, p=0.000). In case of subjects with elevated TAG levels, the TC decreased significantly by 8.8% (p=0.05), TAG reduced by 19% (p=0.02), LDL came down by 6.7% (p=0.08), VLDL reduced by 21% (p=0.02), apo A levels declined by 8.9% (p=0.001), and apo B reduced by 13.4% (p=6.7E-05).

Impact on the Atherogenic Indices Based on The Initial TAG Levels

The data on atherogenic indices when analyzed separately depending upon the initial TAG levels, showed that in the subjects with normal initial TAG levels, the TAG/HDL, AIP and apo A / apo B values did not show much decline, whereas in subjects with elevated TAG levels, all these values saw a non significant reduction- TAG/HDL by 14.6%, AIP by 10.3% and apo A /apo B by 3.4%. The results are depicted in Table 4.80.

Impact on the FBS Levels Based on The Initial TAG Levels

The FBS levels of the experimental subjects who had normal initial TAG levels showed a smaller decline (2.5%, p=0.13) as compared to those who had elevated initial TAG (7.6%, p=0.19). The control group subjects also experienced a mild reduction in the FBS levels after the intervention period.

TABLE 4.79 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE LIPID AND LIPOPROTEIN FRACTIONS OF THE SUBJECTS BASED ON THE INITIAL TAG LEVELS (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	Post	Paired t 2 tailed p value	Pre	Post	Paired t 2-tailed p value
TAG<150 mg/dl	**N=20**			**N=18**		
TC	215 ± 23.6	218 ± 35.1	0.6	221 ± 25.4	214 ± 44.4	0.29
TAG	10 ± 18.8	112 ± 24.6	0.07	113 ± 20.2	113 ± 41.2	0.95
LDL	139 ± 20.2	142 ± 29.7	0.58	141 ± 18.0	136 ± 34.5	0.38
HDL	55 ± 7.5	54.9 ± 7.9	0.43	57.9 ± 10.9	55.7 ± 10.4	0.25
VLDL	20.7 ± 3.8	21.3 ± 5.9	0.58	22.7 ± 4.1	22.6 ± 8.4	0.97
Apo A	139 ± 19.0	127 ± 18.5	**0.001****	147 ± 27.4	135 ± 21.1	**0.02***
Apo B	107 ± 14.1	103 ± 14.1	0.33	110 ± 14.7	96 ± 21.8	**0.000*****
TAG≥150 mg/dl	**N=10**			**N=11**		
TC	216 ± 33.9	214 ± 34.0	0.81	226 ± 34.8	206 ± 31.0	**0.005****
TAG	207 ± 26.0	104 ± 46.3	0.77	200 ± 47.3	162 ± 33.1	**0.02***
LDL	128 ± 38.9	123 ± 30.6	0.74	135 ± 29.9	126 ± 25.5	**0.08**
HDL	47 ± 7.5	46 ± 8.4	0.55	52 ± 11.9	48 ± 9.3	**0.09**
VLDL	45 ± 18.3	44 ± 13.3	0.75	38 ± 12.3	30 ± 8.6	**0.02***
Apo A	139 ± 22.4	124 ± 16.9	**0.01***	146 ± 24.6	133 ± 22.1	**0.001****
Apo B	120 ± 18.6	111 ± 19.9	0.10	112 ± 21.4	97 ± 19.2	**6.7E-05*****

**Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001*

TABLE 4.80 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE SUBJECTS BASED ON THE INITIAL TAG LEVELS

(MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	**Post**	**Paired t 2 tailed p value**	**Pre**	**Post**	**Paired t 2-tailed p value**
TAG<150 mg/dl	**N=20**			**N=18**		
TAG/HDL	1.9 ± 0.4	2.1 ± 0.6	0.07	2.04 ± 0.6	2.12 ± 0.1	0.69
AIP	0.27 ± 0.1	0.30 ± 0.1	0.09	0.29 ± 0.1	0.29 ± 0.2	0.99
Apo B/ Apo A	0.78 ± 0.13	0.83 ± 0.13	0.12	0.77 ± 0.15	0.72 ± 0.14	0.13
TAG≥150 mg/dl	**N=10**			**N=11**		
TAG/HDL	4.46 ± 0.97	4.49 ± 1.21	0.92	4.1 ± 1.8	3.5 ± 1.4	0.28
AIP	0.64 ± 0.10	0.64 ± 0.13	0.90	0.58 ± 0.2	0.52 ± 0.2	0.16
Apo B/ Apo A	0.89 ± 0.19	0.91 ± 0.19	0.60	0.79 ± 0.2	0.76 ± 0.2	0.21

Subjects having normal initial TAG levels experienced a drop of 3.7% and subjects having elevated initial TAG levels experienced a decline of 2.2%. The results are shown in Table 4.81.

Impact on the Inflammation in the Subjects Based on The Initial TAG Levels

Supplementation with wheatgrass powder showed a better decline in the inflammation in experimental subjects with elevated initial TAG levels wherein the hs-CRP levels decreased by 18.7% (p=0.26) after the supplementation (Table 4.82). However, in the subjects with normal initial TAG levels, the hs-CRP levels reduced non-significantly by 3.2% (p=0.41). The control group subjects saw an increase in the hs-CRP levels at the end of the supplementation period as compared to the baseline levels, irrespective of the initial TAG levels.

Impact of Wheatgrass Supplementation on the Hemoglobin Levels of the Subjects Based on The Initial TAG Levels

In case of the hemoglobin values, no specific trend was seen in the subjects with normal initial TAG levels versus those with elevated initial TAG levels (Table 4.83). The experimental subjects with normal TAG levels experienced a decrease of 2.4% in the hemoglobin levels, whereas the hemoglobin values of the subjects with elevated TAG values remained constant at 12.4g/dl before and after the supplementation. In the control group on the other hand, the values remained constant at 12.2g/dl in the subjects with normal TAG as compared to those with elevated TAG values, wherein the mean hemoglobin value decreased by 3.2%.

Influence of Initial TAG Levels on Change in Percent Prevalence of Dyslipidemia in Supplemented Group

The influence of the initial TAG levels was studied on the change in percent prevalence of dyslipidemia in the experimental subjects (Figure 4.43). It was seen that in subjects with initial elevated TAG levels, there were greater

TABLE 4.81 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE FBS LEVELS OF THE SUBJECTS BASED ON THE INITIAL TAG LEVELS (MEAN ± S.D., mg/dl)

	FBS (mg/dl)	
	Control	**Experimental**
TAG<150mg/dl	**N=20**	**N=18**
Pre	80 ± 7.3	80 ± 9.5
Post	77 ± 5.6	78 ± 7.2
Paired t 2 tailed p value	**0.005****	0.13
TAG≥150mg/dl	**N=10**	**N=11**
Pre	89.3 ± 16.6	92 ± 25.5
Post	87.1 ± 15.2	85 ± 12.1
Paired t 2 tailed p value	0.18	0.19

*** Significantly different from baseline at p<0.01*

TABLE 4.82 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE INFLAMMATION IN THE SUBJECTS BASED ON THE INITIAL TAG LEVELS (MEAN ± S.D., mg/dl)

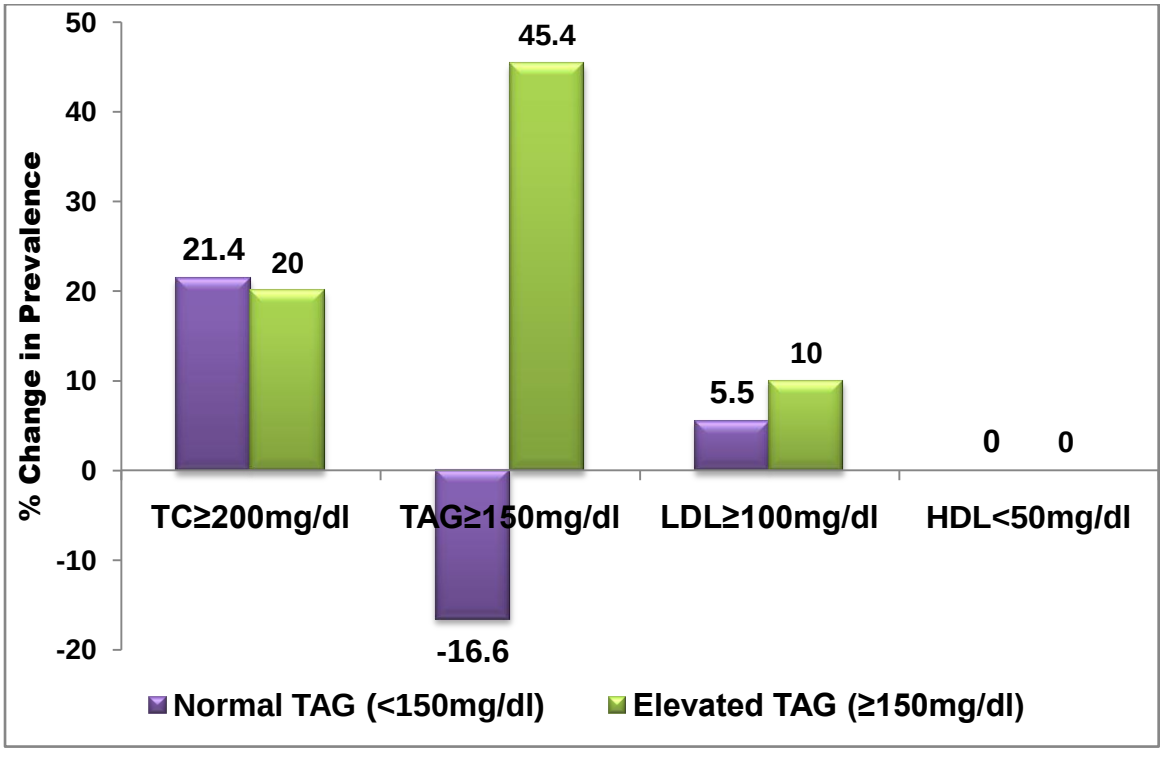
	Control	Experimental
TAG<150mg/dl	**N=20**	**N=18**
Pre	0.21± 0.23	0.19 ± 0.2
Post	0.23 ± 0.24	0.21 ± 0.2
Paired t 2 tailed p value	0.35	0.41
TAG≥150mg/dl	**N=10**	**N=11**
Pre	0.37 ± 0.26	0.48 ± 0.3
Post	0.42 ± 0.34	0.39 ± 0.3
Paired t 2 tailed p value	0.51	0.26

TABLE 4.83 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE HEMOGLOBIN LEVELS OF THE SUBJECTS BASED ON THE INITIAL TAG LEVELS (MEAN ± S.D., g/dl)

	Hb (g/dl)	
	Control	Experimental
TAG<150mg/dl	**N=20**	**N=18**
Pre	12.2 ± 1.2	12.4 ± 1.2
Post	12.2 ± 1.2	12.1 ± 1.1
Paired t 2 tailed p value	0.46	0.23
TAG≥150mg/dl	**N=10**	**N=11**
Pre	12.6 ± 1.5	12.4 ± 1.3
Post	12.2 ± 1.4	12.4 ± 1.1
Paired t 2 tailed p value	**0.02***	0.62

** Significantly different from baseline at p<0.05*

FIGURE 4.43 CHANGE IN PERCENT PREVALENCE OF DYSLIPIDEMIA IN SUPPLEMENTED GROUP STRATIFIED BY INITIAL TAG LEVELS



reductions in TC, TAG and LDL. In case of subjects with normal initial TAG levels, there was an increase increase in the prevalence of elevated TAG following the supplementation. The prevalence of low HDL levels was unchanged in either group.

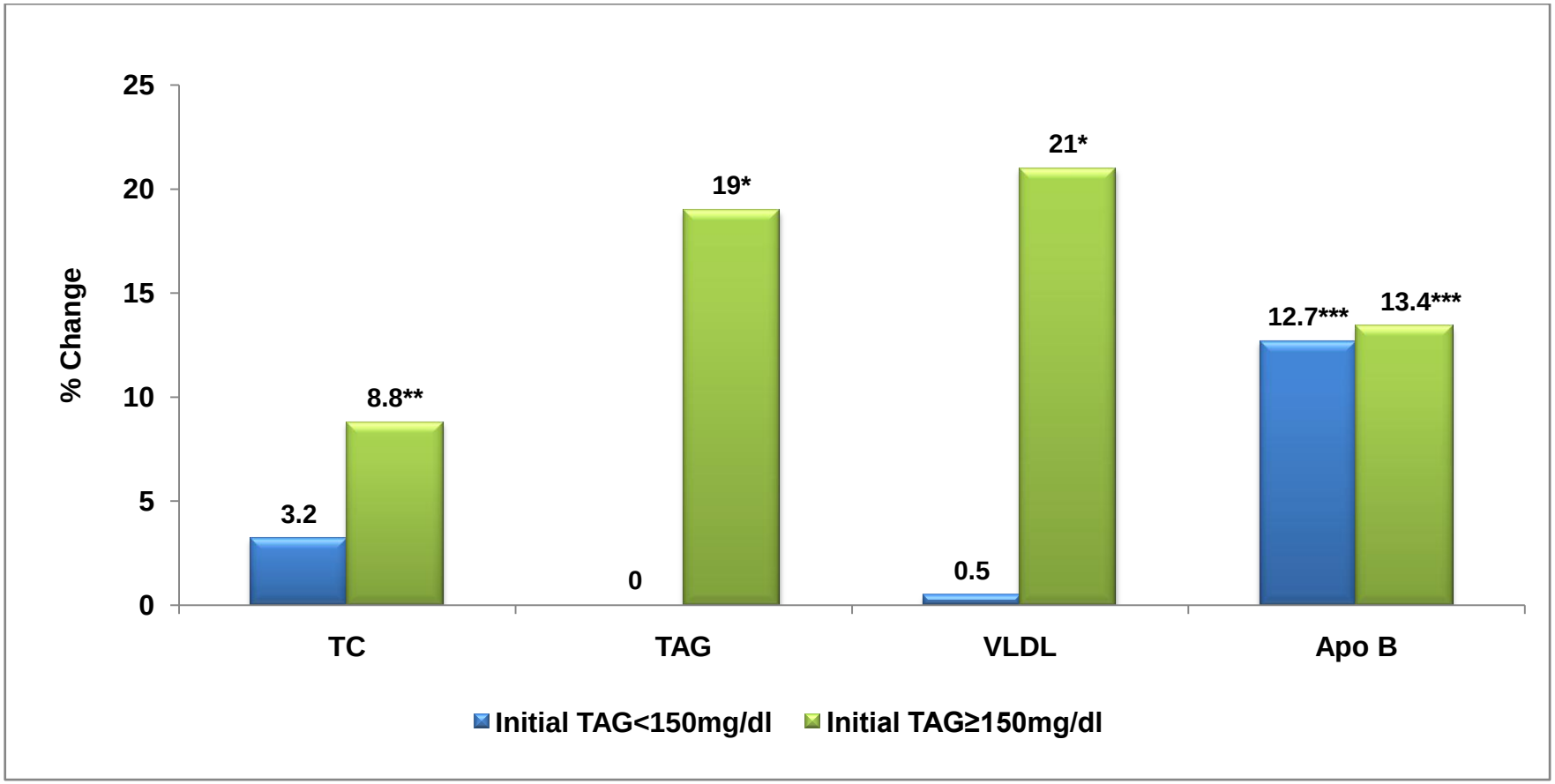
SUMMARY OF THE EFFECT OF INITIAL TAG LEVELS ON IMPACT OF WHEATGRASS SUPPLEMENTATION

- 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 (Figure 4.44).
- The apo B levels were not affected by the initial TAG levels and declined significantly in all the subjects.
- None of the atherogenic indices showed any specific differences with regard to initial TAG levels.
- Impact on FBS levels and hs-CRP levels did not appear to be affected by the initial TAG levels.
- The hemoglobin levels of the all the subjects declined by the end of the supplementation period, except subjects with higher than normal TAG levels, whose hemoglobin levels were maintained.
- To conclude, having higher than normal TAG levels (≥150mg/dl) positively influenced the impact of wheatgrass supplementation on the subjects.

IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE LIPID AND LIPOPROTEIN FRACTIONS OF THE SUBJECTS BASED ON THE INITIAL BMI

Comparison of the supplementation's impact across experimental subjects who had normal BMI at the beginning of the supplementation and those who were in the overweight and/or obese category of BMI ($\geq$23kg/m^2, that is) revealed clear trends of better impact in the individuals who had a higher than normal BMI (Table 4.84). In the experimental group subjects with a normal

FIGURE 4.44 INFLUENCE OF INITIAL TAG LEVELS ON THE % CHANGE IN OUTCOME PARAMETERS IN SUPPLEMENTED SUBJECTS



***Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001**

TABLE 4.84 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE LIPID AND LIPOPROTEIN FRACTIONS OF THE SUBJECTS BASED ON THE INITIAL BMI (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	Post	Paired t 2 tailed p value	Pre	Post	Paired t 2-tailed p value
BMI< 23kg/m^2	**N=3**			**N=8**		
TC	198 ± 11.7	182 ± 32.9	0.32	214 ± 34.8	214 ± 33.5	0.96
TAG	97 ± 19.7	91 ± 25.9	0.79	127 ± 33.5	116 ± 43.1	0.38
LDL	127 ± 14.2	116 ± 30.6	0.46	135 ± 24.2	138 ± 30.3	0.64
HDL	52 ± 5.2	50 ± 4.2	0.16	55 ± 10.5	54 ± 8.8	0.82
VLDL	19.4 ± 4.0	17.0 ± 6.3	0.60	23.1 ± 7.0	21.4 ± 9.2	0.49
Apo A	130 ± 11.9	111 ± 14.5	**0.02***	141 ± 24.0	133 ± 14.0	0.44
Apo B	103 ± 7.5	84 ± 15.8	0.17	105 ± 20.4	95 ± 21.1	**0.004****
BMI≥ 23kg/m^2	**N=27**			**N=21**		
TC	217 ± 27.5	221 ± 32.6	0.42	227 ± 26.2	210 ± 41.1	**0.007****
TAG	142 ± 54.8	148 ± 54.3	0.20	153 ± 58.9	138 ± 44.9	0.13
LDL	135 ± 29.1	138 ± 30.7	0.60	140 ± 22.8	130 ± 32.1	**0.04***
HDL	52 ± 8.7	52 ± 9.2	0.50	56 ± 11.9	52 ± 11.1	**0.02***
VLDL	30 ± 16.4	30.3 ± 14.2	0.81	30 ± 11.8	27 ± 9.0	0.1
Apo A	140 ± 20.4	127 ± 17.5	**0.000*****	149 ± 26.8	134 ± 23.5	**5.8E-05*****
Apo B	112 ± 17.5	108 ± 17.2	0.20	113 ± 15.8	96 ± 20.8	**2.6E-05*****

**Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001*

BMI, the lipoprotein fractions showed only a marginal reduction, whereas in the experimental group, the TC reduced by 7.5% ($p<0.01$), LDL reduced by 7.1% ($p<0.05$), apo B reduced by 15% ($p<0.001$) and an unfavorable decline was also seen in the HDL and apo A levels (HDL declined by 7.1%, $p<0.05$, apo A declined by 10.1%, p=<0.001).

Impact on the Atherogenic Indices Based on The Initial BMI

The segregation of data on atherogenic indices based on initial BMI revealed that in experimental subjects with a normal BMI the TAG/HDL, AIP and apo A / apo B reduced non-significantly (Table 4.85) by 6.5%. The overweight and/or obese subjects saw a significant reduction of 6.3% in the apo A / apo B levels ($p=0.05$), while the TAG/HDL showed a non-significant reduction of 3.4% ($p=0.59$) and AIP by 4.8% ($p=0.66$).

Impact of the Supplementation on the FBS Levels Based on The Initial BMI

The FBS levels saw a moderate decline when analyzed according to initial BMI levels (Table 4.86), in both the control and experimental groups. However, the experimental subjects who had a higher than normal BMI ($\geq 23 kg/m^2$) experienced a significant decline of 6.9% from 87 to 81mg/dl ($p=0.002$), as compared to the normal BMI subjects whose mean FBS levels reduced marginally by 2.5% from 80 to 78mg/dl ($p=0.11$).

Impact on the Inflammation Indicator in the Subjects Based on The Initial BMI

The hs-CRP levels in the experimental subjects went down marginally regardless of their mean BMI (Table 4.87). In subjects with normal BMI, the hs-CRP levels went down by 13% ($p=0.49$), while in the subjects with high BMI (≥23kg/m2), the hs-CRP levels came down by 6% ($p=0.62$). In the control group subjects, the normal BMI individuals saw a mild reduction (7.1%, $p=0.75$), while the overweight/obese individuals saw an increase of 10.7% in the hs-CRP levels from 0.28 to 0.31 ($p=0.27$).

TABLE 4.85 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE SUBJECTS BASED ON THE INITIAL BMI (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	**Post**	**Paired t 2 tailed p value**	**Pre**	**Post**	**Paired t 2-tailed p value**
BMI< 23kg/m^2	**N=3**			**N=8**		
TAG/HDL	1.88 ± 0.5	1.80 ± 0.4	0.87	2.44 ± 0.9	2.28 ± 1.2	0.68
AIP	0.26 ± 0.1	0.25 ± 0.1	0.90	0.36 ± 0.2	0.31 ± 0.2	0.42
Apo B/ Apo A	0.80 ± 0.14	0.76 ± 0.17	0.62	0.76 ± 0.17	0.72 ± 0.18	0.55
BMI≥ 23kg/m^2	**N=27**			**N=21**		
TAG/HDL	2.85 ± 1.4	3.0 ± 1.5	0.16	2.95 ± 1.7	2.81 ± 1.4	0.59
AIP	0.41 ± 0.2	0.43 ± 0.2	0.10	0.42 ± 0.2	0.40 ± 0.2	0.66
Apo B/ Apo A	0.82 ± 0.16	0.86 ± 0.16	0.07	0.79 ± 0.2	0.74 ± 0.2	**0.05***

** Significantly different from baseline at p<0.05*

TABLE4.86 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE FBS LEVELS OF THE SUBJECTS BASED ON THE INITIAL BMI (MEAN ± S.D., mg/dl)

	FBS (mg/dl)	
	Control	**Experimental**
BMI< 23kg/m^2	**N=3**	**N=8**
Pre	77 ± 6.0	80 ± 7.8
Post	75 ± 2.6	78 ± 6.7
Paired t 2 tailed p value	0.58	0.11
BMI≥ 23kg/m^2	**N=27**	**N=21**
Pre	84 ± 12.1	87 ± 20.5
Post	81 ± 11.2	81 ± 10.8
Paired t 2 tailed p value	**0.1**	**0.002****

*** Significantly different from baseline at p<0.01*

TABLE 4.87 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE INFLAMMATION IN THE SUBJECTS BASED ON THE INITIAL BMI (MEAN ± S.D., mg/dl)

	hs-CRP (mg/dl)	
	Control	**Experimental**
BMI< 23kg/m^2	**N=3**	**N=8**
Pre	0.14 ± 0.1	0.23 ± 0.3
Post	0.13 ± 0.1	0.20 ± 0.2
Paired t 2 tailed p value	0.75	0.49
BMI≥ 23kg/m^2	**N=27**	**N=21**
Pre	0.28 ± 0.3	0.33 ± 0.3
Post	0.31 ± 0.3	0.31 ± 0.3
Paired t 2 tailed p value	0.27	0.62

Impact the Supplementation on the Hemoglobin Levels Based on The Initial BMI

The data on hemoglobin levels was also analyzed separately depending on initial BMI values (Table 4.88). The Hb levels remained unaltered in both the groups before and after the supplementation.

Influence of Initial TAG Levels on Change in Percent Prevalence of Dyslipidemia in Supplemented Group

The change in percent prevalence of dyslipidemia in the experimental subjects was stratified according to the initial BMI levels (Figure 4.45). It was seen that overweight/obese subjects experienced consistent reductions in prevalences of elevated TC, TAG, LDL and low HDL levels. In case of subjects with normal BMI, there was a 100% reduction in the prevalence of elevated TAG levels however; there was also a concurrent 50% increase in the prevalence of low HDL levels following the supplementation.

SUMMARY OF THE EFFECT OF INITIAL BMI ON IMPACT OF WHEATGRASS 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. However, the levels of HDL and Apo A also declined in the high BMI group (Figure 4.46).
- 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 reduction in the FBS levels too, was significant in the high BMI group but not in the normal BMI group
- The hs-CRP levels did not show significant decline in either of the groups.

TABLE 4.88 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE HEMOGLOBIN LEVELS OF THE SUBJECTS BASED ON THE INITIAL BMI (MEAN ± S.D., g/dl)

	Hb (g/dl)	
	Control	Experimental
BMI< 23kg/m^2	**N=3**	**N=8**
Pre	12.0 ± 1.3	12.2 ± 1.9
Post	11.4 ± 0.7	12.2 ± 1.4
Paired t 2 tailed p value	0.30	0.85
BMI≥ 23kg/m^2	**N=27**	**N=21**
Pre	12.4 ± 1.3	12.4 ± 0.9
Post	12.3 ± 1.2	12.3 ± 1.0
Paired t 2 tailed p value	0.14	0.25

FIGURE 4.45 CHANGE IN PERCENT PREVALENCE OF DYSLIPIDEMIA IN SUPPLEMENTED GROUP STRATIFIED BY OVERWEIGHT/OBESE STATUS

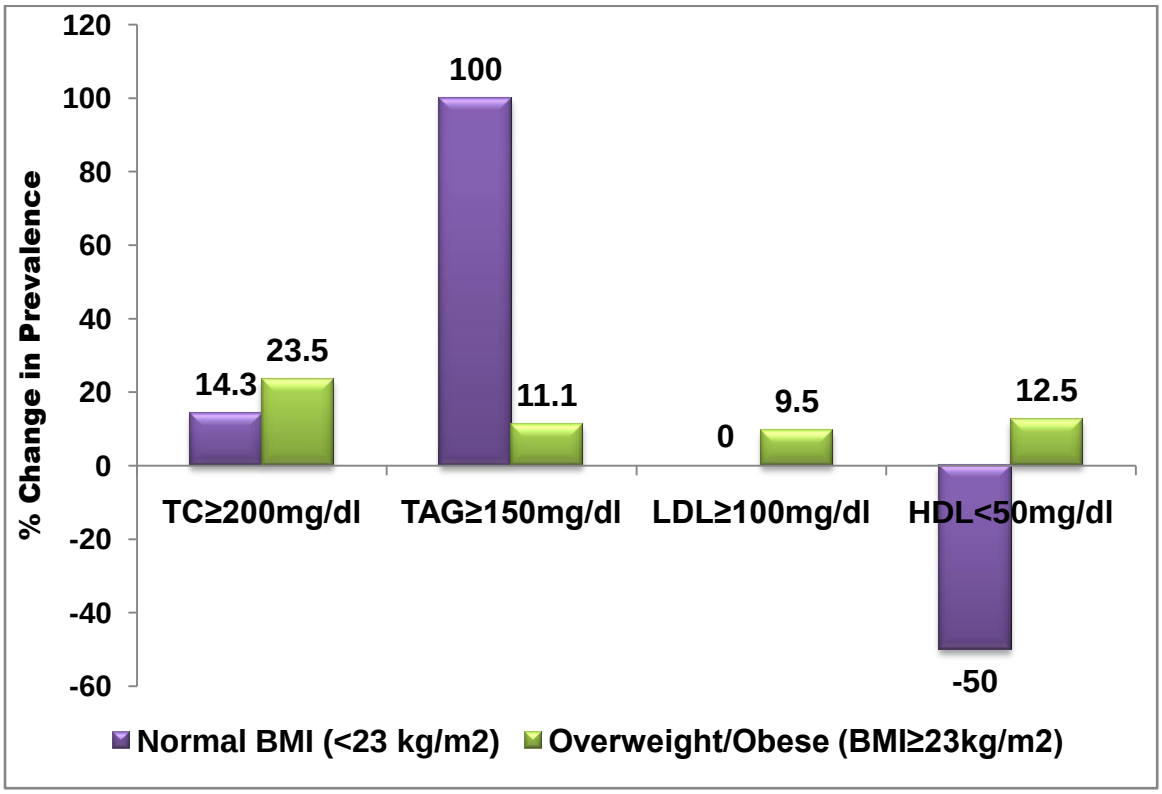
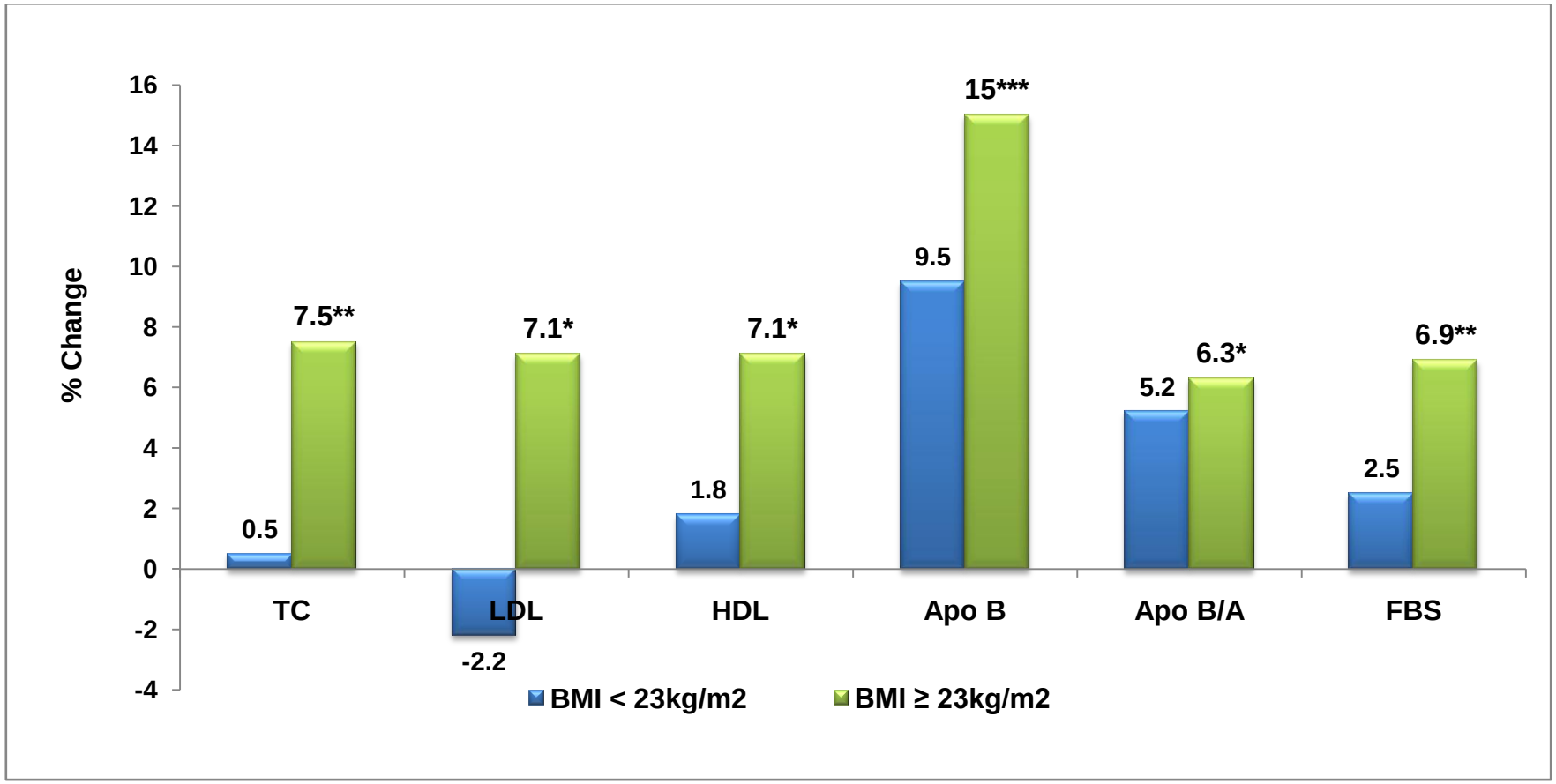


FIGURE 4.46 INFLUENCE OF INITIAL BMI LEVELS ON THE % CHANGE IN OUTCOME PARAMETERS IN SUPPLEMENTED SUBJECTS



***Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001**

Thus, initial BMI was found to influence the effect of supplementation, in the sense that individuals with higher than normal BMI experienced better impact of the supplementation compared to the individuals with a normal BMI.

Impact of Wheatgrass Supplementation on the Lipid and Lipoprotein Fractions of the Subjects With Hypertension as a Complication

An attempt was made to study the role of hypertension as a complication affecting the impact of the supplementation (Table 4.89). It was seen that in the individuals in the experimental group who did not have hypertension, showed significant reductions in TC (7.3%, p=<0.001), LDL (8%, p<0.001) and apo B (14%, p<0.001). In addition to undesirable reductions in apo A (6.4%) and HDL (5.4%).However, in the hypertensive individuals in the experimental group, such a reduction was not seen, except for a significant reduction in apo A (12.1%, p<0.01). The reduction in apo A was seen across the normotensives (9.6%, p=0.000) and hypertensives (9%, p<0.01) in the control group also. This trend therefore, hints to the complicating role of hypertension in the line of therapy in primary hypercholesterolemia.

Impact of the Supplementation on the Atherogenic Indices of the Subjects With Hypertension as a Complication

The atherogenic indices did not show any particular trend of reduction with respect to presence or absence of hypertension (Table 4.90). However, the only significant reduction that was seen was in the apo B / apo A values of normotensive experimental subjects which declined by 7.6% from 0.79 to 0.73 (p=0.02). Other than this, there were moderate reductions in the TAG/HDL ratio and AIP levels in the experimental group subjects regardless of presence of hypertension.

TABLE 4.89 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE LIPID AND LIPOPROTEIN FRACTIONS OF THE SUBJECTS WITH HYPERTENSION AS A COMPLICATION (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	Post	Paired t 2 tailed p value	Pre	Post	Paired t 2-tailed p value
Normo-tensive	**N=18**			**N=18**		
TC	210 ± 30.7	211 ± 39.7	0.88	218 ± 29.6	202 ± 28.2	**5.03 E-05*** **
TAG	138 ± 58.3	146 ± 65	0.20	133 ± 47.9	123 ± 49.1	0.15
LDL	128 ± 33.2	130 ± 36.1	0.76	137 ± 23.4	126 ± 23.5	**0.000*** **
HDL	52 ± 8.1	51± 8.0	0.18	55 ± 12.1	52 ± 10.4	**0.04***
VLDL	30.9 ± 18.8	30.4 ± 16.7	0.78	25.6 ± 9.9	23.6 ± 10.1	0.12
Apo A	135 ± 18.4	122 ± 15.2	**0.00****	140 ± 21.3	131 ± 21.3	**0.005****
Apo B	110 ± 16.8	103 ± 17.9	0.13	107 ± 18	92 ± 19.7	**0.000*** **
Hyper-tensive	**N=12**			**N=11**		
TC	223 ± 18.0	226 ± 22.3	0.63	230 ± 27.1	226 ± 50.9	0.73
TAG	138 ± 49.0	137 ± 36.3	0.87	167 ± 58.5	145 ± 34.0	0.25
LDL	144 ± 13.3	144 ± 19.0	0.99	141 ± 23.0	143 ± 39.9	0.78
HDL	53 ± 9.0	53 ± 10.2	0.99	56 ± 10.6	54 ± 10.8	0.46
VLDL	25.9 ± 10.3	26.8 ± 9.3	0.63	33.4 ± 11.7	29.2 ± 6.8	0.26
Apo A	144 ± 21.5	131 ± 20.4	**0.009****	157 ± 27.3	138 ± 21.1	**0.02***
Apo B	114 ± 16.9	110 ± 15.9	0.36	117 ± 14.2	102 ± 21.3	**0.003****

** Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001*

TABLE 4.90 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE ATHEROGENIC INDICES OF THE SUBJECTS WITH HYPERTENSION AS A COMPLICATION (MEAN ± S.D., mg/dl)

	Control			Experimental		
	Pre	**Post**	**Paired t 2 tailed p value**	**Pre**	**Post**	**Paired t 2-tailed p value**
Normo-tensive	**N=18**			**N=18**		
TAG/HDL	2.73 ± 1.4	2.98 ± 1.6	0.12	2.59 ± 1.3	2.58 ± 1.5	0.9
AIP	0.39 ± 0.2	0.42 ± 0.2	0.19	0.36 ± 0.2	0.35 ± 0.2	0.62
Apo B/ Apo A	0.83 ± 0.15	0.85 ± 0.13	0.43	0.79 ± 0.2	0.73 ± 0.2	**0.02***
Hyper-tensive	**N=12**			**N=11**		
TAG/HDL	2.78 ± 1.5	2.76 ± 1.2	0.89	3.17 ± 1.8	2.79 ± 0.9	0.44
AIP	0.40 ± 0.2	0.40 ± 0.2	0.68	0.46 ± 0.2	0.43 ± 0.1	0.51
Apo B/ Apo A	0.81 ± 0.2	0.86 ± 0.2	0.09	0.77± 0.2	0.74 ± 0.1	0.59

** Significantly different from baseline at p<0.05*

Impact of on the FBS Levels With Hypertension as a Complication

The FBS levels seemed not to be affected by presence of hypertension while reflecting the impact of the supplementation (Table 4.91). The normotensive experimental subjects experienced a decline of 2.3% in the mean FBS levels from 85 to 83mg/dl (p=0.04),and so did the hypertensive experimental subjects whose FBS levels fell by 3.8% from 79 to 76mg/dl (p=0.003). The control group subjects however experienced a marginal decline in the FBS levels.

Impact of Wheatgrass Supplementation on the Inflammation in the Subjects With Hypertension as a Complication

The supplementation had a significant impact on the hs-CRP levels (Table 4.92) in the normotensive experimental subjects whose mean levels came down by 32% from 0.25 to 0.17 (p=0.03). On the other hand, the hs-CRP levels increased by 12.5% in hypertensive experimental subjects from 0.40 to 0.45 (p=0.41). Similarly, the control group subjects also saw an increase in the hs-CRP levels, irrespective of presence of hypertension. Thus, the impact of the supplementation on inflammation also seems to be complicated by the presence of hypertension

Impact on the Hemoglobin Levels With Hypertension as a Complication

With respect to hemoglobin levels (Table 4.93), it was observed that the hypertensive experimental subjects experienced a decline of 2.3% in their mean hemoglobin levels after the supplementation from 13.1 to 12.8g/dl (p=0.05), which was surprising. The normotensive experimental subjects were able to maintain their Hb levels at 11.9g/dl, whereas the control group subjects also saw a decline in their Hb levels, whether hypertensive or not.

TABLE 4.91 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE FBS LEVELS OF THE SUBJECTS WITH HYPERTENSION AS A COMPLICATION (MEAN ± S.D., mg/dl)

	FBS (mg/dl)	
	Control	**Experimental**
Normotensive	**N=18**	**N=18**
Pre	78 ± 8.4	85 ± 13.1
Post	77 ± 4.3	83 ± 11.9
Paired t 2 tailed p value	0.3	**0.04***
Hypertensive	**N=12**	**N=11**
Pre	96 ± 23.8	79 ± 8.7
Post	87 ± 12.9	76.6 ± 7.6
Paired t 2 tailed p value	0.11	**0.003****

** Significantly different from baseline at p<0.05*

.TABLE4.92 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE INFLAMMATION IN THE SUBJECTS WITH HYPERTENSION AS A COMPLICATION (MEAN ± S.D., mg/dl)

	Hs-CRP (mg/dl)	
	Control	**Experimental**
Normotensive	**N=18**	**N=18**
Pre	0.31 ± 0.3	0.25 ± 0.3
Post	0.35 ± 0.3	0.17 ± 0.2
Paired t 2 tailed p value	0.34	**0.03***
Hypertensive	**N=12**	**N=11**
Pre	0.20 ± 0.2	0.40 ± 0.3
Post	0.21 ± 0.2	0.45 ± 0.3
Paired t 2 tailed p value	0.63	0.41

** Significantly different from baseline at p<0.05*

TABLE 4.93 IMPACT OF WHEATGRASS SUPPLEMENTATION ON THE HEMOGLOBIN LEVELS OF THE SUBJECTS WITH HYPERTENSION AS A COMPLICATION (MEAN ± S.D., mg/dl)

	Hb (g/dl)	
	Control	**Experimental**
Normotensive	**N=18**	**N=18**
Pre	12.4 ± 1.4	11.9 ± 1.3
Post	12.2 ± 1.4	11.9 ± 1.1
Paired t 2 tailed p value	0.12	0.97
Hypertensive	**N=12**	**N=11**
Pre	12.3 ± 1.2	13.1 ± 0.6
Post	12.1 ± 1.1	12.8 ± 0.7
Paired t 2 tailed p value	0.28	**0.05***

** Significantly different from baseline at p<0.05*

INFLUENCE OF INITIAL TAG LEVELS ON CHANGE IN PERCENT PREVALENCE OF DYSLIPIDEMIA IN SUPPLEMENTED GROUP

The effect of presence of hypertension was studied on the impact on the prevalence of dyslipidemia in experimental subjects (Figure 4.47). The presence of hypertension was observed to result in better reductions in the prevalence of elevated TC, TAG and LDL. The prevalence of low HDL was not affected by the presence of hypertension and remained unchanged in both the groups.

SUMMARY OF THE EFFECT OF HYPERTENSION ON IMPACT OF WHEATGRASS 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 (Figure 4.48).
- 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.
- The hemoglobin levels experienced a decline in all the groups, except the normotensive experimental subjects whose hemoglobin levels were maintained at the end of the supplementation period
- Thus absence of hypertension was found to positively influence the impact of the supplementation in the experimental subjects.

OVERALL SUMMARY OF IMPACT OF THE SUPPLEMENTATION

Comparing the impact of supplementation overall and after stratifying according the initial levels of TC, TAG, BMI and absence of hypertension, it was seen that TC, Apo A and Apo B decreased in all the cases, while

FIGURE 4.47 CHANGE IN PERCENT PREVALENCE OF DYSLIPIDEMIA IN SUPPLEMENTED GROUP STRATIFIED BY ABSENCE OF HYPERTENSION

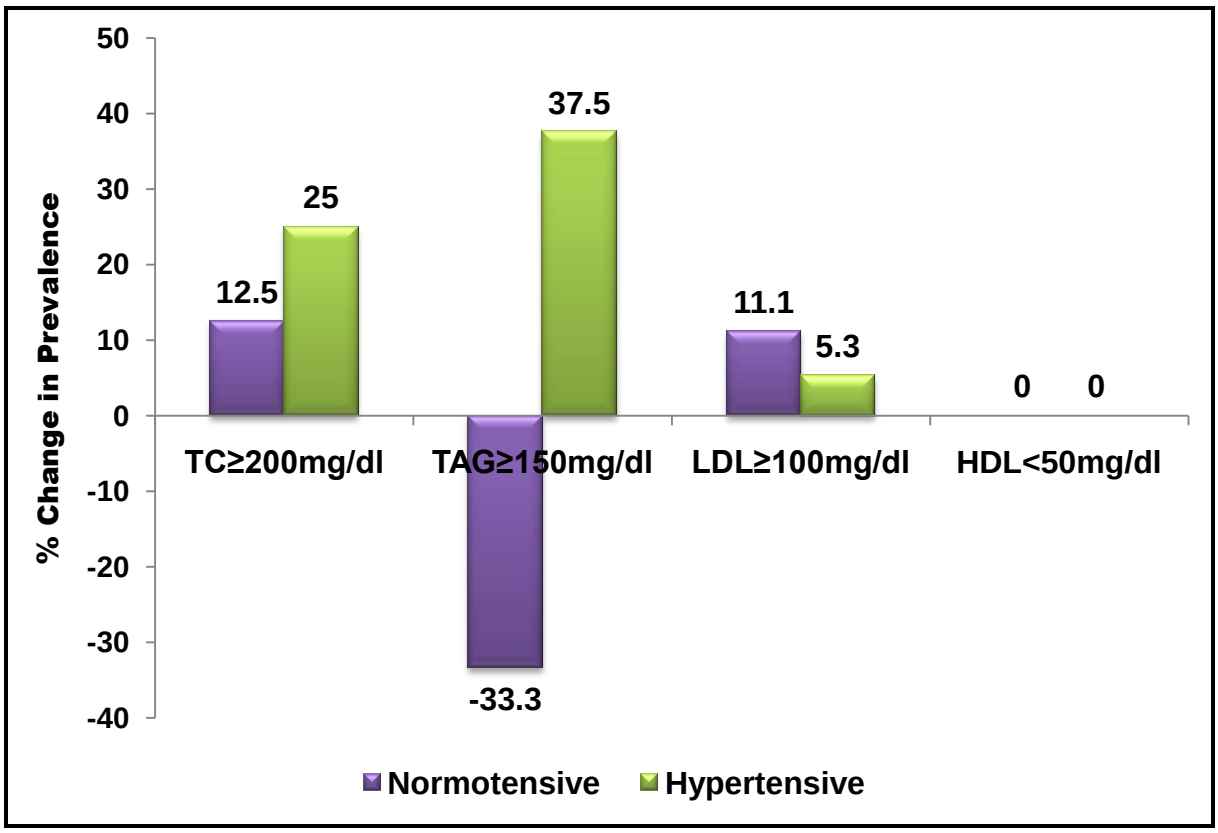
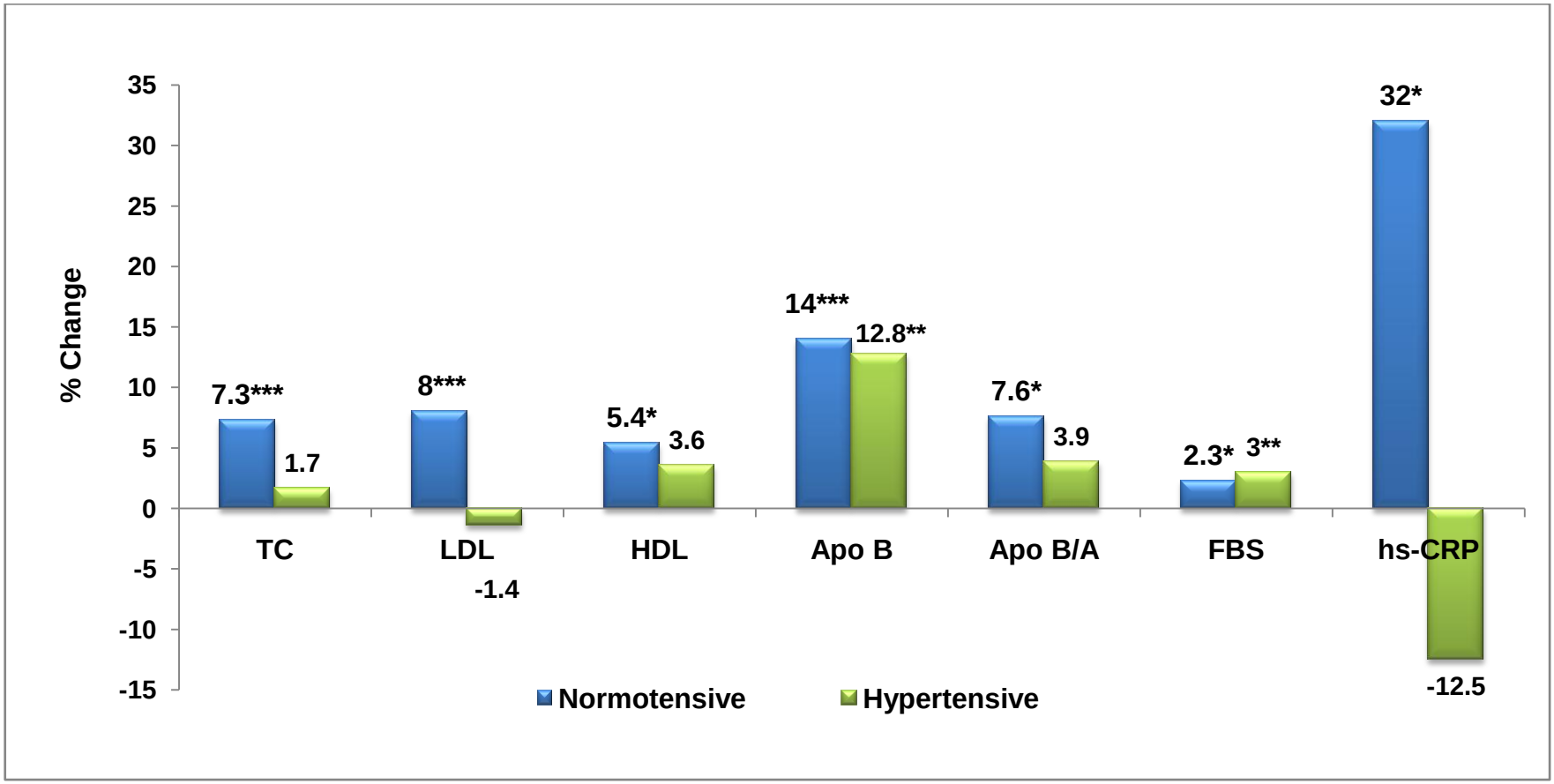


FIGURE 4.48 INFLUENCE OF ABSENCE OF HYPERTENSION ON THE % CHANGE IN OUTCOME PARAMETERS IN SUPPLEMENTED SUBJECTS



**Significantly different from baseline at p<0.05, **p<0.01, ***p<0.001*

TAG/HDL did not in any case. The supplementation was found to be most effective in normotensive individuals who saw reduction in maximum number of parameters. The overall summary has been described in Table 4.94.

DISCUSSION

IMPACT OF WHEATGRASS SUPPLEMENTATION ON MENOPAUSAL SYMPTOMS

Midlife in women sees a plethora of endocrine changes and resulting metabolic and physical changes, the triggering point of this change being decline in ovarian function. The varied effects of estrogen deficiency on the target organs are manifested as collection of symptoms commonly known as the menopausal syndrome. It encompasses symptoms affecting various organ systems including the psychological, vasomotor, urogenital and a host of other systems which are grouped together as somatic symptoms.

As a therapeutic measure, administering pharmacological estrogen, known as Hormone Replacement Therapy (HRT), was considered the main line of therapy till the late nineties, after which in 2002, the dissemination of the results of the landmark large scale primary prevention trial: Women's Health Initiative (WHI) of the National Institute of Health which had more than 16000 participants; suggested that the risks of HRT for post menopausal women outweighed the benefits it bestowed. In 2002, an interim analysis of the WHI (which was supposed to be a 8.5 year long trial) at 5.2 years, revealed that women in the HRT group had increased risk of coronary heart disease, breast cancer, venous thromboembolism and stroke. This caused the investigators to terminate the HRT section of the trial early (Writing group for the WHI 2002). More recently, researchers closed down the ERT arm of the WHI after finding out that estrogen alone increased the risk of stroke in postmenopausal women (The Women's Health Initiative Steering Committee 2004).

Following these turn of events, within 3 months of the WHI, HRT prescriptions reported decline from 12.5% to 9.4%, ($P \leq .0001$) in the United States as assessed from the Medco Healthcare data by Kim and associates (2005).

TABLE 4.94 SUMMARY OF IMPACT OF THE SUPPLEMENTATION AND INFLUENCE OF INITIAL LEVELS OF TC, TAG, BMI AND BLOOD PRESSURE

Parameters	Overall	At the beginning of the Supplementation			
		TC> 200mg/dl	TAG> 150mg/dl	BMI> 23kg/m2	Norm-otensive
FBS	***	***	NS	**	*
Hb	*	NS	NS	NS	NS
hs-CRP	NS	NS	NS	NS	*
TC	**	*	**	**	***
TAG	NS	NS	*	NS	NS
LDL	NS	NS	NS	*	***
HDL	*	*	NS	*	*
Apo A	***	***	**	***	***
Apo B	***	***	***	***	***
Apo B/ A	***	*	NS	*	*
TAG/HDL	NS	NS	NS	NS	NS

**Significantly different from baseline at p< 0.05, **p<0.01, ***p<0.001, NS: Non-significant*

The decline in the HRT use was statistically significant, when stratified by age, with a decline seen in all age groups after the WHI results, with the decline being sharpest in women aged 55 to 64 years (18% to 11%, p<0.0001). With regard to the users who were still using HRT, there was an increase in discontinuation in the year 2002 (67%) when compared to the year 2001 (53%, p<0.0001).Similar results were reported in Australia (Canfell et al 2008), Canada (Guay et al 2008) and Germany (Katalinic and Rawal 2008). To worsen the case against HRT, many studies in the past decade and recently have also reported a marked decrease in the breast cancer rates following the decline in the use of HRT (Katalinic and Rawal 2008, Canfell et al 2008, Verkooijen et al 2009, Lambe et al 2010).Eventually, it gave rise to a pressing need for alternative herbal therapy for managing menopausal symptoms.

The nutraceutical compounds in many natural functional foods like red yeast rice, black cohosh, red ginseng and red clover (Low Dog 2005, Geller and Studee 2005, Young Kim et al 2011, Lipovac et al 2012, Leach and Moore 2012) have been proposed to exert holistic health benefits, particularly with regard to cardio vascular, metabolic and oncogenic events. One such substance which is hypothesized to contain a plethora of nutraceuticals like polyphenols, mucopolysacccharides, 13 vitamins, protective enzymes superoxide dismutase & cytochrome p450 and generous amounts of healthy pigment chlorophyll, is wheatgrass (Rana, Camboj and Gandhi 2011).

Wheatgrass has been observed to possess significant antioxidant properties (Kulkarni et al 2006), and has been shown to decrease oxidative stress, atherogenic indices (Sethi et al 2010, Kothari et 2011, Das, Hakim & Mittal 2012) and have anti-cancerous (Zelina et al 2008, Wheat and Currie 2008) and immunosuppressive properties (Hemalatha et al 2012) in animal model studies, in vitro, molecular and genetic studies. However most of these beneficial effects of wheatgrass, especially on atherogenic indices and menopausal symptoms have not been corroborated by conducting human trials.

It was therefore felt worthwhile to evaluate the multifaceted effect of wheatgrass powder on the atherogenicity, inflammation, glucose control and menopausal symptoms as well, in hypercholesterolemic women.

Confirming the alternate hypothesis, wheatgrass supplementation in this study saw improvements in the menopausal symptoms in the intervened group after the supplementation period. The most marked change was in the prevalence of vasomotor symptoms which decreased 42% after a period of 10 weeks, followed by somatic symptoms which decreased 33%, followed by psychological symptoms which reduces by 50%. Only the prevalence of urogenital symptoms did not experience any decrease which remained unchanged at 13.8% from the beginning till the end of the supplementation period, suggesting that the changes in the urogenital musculature are lasting and might not be reversible.

Natural herbal products that have yielded positive results in relieving menopausal symptoms include red ginseng which in a study by Young et al (2011), reported significant decrease in aggregate menopausal symptom rating ($p<0.05$) from menopause rating scale and Kupperman index, after a 12 week supplementation with 3g red ginseng in a randomized double-blind placebo controlled trial. However, a systematic review on effectiveness of botanical herbal supplements in alleviating menopausal symptoms revealed that majority of studies reviewed indicated that extract of black cohosh (Actaearacemosa L.) improves menopause-related symptoms. The results of the clinical trials of soy isoflavones were mixed, and those of red clover extracts, ginseng (Panax ginseng C.A. Mey), dong quai (Angelica sinensis L.) or evening primrose seed oil were inconclusive and contradictory (Low Dog 2006).

A systematic review by Krebbs et al (2004) studied the effect of soy isoflavones on menopausal symptoms. The review took into account only trials that included symptomatic perimenopausal or postmenopausal women, compared phytoestrogen with placebo or control, reported hot flush frequency or menopausal symptom scores, and were at least 4 weeks in duration. The authors concluded that phytoestrogens available as soy foods, soy extracts or

red clover did not improve hot flushes or other menopausal symptoms in menopausal women.

In the present study though the prevalence of various menopausal symptoms showed a decline, the reduction was not statistically significant, indicating a positive response, but not strong enough to draw strong conclusions and hence replicating the research with a longer supplementation period might yield more interpretable results.

HYPOLIPIDEMIC EFFECT OF WHEATGRASS SUPPLEMENTATION

Dietary interventions for lowering serum cholesterol concentrations and for modifying serum lipoprotein levels are the cornerstone of prevention and treatment plans for coronary heart disease (Krause et al 2000). However in the past few decades, this condition has increased multifold across populations and continues to rise, especially in the Indian continent. The formative research in the present study itself has indicated that hyperlipidemia is the most pressing problem in the Indian menopausal women inspite of drug therapies available, continues to soar. Manifestation of this problem as early late 30's and early 40's in life, has lead the population to turn to alternative therapies for help (Liu et al 2000, Ai and Bolling 2002).

Most of the dietary interventions targeted at hyperlipidemia focus mainly on the action of three classes of bioactive compounds: a] Plant sterols and stanols, collectively known as Phytosterols; b] viscous polysaccharides (soluble fiber) and c] Polyphenols. The major mechanism through which the lipid lowering benefit is effected by these compounds includes the inhibition of intestinal absorption of cholesterol and bile acids at several sites within the intestinal tract. This inhibition has been suggested to take place via various mechanisms including

1. competition for solubilization in dietary mixed micelles,
2. co-crystallization to form insoluble mixed crystals
3. interference with lipase and cholesterol esterase mediated hydrolysis and,

4. Competitive inhibition at the time of incorporation into chylomicrons

There is also rising evidence that plant sterols meddle with transport-mediated method of cholesterol uptake. The outcome of all these events is that intestinal cholesterol absorption is reduced, and in turn, more of cholesterol and bile acids are excreted in the feces, causing the liver to turn to circulating cholesterol for hepatic bile acid production, and thereby effecting a reduction in the circulating cholesterol levels (Tebib, Besancon and Rouanet 1994, Trautwein et 2003).

Wheatgrass is found to be rich in all three of the above mentioned classes of bioactive compounds: Phytosterols, Viscous Polysaccharides and Polyphenols. Phytosterols, namely beta-sitosterol, campesterol, and stigmasterol were found in hexane extracts of wheatgrass, with beta-sitosterol accounting to 74% of the total phytosterols in the extract, which ranged from 834-1206 mg/kg (Dunford, Irmak and Jonnala 2009, Dunford and Edwards 2010). Polyphenol tests revealed the presence of flavonoids, triterpenoids, anthranol, alkaloids, tannins, saponins and sterols in fresh grass juice (Kothari et al 2011).Aqueous extracts of wheatgrass were found to contain gums and mucilages also, which belong to the family of viscous polysaccharides (Shirude 2011).

The supplementation in the present study yielded a favorable impact on the atherogenic indices as gauged by significant reductions in TC which reduced by (5.3%, $p<0.01$), Apo B which reduced by 13% ($p<0.001$) and near significant reduction in TAG of 9.7% ($p=0.07$) in the supplemental group. The LDL levels decreased non-significantly by 4.5% and the VLDL levels saw a near significant reduction of 10.1% ($p=0.07$).

This marked hypolipidemic effect can be attributed to the synergistic actions of the variety of phytonutrients mentioned above. To start with, the role of phyosterols, especially beta-sitosterol can be considered to interact with both the types of estrogen receptors and effect reduction in the cholesterol, because of well documented effects of estrogen on lipid metabolism (Walsh et

al 1991). The mechanism has been found to be reduction in the intestinal absorption of cholesterol as well as reducing the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity (Laraki et al 1993).

Further the presence of various types of saponins, triterpenes and steroid glycosides have been found to exert hypolipidemic activity by forming complexes with cholesterol in the gut and resultant precipitation of cholesterol causes exclusion of cholesterol and bile acids from incorporated into the micelles (Trautwein et al 2003).

Another causative factor that can be considered is the amino acid composition of wheatgrass, which has also been known to affect lipid metabolism. It has been found that wheatgrass has a lysine arginine ratio of 0.7, considered to be low compared to animal protein, with the value for casein being 1.2; and also a low methionine content of 15mg per 3.5g of wheatgrass, which is abysmally low compared to 86mg of 100ml cow's milk or other proteins of animal origin. A low lysine-arginine ratio and low methionine content have found to exert hypocholesterolemic effects (Kritchevsky 1979).The underlying mechanisms seem to be reduced absorption of cholesterol, increase in glucagon secretion and inhibition of insulin production (Sanchez 1991). The other mechanism can be suppressing the HMG CoA reductase and 7-α-hydroxylase activities through regulating hepatic glutathione concentrations (Potter and Kies 1990).

Other possible mechanism of hypolipidemic effect of wheatgrass supplementation can be action of tannic acid, which also binds cholesterol in the gut and prevents absorption. Additionally, in in vitro studies, tannic acid has been shown to suppress insulin-induced lipogenesis in mouse- adipose tissue cultures. The causal pathway was identified to be phosphorylation of beta subunit of the insulin receptor, and additionally, inhibition of tyrosine kinase activity (Ong, Khoo and Das 1995). Furthermore, tannic acid also increases the activity of endothelium bound lipoprotein lipase (LPL) which is responsible for clearing TAG from the circulation (Tebib, Besancon and Rounanet 1994).

The dynamics of endocrine interrelationships of metabolic hormones also govern the cholesterol concentrations: insulin, glucagon, glucocorticoids, and thyroid hormones are known to be involved in cholesterol metabolism. Thus it is possible that the synergistic effects these essential non-nutrients exert on the endocrine system, probably results in a favorable variation in the hormone concentrations which also plays a role in the hypocholesterolemic effect of active compounds that have both hypoglycemic and hypocholesterolemic effects. The present study also found a significant reduction in the mean FBS levels from 85.3mg/dl to 80.9mg/dl (paired t test 2 tailed $p<0.001$) in the experimental group at the end of the intervention period. So, among the various hypolipidemic mechanisms being considered here, reduction in FBS also seems to be at play.

Diets rich in phytonutrients more often than not, effected improved control of carbohydrate and lipid metabolism, particularly in patients with diabetes mellitus or dyslipidemia. Diminished and/or delayed postprandial glucose absorption results in reduced insulin concentrations. Insulin has been found to increase lipoprotein lipase (LPL) synthesis and also stimulate the HMG CoA reductase activity. This results in increase in the de novo cholesterol synthesis (Ness, Zhao and Wiggins 1994). Furthermore, insulin has also been found to cause a marked and rapid increase in the fatty acid synthase gene transcription rates and also the mRNA amounts of the enzyme fatty acid synthase. In addition to this, insulin down-regulates cholesterol 7a-hydroxylase and sterol 27-hydroxylase gene transcription thereby impairing the synthesis of bile acids (Soncini et al 1995, Twisk et al 1995).

Hypolipidemic action of wheatgrass has been corroborated in animal model studies, which are the only volume of studies conducted to evaluate the effect of wheatgrass on atherogenicity. Results from a recent mouse model study (Kothari et al 2011) on wheatgrass were similar to found in this research, wherein wheatgrass juice was administered at 5 mL/kg and 10 mL/kg in hypercholesterolemia induced Wistar rats for a period of 14 days. The supplementation resulted in dose dependent significant ($p<0.05$) decline in TC, TAG, LDL and VLDL levels. The researchers also looked at the fecal

cholesterol excretion which was significantly enhanced ($p<0.05$) upon wheatgrass supplementation.

Another study in rabbit model (Das, Hakim and Mittal 2012) evaluated the effect of ethanol extract of wheatgrass hyperlipidemic as well as normal animals. The experimental animals were fed 500mg/kg/day of wheatgrass extract orally for a period of 12 weeks, after which the authors found a significant ($p<0.05$) decline in the serum TG, TAG, LDL and MDA levels of the animals in both the normal and hypercholesterolemic groups. Interestingly, the HDL cholesterol had increased in the normal group but decreased in the hypercholesterolemic group. In the present study too, the supplemental group, all of whom were hypercholesterolemics, saw a decline in the HDL levels.

Experiments on the glycemic and lipemic index of wheatgrass containing recipes (Iyer, Sharma, Dhruv and Mani 2009) have reported that incorporation of wheatgrass into recipes reduced the glycemic index and the TAG level response of the recipes as compared to without addition of wheatgrass.

Other interventions focusing on the hypolipidemic potential of phenolic compounds and plant sterols have also found promising results. One such intervention is barley grass powder. A recent study (Venugopal and Iyer 2010) with subatmospheric dried barley grass powder capsules in stable diabetic subjects for 60 days saw a significant reduction in the serum TC, LDL and non-HDL and a significant decrease in HDL, which can be attributed to presence of hypocholesterolemic beta-sitosterol, anthocyanins and flavone-C glycosides in the barley grass powder.

The stereoisomers E- and Z-guggulsterone found in the resin of the Commiphora mukul tree, are the active agents that have been proposed to act as antagonististic ligands for a specific bile acid receptor. This receptor, called farnesoid X receptor (FXR), is an important step in regulation of cholesterol homeostasis. It is likely that this effect confers hypolipidemic activity to the phytosteroids in this resin (Urizar and Moore 2003).To corroborate this, a recent review on role of guggul in hyperlipidemia by Ulbricht et al (2005), reported that 11 small scale trials observed significant reductions in serum

TC, LDL and TAG and elevations in HDL following guggul supplementation, indicating a consistent hypolipidemic action of the resin.

Nuts is a food group that contains significant presence of phytosterols, lignans & fiber and have been observed to apply cholesterol lowering effects in epidemiological, in vitro, in vivo, animal model and small scale trials all alike. Also, a recent systematic review by Grieland Kris-Etherton (2008) reviewed trials on interventions on nuts and reported that 10 of 17 studies on nuts established a reduction in LDL that was greater than that predicted using predictive equations for blood cholesterol. The predicted mean decrease in LDL for these 17 controlled feeding studies was found to be 20.23 mmol/L, with an observed decrease of 20.29 mmol/L while comparing the nut rich diet to the control diet. More recently, a meta-analysis by Sabate (2010) analyzed 25 nut consumption trials in hyper and normolipidemics and found that a mean nut consumption of 67g/day improves blood lipid levels in a dose-related manner, particularly among subjects with higher LDL-C or with lower BMI.

Nigella sativa seeds, commonly known as niger seeds, are found to contain a large amount of phenyl propanoid compounds which accounted to about 46% of total volatile oil from the seeds; this extract approximately consists 40% of monoterpenoid compounds, in addition to presence of thymoquinone, thymol, tocopherols, tocopherol, trans-retinols, all of which have speculated to exert cardiovascular health benefits (Nickavar et al 2003). Sabzghabaee et al (2012) evaluated the impact of Nigella sativa seeds on the blood lipid levels of 88 hypercholesterolemic individuals in a randomized placebo controlled trial where the subjects were supplemented with 2g of Nigella sativa seeds per day for a period of 4 weeks. The authors observed a decline in TC (4.78%), LDL (7.6%) and TAG (16.65%), with the decrease being more significant for TAG concentration. However, similar to the results found in the present research, this study too, did not observe any appreciable reductions in the HDL fraction.

Another intervention that has showed substantial hypolipidemic effect is artichoke leaf whose alcohol extract possesses a number of active

hypolipidemic phenolic constituents, including chlorogenic acid, cynarin, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid , and the flavonoidsluteolin-7-rutinoside, cynaroside, apigenin-7-rutinoside, and apigenin-7-O-β-D-glucopyranoside.The main active compound among these has been identified as Cynarin, a hydroxycinnamic acid, which has been speculated to stimulate the bile flow (Zhu, Zhang and Lo 2004, Gebhardt 2001). A recent Cochrane systematic review (Wider et al 2009) summarized the available quality evidence on artichoke leaf extract which included 3 randomized controlled trials evaluating the effect of artichoke leaf extract as a mono-combination and not in conjunction to any other interventions and found that the intervention significantly reduced serum total cholesterol compared to placebo and the adverse events if any were mild and transient. Another recent rat-model study (Kiraz et al 2010) which evaluated the effect of 1.5g/kg/day artichoke leaf extract intervention to hypercholesterolemic rats for 2 weeks, reported that it resulted in reduced TC, TAG and cholesterol to HDL ratio.

Consumption of tea, specifically green tea has been associated with better cardiovascular outcomes, specifically slowing or inhibiting the progression of atherosclerotic lesions in various large scale epidemiological studies, including the Rotterdam study (Geleijnse et al 1999, Sasazuki et al 2000, Nakachi et al 2000). The prinicipal cardio-protective agent in tea has been identified as a monomeric flavan-3-ol called epigallocatechingallate (EGCG). Administration of 100mg/kg of EGCG in hypercholesterolemic rats for 15 days has been found to result in significantly lower levels of TC, LDL, TAG and higher levels of HDL (Ramesh et al 2008). Several other rat model studies have demonstrated similar hypolipidemic effect of EGCG after intervention with green, black, pu-erh tea (Huang and Lin 2012, Kim et al 2012). However, the saponin extract of green tea has also resulted in similar lowering of serum lipids as found by Matsui et al (2009), where after quantification of the saponins fraction in the green tea by liquid chromatography and time-of-flight mass spectroscopy, the extract was administered to mice and the authors reported an increased fecal cholesterol excretion and decreased serum lipids. A small scale human trial conducted on healthy human female subjects

(Gomikawa et al 2008) reported that two weeks of drinking green tea, reduced the mean cholesterol levels of the subjects by 10mg/dl.

Fruhbeck, Monreal, and Santidrian (2010) evaluated the impact of consumption of Viciafaba beans commonly known as field beans after finding the composition of the beans to be exceptionally rich in non-starch polysaccharides and sitosterol, which were found to be in the order of 166g/kg dry matter and 2% of the total unsaponifiable matter respectively. Other non-nutrients like tannins and saponins were also present in considerable amounts. The trial included 40 hypercholesterolemic individuals as well as individuals with borderline cholesterol levels, who received raw or cooked Vicia faba flour for a period of 30 days with excellent compliance. The study reported highly significant reductions in TC, TAG, LDL and VLDL fractions irrespective of initial cholesterol levels or extent of cooking of the flour. The observed hypolipidemic effect has been attributed to presence of significant amount of saponins which are observed to increased bile acid excretion through precipitation and hence prevention from micelle formation.

Impact on Inflammation

In the present study, the mean hs-CRP levels showed a decreasing trend from initial 0.30mg/dl to 0.27mg/dl, however, the difference was not statistically significant. Here difference between pre and post intervention values in the experimental group was significant, however the difference across post intervention values across control and experimental groups were not.

The anti-inflammatory effect of wheatgrass can be attributed partly to the presence of beta sitosterol which has been found to exert protective effects against endothelial inflammation. Specifically, beta-sitosterol has been found to prevent inflammatory changes by suppressing vascular adhesion molecule 1 and intracellular adhesion molecule 1 expression in Tumor Necrosis Factor alpha (TNF-α)-stimulated human aortic endothelial cells in addition to inhibiting binding of U937 cells to TNF-α-stimulated human aortic endothelial

cells. It also attenuates the phosphorylation of nuclear factor-kappa B (Loizou 2010).

Other active compounds that render wheatgrass anti-inflammatory can be polyphenols, which have been known to suppress inflammation cascade. The mechanisms are several, since inflammation is a result of a myriad of interactions. One way is through modulation of the gene expression of several pro-inflammatory molecular targets such as lipooxygenase, nitric oxide synthases, inflammatory cytokines and cyclooxygenase. The mediatory mechanism is thought to be inhibition of nuclear factor-kappa B signaling and mitogen-activated protein kinase signaling (Biesalski 2007, Santangelo et al 2007, Gonzalez et al 2011).

The flavanol in green tea, called as Epigallocatechingallate (EGCG) is also known to exert anti inflammatory effects. A rat model study by Bornhoeft et al (2012) also demonstrated anti inflammatory effects of green tea polyphenols in dextran sodium sulfate induced hypercholesterolemic rats. Specifically, addition of green tea polyphenols extract to the diet of these rats reduced the hs-CRP levels and also improved markers of antioxidant status including enzymes catalase, superoxide dismutase and glutathione peroxidase. A recent crossover trial by Stote et al (2012) reported that intake of 30-900mg of flavonols from either cocoa or green tea resulted in significantly decreased markers of inflammation, including hs-CRP, interleukin 6 and fibrinogen. However, a randomized controlled trial evaluating the anti inflammatory role of green tea as beverage and as green tea extracts for a period of 8 weeks, found no effect on biomarkers of inflammation including adiponectin, hs-CRP, IL-6, IL-1β or soluble VCAM1 in obese subjects with metabolic syndrome.

Kris-Etherton et al (2007) reviewed interventions with nuts in hypercholesterolemic subjects and reported that two of the three trials studied reported a decrease in the hs-CRP levels after the intervention period. Two of these studies were done on almonds, and one on walnuts, both of which contain fairly good amounts of ALA, which have studied to exert an anti-inflammatory effect (Zhao et al 2005).

The only human trial conducted with wheatgrass is by Shyam et al (2007), where the authors evaluated the effect of wheatgrass supplementation on oxidative stress parameters malondialdehyde (MDA), Superoxide dismutase (SOD) and vitamin C in healthy subjects. The study found that after supplementation with 500mg wheatgrass for 30 days, there was significant reduction ($p<0.05$) in MDA and significant enhancements in SOD and vitamin C levels in the subjects

The above observed effects of wheatgrass on oxidative stress can be attributed to its high antioxidant activity as reported by Kulkarni et al (2006). Wheatgrass extracts have been found to significantly inhibit lipid peroxidation induced by ascorbate and Fe2+ in liver mitochondria in rat model and its free radical scavenging ability is reported to be higher than those of many natural extracts or vegetables, as indicated by ORAC values of 39.9 for aqueous extracts and 48.2 for ethanol extracts. The antioxidant activity, reported in terms of FRAP values, were found to be 0.463 and 0.573 mmol of ascorbic acid and Trolox equivalents/100 g fresh wheatgrass.

Thus, two points are evident at this juncture: one that wheatgrass possesses a myriad of bio-active substances, which have shown to have a beneficial effect on the atherogenic status, inflammation and menopausal symptoms in several in vitro and in vivo animal and human trials. The second thing is since, after supplementation with wheatgrass for a period of 10 weeks with no significant change in the dietary patterns or intake of lipid lowering or anti-hyperglycemic medication or HRT during the course of the intervention period, the hypolipidemic effect and reduction in menopausal symptoms seen in the intervened subjects compared to the control group, is most likely the result of the effects of wheatgrass on the endocrine and metabolic functions of the subjects.

From the results of the study and the supporting evidence discussed above, **wheatgrass can said to be effective in alleviating the menopausal symptoms and improving the cardio-metabolic profile of menopausal**

women. Furthermore, this research, being the only documented human trial evaluating the hypolipidemic effects of wheatgrass, constitutes scientific evidence supporting the aforementioned features of wheatgrass, in freeze-dried form.

SALIENT OBSERVATIONS

1. Wheatgrass powder supplementation in freeze dried form, in the dosage of 1.4g per day for a period of 10 weeks appears to reduce the psychological, vasomotor symptoms and somatic in menopausal women
2. Wheatgrass supplementation also significantly improved the lipid profile in that; it reduced the TC and Apo B levels compared to baseline at the end of 10 weeks. It also appears to result in marginally significant reductions in TAG levels.
3. Though the beneficial apo A and HDL levels also declined, the decline in atherogenic parameters e.g. apo B/ A was more, resulting in a net favorable response.
4. The response of the supplementation was found to be better in subjects with elevated levels of TC and TAG.
5. The hs-CRP levels decreased non-significantly after the supplementation period.

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, which is sufficient grounds for recommending this antioxidant and phytonutrient-rich nutraceutical for the management of marginal hyperlipidemia and menopausal syndrome in middle aged women, who have been documented to largely depend on alternative therapies for these problems.

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 adversely 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 scientific evidence in favor of hypolipidemic effect of herbal and food based interventions like fruits, nuts, oil seeds, green tea, and various powdered leaves is substantial (Chong et al 2010, Kim et al 2012, Sabate et al 2010, Pan et al 2009, Venugopal et al 2012, Kumar et al 2010, Nambiar et al 2010).

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 from 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 symptos-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 2years
- 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 4.39) at baseline compared 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

REFERENCES

1. Abbasi S, Ponce De Leon A, Kassaian S et al. Gender Differences in the Risk of Coronary Artery Disease in Iran. Iranian J Publ Health, Vol. 41, No.3, 2012, pp.36-47.
2. Adams MR, Washburn SA, Wagner JD, et al. Arterial changes. Estrogen deficiency and effects of hormone replacement. In: Lobo RA, ed. Treatment of the Postmenopausal Woman. Raven, New York, 1994,pp.243250.
3. Aggarwal N, Raveendran A, Khandelwal N et al. Prevalence and related risk factors of osteoporosis in peri- andpostmenopausal Indian women. J Midlife Health. 2011 Jul; 2(2):81-5. doi: 10.4103/0976-7800.92537.
4. Ahmed M, Hassanein K. Effects of estrogen on hyperglycemia and liver dysfunction in diabetic male rats. Int J Physiol Pathophysiol Pharmacol 2012;4(3):156-166.
5. Ahmed-Sorour H, Bailey CJ. Role of ovarian hormones in the long-term control of glucose homeostasis, glycogen formation and gluconeogenesis. Ann Nutr Metab. 1981; 25:208–12.
6. Alghasham A, Settin AA, Ali A, Dowaidar M, Ismail H. Association of MTHFR C677T and A1298C gene polymorphisms with hypertension. Int J Health Sci (Qassim). 2012 Jan; 6(1):3-11.
7. Allain CC, Poon LS, Chan CSG, Richmond W and Pu FC, 1974. Enzymatic Determination of Total Serum Cholesterol, Clinical Chemistry 20: 470.
8. Allison M, Manson J, Aragaki A et al. Vasomotor symptoms and coronary artery calcium in postmenopausal women. Menopause. 2010; 17(6): 1136–1145. Doi:10.1097/gme.0b013e3181e664dc.
9. Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, Gauthier BR, Nef S, Stefani E, Nadal A. Pancreatic insulin content regulation by the estrogen receptor ER alpha. PLoS One. 2008; 3:e2069.

10. American Diabetes Association. Standards of Medical Care in Diabetes—2011**.** Diabetes Care, January 2011 vol. 34 no. Supplement 1 S11-S61. doi: 10.2337/dc11-S011.
11. American Heart Association (2004). Heart Disease and Stroke Statistics: 2004 Update. Available from: http://americanheart.org/downloadable/heart/1072969766940HSStats2004Update.pdf. Accessed on 22 May. 2011.
12. American Hospital Formulary Service. Drug information 1998. Bethesda, Md: American Society of Health-System Pharmacists, 1999.
13. Ammann P et al. Transgenic mice expressing soluble tumor necrosis factor receptor are protected against bone loss caused by oestrogen deficiency. Journal of Clinical Investigation, 1997, 99:1699–1703.
14. Anderson TJ, Meredith IT, Charbonneau F, et al. Endothelium-dependent coronary vasomotion relates to the susceptibility of LDL to oxidation in humans. Circulation 1996;93:16471650.
15. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26:12351241.
16. Andersson K, Hellstrand P. Dietary oats and modulation of atherogenic pathways. Mol Nutr Food Res. 2012 Jul;56(7):1003-13. doi: 10.1002/mnfr.201100706.
17. Artinian N, Fletcher G, Mozaffarian D et al. Interventions to promote Physical Activity and Dietary Lifestyle Changes for Cardiovascular Risk Factor Reduction in Adults. Circulation.2010; 122: 406-441**.**
18. Available from the URL: http://www.34-menopause-symptoms.com/headaches/articles/menopause-migraine-headaches.htm
19. Aviram M, Dornfeld L, Kaplan M et al. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs Exp Clin Res. 2002;28(2-3):49-62.
20. Ayranci U, Orsal O, Orsal O, Arslan G, Emeksiz D. Menopause status and attitudes in a Turkish midlife female population: an epidemiological study. BMC Women's Health 2010, 10:1.

21. Badawy A, State O, Sherief S. Can thyroid dysfunction explicate severe menopausal symptoms? J Obstet Gynaecol. 2007 Jul;27(5):503-5.
22. Barlow JJ, Emerson J, Saxena BN. Estradiol production after ovariectomy for carcinoma of the breast. N Engl J Med 1969; 280:633.
23. Baron R. Molecular mechanisms of bone resorption: therapeutic implications. Revue du Rhumatisme (English Edition), 1996, 63:633–638.
24. Barros R, Machado U, Gustafsson J. Estrogen receptors: new players in diabetes mellitus. TRENDS in Molecular Medicine 2006, Vol.12 No.9.
25. Barros RP, Gustafsson JÅ 2011 Estrogen receptors and the metabolic network. Cell Metab 14:289–299.
26. BarSela G, Tsalic M, Fried G, Goldberg H: Wheat grass juice may improve hematological toxicity related to chemotherapy in breast cancer patients. Nutr Cancer. 2007, 58(1): 43-48.
27. Bartoli C, Simontacchi M, Tambussi E et al. Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. Journal of Experimental Botany 1999, Volume 50, Issue 332, Pp. 375-383. doi: 10.1093/jxb/50.332.375.
28. Baskin et. al 2002. "AACE Medical Guidelines for Clinical Practice for Evaluation and Treatment of Hyperthyroidism and Hypothyroidism". American Association of Clinical Endocrinologists; 462, 465.
29. Batiste MC, Cartledge TP, Zellmer AW, Merino MJ, Axiotis C, Bremner WJ, Nieman LK. Effects of aging on menstrual cycle hormones and endometrial maturation. Fertil Steril 1995;64:492499.
30. Bauld R, Brown R. Stress, psychological distress, psychosocial factors, menopause symptoms and physical health in women. Maturitas 62 (2009) 160–165.
31. Beale C, Collins P. Estrogen and cardiovascular dynamics. Seminars in Reproductive Endocrinology 1996;14(1):7177.
32. Bechlioulis A, Kalantaridou SN, Naka KK, et al. Endothelial function, but not carotid intima media thickness, is affected early in menopause and is associated with severity of hot flushes. J Clin Endocrinol Metab. 2010; 95:1199–1206.

33. Berger JJ, Barnard RJ. Effect of diet on fat cell size and hormone-sensitive lipase activity. J Appl Physiol (1999) 227–232.
34. Bhagat m, Ghosh A. Obesity measures, metabolic profiles, blood pressure and intake of dietary fatty acids in rural women of Asian Indian origin: Santiniketan women study. J Cardiovasc Dis Res. 2011 Jan-Mar; 2(1): 61–67. doi: 10.4103/0975-3583.78599.
35. Bharati DR, Pal R, Kar S, Rekha R, Yamuna TV, Basu M. Prevalence and determinants of diabetes mellitus in Puducherry, South India. J Pharm Bioallied Sci. 2011 Oct-Dec; 3(4): 513–518.
36. Bharti D, Pal R, Kar S et al. Prevalence and determinants of diabetes mellitus in Puducherry, South India. J Pharm Bioallied Sci. 2011 Oct-Dec; 3(4): 513–518.
37. Bhatia A, Wade G. Energy balance in pregnant hamsters: a role for voluntary exercise? Am J Physiol 1993; 265:R563–567.
38. Bhatt SP, Nigam P, Misra A, Guleria R, Luthra K, et al. (2012) Association of the *Myostatin* Gene with Obesity, Abdominal Obesity and Low Lean Body Mass and in Non-Diabetic Asian Indians in North India. PLoS ONE 7(8): e40977. doi:10.1371/journal.pone.0040977.
39. Blake EJ, Adel T, Santoro NS. Relationships between insulin-like growth hormone factor-1 and estradiol in reproductive aging. Fertil Steril 1997; 67:697701.
40. Boden G, Cheung P, Stein TP, Kresge K, Mozzoli M. FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am J Physiol Endocrinol Metab (2002) 283:E12–E19.
41. Boden G, Shulman GI: Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Invest 2002, 32 (Suppl 3):14-23.
42. Borochov-Neori H, Judeinstein S, Greenberg A, et al. Phenolic antioxidants and antiatherogenic effects of Marula (Sclerocarrya birrea Subsp. caffra) fruit juice in healthy humans. J Agric Food Chem 2008, 56: pg 9884–9891.
43. Bozkurt N, Ozkan S, Kaucuoglu U et al. Urogenital symptoms of postmenopausal women in Turkey. Menopause. 2007 Jan-Feb;14(1):150-6.

44. Brenta G, Berg G, Arias P et al. Lipoprotein alterations, hepatic lipase activity, and insulin sensitivity in subclinical hypothyroidism: response to L-T(4) treatment. Thyroid. 2007 May; 17(5):453-60.
45. Brincat M, Calleja-Agius J. Urogenital Atrophy. European Society of Gynecology, August 2009. Available from the URL: http://www.seg-web.org/index.php/lang-en/component/content/article/101-atrophie-urogeni.
46. Bryzgalova G, Gao H, Ahren B, Zierath JR, Galuska D, Steiler TL, Dahlman-Wright K, Nilsson S, Gustafsson JA, Efendic S, Khan A. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. Diabetologia. 2006; 49:588–97.
47. Buccolo and David 1973. Quantitative Determination of Serum Triglycerides by the Use of Enzymes. Clinical Chemistry May 1973 vol. 19 no. 5 476-482.
48. Buckler HM, Evans CA, Mamtora H, et al. Gonadotropin, steroid, and inhibin levels in women with incipient ovarian failure during anovulatory and ovulatory rebound cycles. J Clin Endocrinol Metab 1991;72:116.
49. Bulbrook RD, Grenwood FC. Persistence of urinary oestrogen excretion after oophorectomy and adrenalectomy. Br Med J 1957; 1:662.
50. Burger H. The menopausal transition—Endocrinology. Journal of Sexual Medicine 2008;5:2266–2273.
51. Burger HG, Dudley EC, Hopper JL, Shelley JM, Green A, Smith A, Dennerstein L, Morse C. The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. J Cline Endocrine Me tab 1995; 80:35373545.
52. C Chandramouli (23 August 2011). "Census of India 2011 – A Story of Innovations". Press Information Bureau, Government of India.
53. Cagnacci A, Cannoletta M, Palma F et al. Menopausal symptoms and risk factors for cardiovascular disease in postmenopause. Climacteric. 2012 Apr;15(2):157-62. doi: 10.3109/13697137.2011.617852.
54. Cagnacci A, Cannoletta M, Palma F et al. Menopausal symptoms and risk factors for cardiovascular disease in postmenopause. Climacteric. 2012 Apr;15(2):157-62. doi: 10.3109/13697137.2011.617852.

55. Canner PL, Berge KG, Wenger NK, Stamler J, Fried-man L, Prineas RJ, et al. Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. J Am Coll Cardiol. 1986; 8:1245–55.
56. Carpita N, Kanabus J, Housley T. Linkage Structure of Fructans and Fructan Oligomers from*Triticum aestivum* and *Festuca arundinacea* Leaves. Journal of Plant Physiology, Volume 134, Issue 2, March 1989, Pages 162–168.
57. Casiglia E, d'Este D, Ginocchio G, Colangeli G, Onesto C, Tramontin P, Ambrosio GB, Pessina AC. Lack of influence of menopause on blood pressure and cardiovascular risk profile: a 16-year longitudinal study concerning a cohort of 568 women. J Hypertens. 1996; 14:729 –736.
58. Celermajer DS, Sorensen KE, Spiegelhalter DJ, et al. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 1994; 24:471476.
59. Chari P. Sushruta: The first Plastic Surgeon in 600 B.C. Internet Journal of Plastic Surgery 2003, 4 (2). ISSN 1528-8293.
60. Chen J, Brown T, Russo J. Regulation of Energy Metabolism Pathways by Estrogens and Estrogenic Chemicals and Potential Implications in Obesity Associated with Increased Exposure to Endocrine Disruptors. *Biochim Biophys Acta*. 2009 July ; 1793(7): 1128–1143. doi:10.1016/j.bbamcr.
61. Chen JF, Wang HR, Yang S, Zhao YP, Zhao XH, Chen YC, Du QL, Liu SJ, Shen C, Xu YC. An association study between transforming growth factor-β1 receptor 2gene polymorphisms and essential hypertension. Zhonghua Yu Fang Yi Xue Za Zhi. 2012 Sep; 46(9):825-30.
62. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the Joint National Committee on Prevention, Detection, evaluation, and Treatment of High Blood Pressure. Hypertension, 2003; 42:1206–1252.
63. Chowdhury B, Lantz H, Sjostrom L (1996) Computed tomography-determined body composition in relation to cardiovascular risk factors in Indian and matched Swedish males. Metabolism 45(5):634–44.

64. Chunni N, Sreeramareddy C. Frequency of symptoms, determinants of severe symptoms, validity of and cut-off score for Menopause Rating Scale (MRS) as a screening tool: A cross-sectional survey among midlife Nepalese women. BMC Womens Health. 2011; 11: 30. doi: 10.1186/1472-6874-11-30.
65. Clegg D. Minireview: The Year in Review of Estrogen Regulation of Metabolism. Mol Endocrinol, December 2012, 26(12):1957–1960.
66. Cohen LS, Soares CN, Vitonis AF, et al. Risk for new onset of depression during the menopausal transition. Arch Gen Psychiatry 2006;63:385–90.
67. Collins P, Rosano G, Jiang C, et al. Cardiovascular protection by oestrogena calcium antagonist effect? Lancet 1993;341:12641265.
68. Compston J, Watts N, Chapurlat R et al. Glow Investigators. Obesity is not protective against fracture in postmenopausal women: GLOW. Am J Med 2011; 124: 1043 – 50.
69. Copeland JC. Textbook of gynecology. Philadelphia, PA: Saunders, 1993; 619.
70. Coppack SW, Jensen MD, Miles JM. In vivo regulation of lipolysis in humans. J Lipid Res (1994) 35:177–193.
71. CreagerMA, Gallagher SJ, GirerdXJ, et al. $_{L}$-Arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. J Clin Invest 1992; 90:12481253.
72. Cuadros J, Fernandez-Alonzo A, Cuadros-Celorrio A. Perceived stress, insomnia and related factors in women around the menopause. Maturitas 72 (2012) 367–372.
73. Daniel WW (1999). Biostatistics: A Foundation for Analysis in the health Sciences. 7th edition. New York: John Wiley & Sons.
74. Darne B, Girerd X, Safar M, et al. Pulsatile versus steady component of blood pressure: a cross-sectional analysis on cardiovascular mortality. Hypertension 1989;13(4):392400.
75. Das M, Pal S, Ghosh A. Prevalence of cardiovascular disease risk factors by habitat: a study on adult Asian Indians in West Bengal, India. Anthropol Anz. 2011;68(3):253-64.

76. Davis S, Castelo-Branco C, Chedrau P et al. Understanding weight gain at menopause. Climacteric 2012; 15:419–429.
77. Deepa m, Farooq S, Deepa R et al. Prevalence and significance of generalized and central body obesity in an urban Asian Indian population in Chennai, India (CURES: 47). Eur J Clin Nutr. 2009 Feb;63(2):259-67.
78. Deepa M, Farooq S, Deepa R, Manjula D, Mohan V. Prevalence and significance of generalized and central body obesity in an urban Asian Indian population in Chennai, India (CURES: 47). Eur J Clin Nutr. 2009 Feb;63(2):259-67. Epub 2007 Oct 10.
79. Deepa R, Shanthirani C, Premalatha G, Sastry N, Mohan V. Prevalence of insulin resistance syndrome in a selected south Indian population--the Chennai urban population study-7 [CUPS-7]. Indian J Med Res. 2002 Mar; 115:118-27.
80. Deng B, Luo T, Huang Y, Shen T, Ma J. Prevalence and determinants of hyperlipidemia in moderate altitude areas of the Yunnan-Kweichow plateau in Southwestern China. High Alt Med Biol. 2012 Mar;13(1):13-21. doi: 10.1089/ham.2011.1037.
81. Department of Health and Ageing, Office of Gene Technology Regulator, Government of Australia. The Biology of Triticum aestivum L. em Thell. (Bread Wheat). 2008.
82. Dessapt A, Gourdy P. Menopause and cardiovascular risk. J Gynecol Obstet Biol Reprod (Paris). 2012 Nov; 41 (7 Suppl) : F13-9. doi: 10.1016/j.jgyn.2012.09.003.
83. Devi P, Rao M, Faraqui A et al. Prevalence, risk factors and awareness of hypertension in India: a systematic review. Journal of Human Hypertension , (13 September 2012) | doi:10.1038/jhh.2012.33.
84. Devi R, Kaur N, Gupta A. Potential of antioxidant enzymes in depicting drought tolerance of wheat (Triticum aestivum L.). Indian J Biochem Biophys. 2012 Aug; 49(4):257-65.
85. Dey S, Sarkar R, Ghosh P, Khatun R, Ghorai K, Choudhury R, Ahmed R, Gupta, P, Mukhopadhyay S, Mukhopadhyay A: Effect of Wheat Grass Juice in Supportive

Care of Terminally Ill Cancer Patients — A Tertiary Cancer Centre Experience from India. Journal of Clinical Oncology 2006, 24: s18.

86. Dhruv S, Iyer U, Bhatt K. Assessment of Cardio-Metabolic Risk Factors among Young Adult Females. Am. J. Infect. Dis., 8 (1): 34-40, 2012.
87. Dixit V, Jain P, Joshi S. Hypolipidaemic effects of Curcuma longa L and Nardostachys jatamansi, DC in triton-induced hyperlipidaemic rats. Indian J Physiol Pharmacol. 1988 Oct-Dec; 32(4):299-304.
88. Douchi T, Yamamoto S, Yoshimitsu N et al. Relative contribution of aging and menopause to changes in lean and fat mass in segmental regions. Maturitas 42 (2002), pg: 301-306.
89. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atheroscloeosis Prevention Study. JAMA. 1998; 279:1615–22.
90. Duke J. Phytochemical and Ethnobotanical Databases (Online Datbase), 2013. Available from the URL: http://sun.ars-grin.gov:8080/npgspub/xsql/duke/plantdisp.xsql?taxon=1025.
91. Dwivedi S. *Terminalia arjuna* —A useful drug for cardiovascular disorders. Journal of Ethnopharmacology, Volume 114, Issue 2, 1 November 2010, Pages 114–129.
92. Ebrahim S, Kinra S, Bowen L et al. The Effect of Rural-to-Urban Migration on Obesity and Diabetes in India: A Cross-Sectional Study. PLoS Med. 2010 April; 7(4): e1000268. doi: 10.1371/journal.pmed.1000268.
93. Ebrahim S, Kinra S, Bowen L, Andersen E, Ben-Shlomo Y, Lyngdoh T et al. The Effect of Rural-to-Urban Migration on Obesity and Diabetes in India: A Cross-Sectional Study. PLoS Med. 2010 April; 7(4): e1000268. doi: 10.1371/journal.pmed.1000268.
94. El-Bishbishy H, Singab A, Sinkkonen J et al. Hypolipidemic and antioxidant effects of Morus alba L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats. Life Sci. 2006 May 1;78(23):2724-33.
95. Epstein AM, Weissman JS, Schneider EC, Gatsonis C, Leape LL, Piana RN (2003). Race and gender disparities in rates of cardiac revascularization: do they

reflect appropriate use of procedures or problems in quality of care? *Med Care*, 41(11): 1240–55.

96. Erem C, Hacihasanogulu A, Deger O, Kocak M, Topbas M. Prevalence of dyslipidemia and associated risk factors among Turkish adults: Trabzon lipid study. Endocrine. 2008 Aug-Dec;34(1-3):36-51. doi: 10.1007/s12020-008-9100-z.
97. Eshtiaghi R, Esteghamati A, Nakhjavani M. Menopause is an independent predictor of metabolic syndrome in Iranian women. Maturitas, Volume 65, Issue 3, March 2010, Pages 262–266.
98. Espeland MA, Applegate W, Furberg CD, et al. Estrogen replacement therapy and progression of intimal-medial thickness in the carotid arteries of postmenopausal women. Am J Epidemiol 1995; 142:10111019.
99. Estiarte M, Penuelas J, Kimball B et al. Free-air CO_2 enrichment of wheat: leaf flavonoid concentration throughout the growth cycle. Physiol. Plant, 105 (1999), pp. 423–433.
100. Evans, W 2006. Overview of Methods and Instruments for Bone Densitometry. UK, National Osteoporosis Society. National Training Scheme for Bone Densitometry.
101. Expert Panel On Detection, Evaluation, And Treatment Of High Blood Cholesterol In Adults, May 2001. "Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)". JAMA: the Journal of the American Medical Association. 2001; **285** (19): 2486–97. doi:10.1001/jama.285.19.2486
102. Farzadfar F, Funucane M, Danaei G, Pelizzari P, Cowan M, Paciorek C et al. National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3·0 million participants. Lancet. 2011 Feb 12; 377(9765):578-86. doi: 10.1016/S0140-6736(10)62038-7.
103. Fernandez-Guasti A, Fiedler J, Herrera L et al. Sex, Stress, and Mood Disorders: At the Intersection of Adrenal and Gonadal Hormones. Horm Metab Res 2012; 44(08): 607-618. DOI: 10.1055/s-0032-1312592.

104. Ferreira de Araujo P, da Silva Santos V, Machado A et al. Benefits of blackberry nectar (Rubus spp.) relative to ypercholesterolemia and lipid peroxidation. Nutr Hosp. 2011; 26 (5): 984-990.
105. Fletcher B, Berra K, Braun L et al. Managing Abnormal Blood Lipids. A Collaborative Approach. Circulation.2005; 112: 3184-3209.
106. Foo-Hoe L. Menopause and the Asian woman: Is she different from women of other ethnicities? Menopause Medicine Vol. 15, No. 1 / May 2007.
107. Foryst-Ludwig A, Clemenz M, Hohmann S, Hartge M, Sprang C, Frost N, Krikov M, Bhanot S, Barros R, Morani A, Gustafsson JA, Unger T, Kintscher U. Metabolic actions of estrogen receptor beta (ERbeta) are mediated by a negative cross-talk with PPARgamma. PLoS Genet. 2008; 4:e1000108.
108. Franke AA, Cooney RV, Henning SM, et al. Bioavailability and antioxidant effects of orange juice components in humans. J Agric Food Chem 2005, 53; pg 5170–5178.
109. Franklin R, Ploutz-Synder L and Kanaley J. Longitudinal changes in abdominal fat distribution with menopause. Metabolism, 58 (3), March 2009, Pg: 311–315.
110. Freedman R, Woodward S. Altered shivering threshold in postmenopausal women with hot flashes. Menopause 1995; 2: 163-8.
111. Freedman RR. Physiology of hot flashes. Am J Human Biol Jul-Aug; 2001 13(4):453–464.
112. Freidewald W, Levy R and Fredrickson D, 1972. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, Vol. 18, No. 6, pg 499-502.
113. From: Giesbrecht F and Gumpertz M, 2004. Completely Randomized Design (Chapter 2, pg 13-15), in Planning, Construction and Statistical Analysis of Comparative Experiments. Wiley Series in Probability and Statistics. John Wiley and Sons, 2004 edition.
114. Fuhrman B, Volkova N, Kaplan M et al. Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of

LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. Nutrition, Volume 18, Issue 3 , Pg 268-273, March 2002.

115. Gallet M, Saidi S, Hay E et al. Repression of Osteoblast Maturation by ERRα Accounts for Bone Loss Induced by Estrogen Deficiency. PLoS One. 2013; 8(1): e54837.
116. Gallicchio L, Miller SR, Zacur H, Flaws JA. Hot flashes and blood pressure in midlife women. Maturitas. 2010 Jan; 65(1):69-74. doi: 10.1016/j.maturitas.2009.10.013. Epub 2009 Nov 28.
117. Gangar KF, Vyas S, Whitehead M, et al. Pulsatility index in internal carotid artery in relation to transdermal oestradiol and time since menopause. Lancet 1991; 338:83984.
118. Gebara O, Mittleman MA, Suterland P, Lipinska I, et al. Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation 1995; 91:19521958.
119. Geloneze B, Vasques ACJ, Stabe CFC et al 2009. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome – Brazilian Metabolic Syndrome Study (BRAMS). Arq Bras Endocrinol Metab. 2009;53(2):281-287.
120. Gerber LM, Sievert LL, Warren K, Puckering TG, Schwartz JE. Hot flashes are associated with increased ambulatory systolic blood pressure. Menopause. 2007 Mar-Apr; 14(2):308-15.
121. Ghosh A, Bhagat M, Das M et al. Prevalence of cardiovascular disease risk factors in people of Asian Indian origin: Age and sex variation. J Cardiovasc Dis Res. 2010 Apr-Jun; 1(2): 81–85. doi: 10.4103/0975-3583.64441.
122. Ghosh A, Bhagat M. Anthropometric and body composition characteristics in pre- and postmenopausal Asian Indian women: Santiniketan women study. Anthropol Anz. 2010; 68(1):1-10.
123. Gibbons LW, Gonzalez V, Gordon N, Grundy S. The prevalence of side effects with regular and sustained-release nicotinic acid. Am J Med. 1995; 99:378–85.

124. Gigoriou V, Augoulea A, Armeni E et al. Prevalence of vasomotor, psychological, psychosomatic and sexualsymptoms in perimenopausal and recently postmenopausal Greek women: association with demographic, life-style and hormonal factors. Gynecol Endocrinol. 2013 Feb; 29(2):125-8. doi: 10.3109/09513590.2012.708801.
125. Gilligan DM, Badar DM, Panza JA, et al. Effects of estrogen replacement therapy on peripheral vasomotor function in postmenopausal women. Am J Cardiol 1995; 75:264268?
126. Glazer G, Zeller R, Delumba L, et al. The Ohio Midlife Women's Study. Health Care Women Int 2002;2:612–30.
127. Global Health Observatory 2010. Women and Health. WHO 2010. Available from the URL: http://www.who.int/gho/women_and_health/en/.
128. Gluer CC (1997). Quantitative ultrasound techniques for the assessment of osteoporosis .Expert agreement on current status. J Bone Mineral Res, 12(8): 1280-88.
129. Golay A, Swislocki AL, Chen YD, Reaven GM. Relationships between plasma-free fatty acid concentration, endogenous glucose production, and fasting hyperglycemia in normal and non-insulin-dependent diabetic individuals. Metabolism (1987) 36:692–696.
130. Goodman & & Gilman's, The Pharmacological Basis Of Therapeutics, 11th Edition, Pg No. 933-965.
131. Gosden RG. Follicular status at menopause. Hum Reprod 1987; 2:617.
132. Govil D. Health needs of middle aged population: an unaddressed link. Presented in Poster Session 2 of European Population Conference 1-4th September, 2010, Vienna, Austria. Available from the URL: http://epc2010.princeton.edu/abstracts/100861.
133. Gowenlock A, 1988. Varley's Practical Clinical Biochemistry, 6th edition, 664.
134. Greenblum C, Rowe M, Neff D et al 2013. Midlife women: symptoms associated with menopausal transition and early postmenopause and quality of life. Menopause. 2013 Jan; 20(1):22-7. doi: 10.1097/gme.0b013e31825a2a91.

135. Groome NP, Illingworth PJ, O'Brien M, Cooke I, Ganesan TS, Baird DT, McNeilly AS. Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. Clin Endocrinol 1994; 40:717723.
136. Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS. Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 1996; 81:14011405.
137. Groop LC, Bonadonna RC, Shank M, Petrides AS, DeFronzo RA. Role of free fatty acids and insulin in determining free fatty acid and lipid oxidation in man. J Clin Invest (1991) 87: 83–89.
138. Guay M, Dragmomir A, Pilon D et al. Changes in pattern of use, clinical characteristics and persistence rate of hormone replacement therapy among postmenopausal women after the WHI publication. Pharmacoepidem. Drug Safe. 2007, 16: 17–27. doi: 10.1002/pds.1273.
139. Gupta PC, Gupta R, Pednekar MS. Hypertension prevalence and blood pressure trends in 88 653 subjects in Mumbai, India. Journal of Human Hypertension (2004) 18, 907–910. doi:10.1038/sj.jhh.1001763 Published online 12 August 2004
140. Gupta R, Deedwania P, Achari V et al. Normotension, Prehypertension, and Hypertension in Urban Middle-Class Subjects in India: Prevalence, Awareness, Treatment, and Control. American Journal of Hypertension, Volume 26, Issue 1, pp. 83-94.
141. Gupta R, Deedwania P, Sharma K et al. Association of educational, occupational and socioeconomic status with cardiovascular risk factors in Asian Indians: a cross-sectional study. PLoS One. 2012; 7(8):e44098. doi: 10.1371/journal.pone.0044098.
142. Gupta R, Gupta R, Agrawal A, Misra A, Guptha S, Pandey R et al. Migrating husbands and changing cardiovascular risk factors in the wife: a cross sectional study in Asian Indian women. J Epidemiol Community Health. 2012 Oct;66(10):881-9. doi: 10.1136/jech-2011-200101.

143. Gupta R, Sharma K, Gupta A et al. Persistent High Prevalence of Cardiovascular Risk Factors in the Urban Middle Class in India: Jaipur Heart Watch-5. JAPI March 2012, Vol 60.
144. Gupta, Anil K. (2004). Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. Current Science, **87** (1), Indian Academy of Sciences.
145. Hans D, Hartl F and Krieg MA 2003. Device-specific weighted T-score for two quantitative ultrasounds: operational propositions for the management of osteoporosis for 65 years and older women in Switzerland. Osteoporos Int (2003) 14: 251–258 DOI 10.1007/s00198-002-1358-z
146. Hart E, Charkoudian N, Miller V. Sex, hormones and neuroeffector mechanisms. Acta Physiol (Oxf). 2011 September; 203(1): 155–165. doi:10.1111/j.1748-1716.2010.02192.x.
147. Harvard Women's Health Watch. Perimenopause, hormones and midlife health. November 2006.
148. He L, Tang X, Li N et al. Menopause with cardiovascular disease and its risk factors among rural Chinese women in Beijing: A population-based study. Maturitas 72 (2012) pg: 132–138.
149. Hee J, MacNaughton J, Bangah M, Burger HG. Perimenopausal patterns of gonadotrophins, immunoreactive inhibin, oestradiol and progesterone. Maturitas 1993; 18:920.
150. Heikkinen T, Puolivali J, Liu l et al. Effects of Ovariectomy and Estrogen Treatment on Learning and Hippocampal Neurotransmitters in Mice. Volume 41, Issue 1, February 2002, Pages 22–32.
151. Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance, and type II diabetes mellitus. Int J Obes Relat Metab Disord (2004) 28 (Suppl 4):S12–S21.
152. Herd JA, Ballantyne CM, Farmer JA, Ferguson JJ 3d, Jones PH, West MS, et al. Effects of fluvastatin on coronary atherosclerosis in patients with mild to

moderate cholesterol elevations (Lipoprotein and Coronary Atherosclerosis Study [LCAS]). Am J Cardiol. 1997; 80:278–86.

153. Hernandez M, Perez N, Zarate A et al. Hypothyroidism associated to menopause symptoms worsening change with thyroid substitution therapy. Ginecol Obstet Mex. 2008 Oct; 76(10):571-5.
154. Hill K. The demography of menopause. Maturitas. 1996 Mar; 23(2):113-27.
155. History of Medicine 2013. In Encyclopedia Britannica. Retrieved fromhttp://www.britannica.com/EBchecked/topic/372460/history-of-medicine.
156. Hodson M, Sangster A. Observations on the Distribution of Mineral Elements in the Leaf of Wheat (*Triticum aestivum* L.), with Particular Reference to Silicon. Annals of Botany 1988, Volume 62, Issue 5 Pp. 463-471.
157. Holte A. Influences of natural menopause on health complaints: a prospective study of healthy Norwegian women. Maturitas 1992; 14:127141.
158. Horowitz MC. Cytokines and oestrogen in bone: anti-osteoporotic effects. Science, 1993, 260:626–627.
159. Huang A, Moore E, Boyko E et al. Vaginal symptoms in postmenopausal women: self-reported severity, natural history, and risk factors. Menopause. 2010 Jan-Feb; 17 (1): 121-6. doi: 10.1097/gme.0b013e3181acb9ed.
160. Hunninghake D, Bakker-Arkema RG, Wigand JP, Drehobl M, Schrott H, Early JL, et al. Treating to meet NCEP-recommended LDL cholesterol concentrations with atorvastatin, fluvastatin, lovastatin, or simvastatin in patients with risk factors for coronary heart disease. J Fam Pract. 1998; 47:349–56.
161. Hunter GR, Kekes-Szabo T, Treuth MS, Williams MJ, Goran M, Pichon C. Intra-abdominal adipose tissue, physical activity and cardiovascular risk in pre- and post-menopausal women. Int J Obes 1996; 20:860-5.
162. Inkster SE, Brodie AMH. Expression of aromatase cytohrome P-450 inpremenopausal and postmenopausal ovaries: an immunocytochemical study. J Clin Endocrinol Metab 1991; 73:717.
163. International Institute for Population Sciences (IIPS) and Macro International. 2007. National Family Health Survey (NFHS-3), 2005–06: India: Volume II. Mumbai: IIPS.

164. Ismael NN. A study on the menopause in Malaysia. Maturitas. 1994 Oct; 19(3):205-9.
165. Iyer U, Dhruv S, Elayath N, Khalsa A. Development, Acceptability, Physico-Chemical Properties and Tolerance Studies on an Antioxidant Rich Health Drink Enriched with Wheatgrass(*Triticum aestivum*) and Indian Gooseberry (*Emblica officinalis*). Inventi Impact: Nutraceuticals Vol. 2011, Issue 3, pg 120-123.
166. Iyer U, Joshi A, Dhruv S. Impact of Amla (*Embilica officinalis*) supplementation on the glycemic and lipidemic status of type 2 diabetic subjects. J Herbal Med Toxicol 2009; 3:15-21.
167. Iyer U, Mani UV. Studies on the effect of curry leaves supplementation (Murraya Koenigi) on lipid profile, glycated proteins and amino acids in non-insulin-dependent diabetic patients. Plant Foods Hum Nutr. 1990 Oct; 40(4):275-82.
168. Iyer U, Sharma M, Dhruv S, Mani UV. Glycemic and lipemic response of wheatgrass incorporated recipes. J Herb Med Toxicol 2010, vol 4, Issue 1, pg 26.
169. Iyer UM, Desai PA, Venugopal S. Impact of panchratna juice in the management of diabetes mellitus: Fresh vs. processed product. Int J Green Pharm 2010; 4:122-8.
170. James G, Sievert L, Flanagan E. Ambulatory blood pressure and heart rate in relation to hot flash experience among women of menopausal age. Ann Hum Biol 2004; 31(1): 49-58.
171. Jiang C, Poole-Wilson PA, Sarrell PM, et al. Effect of 17-b-oestradiol on contraction, calcium current and intracellular free calcium in guinea-pig isolated cardiac myocytes. Br J Pharmacol 1992; 106:739745.
172. Jilka RL. Cytokines, bone remodeling, and oestrogen deficiency: a 1998 update. Bone, 1998, 23:75–81.
173. Jin S, Hong J, Jung S et al. Turmeric and laurel aqueous extracts exhibit in vitro anti-atherosclerotic activity and in vivo hypolipidemic effects in a zebrafish model. J Med Food. 2011 Mar;14(3):247-56. doi: 10.1089/jmf.2009.1389.
174. Jonusiene G, Zilaitiene B, Adomaitiene V et al. Sexual function, mood and menopause symptoms in Lithuanian postmenopausal women. Climacteric. 2013 Feb; 16(1):185-93. doi: 10.3109/13697137.2012.682746.

175. Judd HL, Shamonki IM, Frumar AM, Lagasse LD. Origin of serum estradiol in postmenopausal women. Obstet Gynecol 1982; 59:680.
176. Jyothi R, Nayak B. A case-control study of dietary habits, physical activity and risk for abdominal obesity among working women. Calicut Medical Journal 2010; 8(2):e2.
177. Kadam N, Chiplonkar S, Khadilkar A, Divate U, Khadilkar V. Low bone mass in urban Indian women above 40 years of age: prevalence and risk factors. Gynecol Endocrinol. 2010 Dec;26(12):909-17. doi: 10.3109/09513590.2010.487604. Epub 2010 Sep 17.
178. Karli N, Baykan B, Ertas M et al. Impact of sex hormonal changes on tension-type headache and migraine: a cross-sectional population-based survey in 2,600 women. J Headache Pain. 2012 Oct;13(7):557-65. doi: 10.1007/s10194-012-0475-0.
179. Karpanou EA, Vyssoulis GP, Papakyriakou SA, et al. Effects of menopause on aortic root function in hypertensive women. J Am Coll Cardiol 1996; 28:15621566.
180. Katsuya T, Morishita R. Gene polymorphism of angiotensin II type 1 and type 2 receptors. Curr Pharm Des. 2012 Nov 21. [Epub ahead of print].
181. Kelly T, Yang W, Chen C et al. Global burden of obesity in 2005 and projections to 2030. International Journal of Obesity (2008) 32, 1431–1437; doi:10.1038/ijo.2008.102.
182. Keramat A, Patwardhan B, Larijani B et al. The assessment of osteoporosis risk factors in Iranian women compared with Indian women. BMC Musculoskelet Disord. 2008 Feb 27;9:28. doi: 10.1186/1471-2474-9-28.
183. Kharitonov SA. Longan-Sinclair RB, Busset CM, et al. Peak expiratory nitric oxide differences in men and women: relation to the menstrual cycle. Br Heart J 1994; 72:243245.
184. Khokhar K, Kaur G, Sidhu S. Prevalence of Obesity in Working Premenopausal and Postmenopausal Women of Jalandhar District, Punjab. J Hum Ecol, 29(1): 57-62 (2010).

185. Khoo CM, Sairazi S, Taslim S, Gardner D, Wu Y, Lee J et al. Ethnicity modifies the relationships of insulin resistance, inflammation, and adiponectin with obesity in a multiethnic Asian population. Diabetes Care. 2011 May; 34(5):1120-6. doi: 10.2337/dc10-2097.

186. Khouri N. Evidence that curcuma longa possesses an active hypolipidemic effects in rabbits. Saudi Med J. 2006 Feb;27(2):264-6.

187. Kireev R, Tresguerres A, Garcia C et al. Hormonal regulation of pro-inflammatory and lipid peroxidation processes in liver of old ovariectomized female rats. Biogerontology, vol. 11, no. 2, pp. 229–243, 2010.

188. Klapholz M, Buttrick P. Myocardial Function and Cardiomyopathy. In: Douglas PS, ed. Heart Disease in Women. FA Davis, Philadelphia, 1989, pp. 105115.

189. Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremner WJ, Soules MR. Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. J Clin Endocrinol Metab 1996; 81:10381045.

190. Klein NA, Battaglia DE, Miller PB, Branigan EF, Guidice LC, Soules MR. Ovarian follicular development and the follicular fluid hormones and growth factors in normal women of advanced reproductive age. J Clin Endocrinol Metab 1996; 81:19461951.

191. Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE, Soules MR. Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. J Clin Endocrinol Metab 1996; 81:27422745.

192. Koh KK, Mincemoyer R, Bui M, et al. Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. N Engl J Med 1997; 336:683690.

193. Kong H, Li X, Zhang S, Guo S, Niu W. The β1-drenoreceptor gene Arg389Gly and Ser49Gly polymorphisms andhypertension: a meta-analysis. Mol Biol Rep. 2012 Dec 28.

194. Kothari S, Jain A, Mehta S et al. Effect of fresh *Triticum aestivum* grass juice on lipid profile of normal rats. Indian J Pharmacol. 2008 October; 40(5): 235–236. doi: 10.4103/0253-7613.44157.
195. Koutsari C, Jensen MD. Free fatty acid metabolism in human obesity. J Lipid Res (2006) 47:1643–1650.
196. Kronenberg F. Hot flashes: epidemiology and physiology. Ann NY Acad Sci 1990; 592586.
197. Kronenberg F. Menopausal Hot Flashes: A Review of Physiology and Biosociocultural Perspective on Methods of Assessment. J Nutr. 2010 Jul;140 (7):1380S-5S. doi: 10.3945/jn.109.120840.
198. Kulkarni B, Shatrugna V, Nagalla B et al. Regional body composition of Indian women from a low-income group and its association with anthropometric indices and reproductive events. Ann Nutr Metab. 2010; 56 (3):182-9. doi: 10.1159/000276597.
199. Kulkarni S, Tilak H, Acharya R et al. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. Phytotherapy Research, 20: 218–227. doi: 10.1002/ptr.1838.
200. Kumar A, Mittal S, Orito S, Ishitani K, Ohta H. Impact of dietary intake, education, and physical activity on bone mineral density among North Indian women. J Bone Miner Metab. 2010 Mar; 28(2):192-201. doi: 10.1007/s00774-009-0118-y.
201. Kumar SN, Mani UV, Mani I. An open label study on the supplementation of Gymnema sylvestre in type 2 diabetics. J Diet Suppl. 2010 Sep; 7(3):273-82. doi: 10.3109/19390211.2010.505901.
202. Kurowska EM, Spence JD, Jordan J, et al. (2000) HDLcholesterol-raising effect of orange juice in subjects with hypercholesterolemia. Am J Clin Nutr 2000, 72; pg 1095–1100.
203. Kwon S, Ahn I, Kim S et al. Anti-obesity and hypolipidemic effects of black soybean anthocyanins. J Med Food. 2007 Sep; 10(3):552-6.
204. Lambrinoudaki I, Kaparos G, Rizos D et al. Apolipoprotein E and paraoxonase 1 polymorphisms are associated with lower serum thyroid hormones in

postmenopausal women. Clin Endocrinol (Oxf). 2009 Aug; 71(2):284-90. doi: 10.1111/j.1365-2265.2008.03476.x.

205. Last AR, Ference JD, Falleroni J. Pharmacologic treatment of hyperlipidemia. Am Fam Physician. 2011 Sep 1; 84(5):551-8.
206. Lee SJ, Lenton EA, Sexton L, Cooke ID. The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. Hum Reprod 1988; 3:851855.
207. Legendre G, Fritel X, Ringa V et al. Urinary incontinence and menopause. Prog Urol. 2012 Oct;22(11):615-21. doi: 10.1016/j.purol.2012.08.267.
208. Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Alméras N, Bergeron J, Gaudet D, Tremblay G, Prud'homme D, Nadeau A, Després JP: Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? Circulation 2000, 102:179-184.
209. Length, R. V. (2006-9). Java Applets for Power and Sample Size [Computer software]. Retrieved February 22nd, 2011, from http://www.stat.uiowa.edu/~rlenth/Power.
210. Lenton EA, Sexton L, Lee S, Cooke ID. Progressive changes in LH and FSH and LH: FSH ratio in women throughout reproductive life. Maturitas 1988; 10:3543.
211. Lenton EA, Sexton L, Lee S, Cooke ID. Progressive changes in LH and FSH and LH: FSH ratio in women throughout reproductive life. Maturitas 1988; 10:3543.
212. Lerman A, Edwards BF, and Hallett JW, et al. Circulating and tissue endothelin immuno-1. reactivity in advanced atherosclerosis. N Engl J Med 1991;325:9971001.
213. Levy JC, Matthews DR, Hermans MP. Correct Homeostasis Model Assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998; 21: 2191-92.
214. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev (2002) 23:201–229.

215. Ley CJ, Lees B, Stevenson JC. Sex- and menopause-associated changes in body-fat distribution. Am J Clin Nutr 1992;55:950-4.
216. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med 1994; 121:936941.
217. Liu H, Liu K, Bodenneer D. Estrogen receptor inhibits interleukin-6 gene expression by disruption of nuclear factor kappa B transactivation. Cytokine 2005; 31:251–257.
218. Liu M, Wang Y, Li X ey al 2013. A health survey of Beijing middle-aged registered nurses during menopause. Maturitas. 2013 Jan;74(1):84-8. doi: 10.1016/j.maturitas.2012.10.006.
219. Liu Z, Shen H, Huang J et al. Butyl 4-(butyryloxy)benzoate functions as a new selective estrogen receptor β agonist and induces GLUT4 expression in CHO-K1 cells. The Journal of Steroid Biochemistry and Molecular Biology. Volume 110, Issues 1–2, May 2008, Pages 150–15.
220. London GM, Guerin AP, Pannier B, et al. Influence of sex on arterial hemodynamics and blood pressure. Role of body height. Hypertension 1995; 26:514519.
221. Longcope C, Franz C, Morello C, et al. Steroid and gonadotropin levels in women during the perimenopausal years. Maturitas 1986; 8:189.
222. Lovejoy JC, Champagne CM, DeJorge L, Xie H, Smith RS. Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes 2008;32:949-58.
223. Lucas E, Mahajan S, Soung Y. Flaxseed but not flaxseed oil prevented the rise in serum cholesteroldue to ovariectomy in the Golden Syrian hamsters. J Med Food. 2011 Mar; 14(3):261-7. doi: 10.1089/jmf.2009.0192.
224. Lucchesi L, Hachul H, Yagihara F et al. Does menopause influence nocturnal awakening with headache? Climacteric. 2012 Nov 1. [Epub ahead of print].
225. Lucchesi L, Hachul H, Yagihara F et al. Does menopause influence nocturnal awakening with headache? Climacteric. 2012 Nov 1.

226. Luoto R, Sharrett AR, Schreiner P, Sorlie PD, Arnett D, Ephross S. Blood pressure and menopausal transition: the Atherosclerosis Risk in Communities study (1987–95). J Hypertens. 2000;18:27–33.
227. Maatta KM, Nikkari ST, Lahteela KH et al. A functional variant in the serine-threonine kinase coding gene is associated with hypertension: a case-control study in a Finnish population, the Tampere adult population cardiovascular risk study. J Hypertens. 2012 Dec 11. [Epub ahead of print].
228. Mani U, Mani I, Biswas M et al. An open-label study on the effect of flax seed powder (Linum usitatissimum) supplementation in the management of diabetes mellitus. J Diet Suppl. 2011 Sep;8(3):257-65. doi: 10.3109/19390211.2011.593615.
229. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling: emerging insights into the pathophysiology of osteoporosis. New England Journal of Medicine, 1995, 332:305–311.
230. Manolio TA, Furburg CD, Shemanski L, et al. Associations of postmenopausal estrogen use with cardiovascular disease and its risk factors in older women. Circulation 1993; 88:11131117.
231. Maron D, Lu G, Cai N et al. Cholesterol lowering effect of a theaflavin enriched green tea extract. A randomized controlled trial. Arch Intern Med. 2003; 163: 1448-1453.
232. Martin TJ, Udagawa N. Hormonal regulation of osteoclast function. Trends in Endocrinology & Metabolism, 1998, 9:6–12.
233. Marwaha R, Tandon N, Ganie M et al. Status of thyroid function in Indian adults: two decades after universal salt iodization. J Assoc Physicians India. 2012 Apr;60:32-6.
234. Marwaha RK, Bansal D, Kaur S, Trehan A: Wheat grass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. Indian Pediatrics 2004, l 41: 716-720.
235. Matsuzawa Y: The metabolic syndrome and adipocytokines. FEBS Lett 2006, 580:2917-2921.

236. Matthews KA, Wing RR, Kuller LH, et al. Influence of the perimenopause on cardiovascular risk factors and symptoms of middle-aged healthy women. Arch Intern Med 1994; 154:23492355.
237. Maulik S, Talwar K. Therapeutic potential of Terminalia arjuna in cardiovascular disorders. Am J Cardiovasc Drugs. 2012 Jun 1; 12(3):157-63. doi: 10.2165/11598990-000000000-00000.
238. MayerEL, JacobsenDW, Robinson K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol 1996;27:517527.
239. McAuley KA, Williams SM, Mann JI, Walker RJ, Ledwis-Barned NJ, Temple LA, Duncan AS: Diagnosing insulin resistance in the general population. Diabetes Care 24:460–464, 2001.
240. McCrohon JA, Adams MR, McCredie RJ, et al. Hormone replacement therapy is associated with improved arterial physiology in healthy post-menopausal women. Clin Endocrinol 1996; 45:435441.
241. McEwen B. Genome and Hormones: Gender Differences in Physiology Invited Review: Estrogens effects on the brain: multiple sites and molecular mechanisms. J Appl Physiol, 91: 2785–2801, 2001.
242. McEwen. The Molecular and Neuroanatomical Basis for Estrogen Effects in the Central Nervous System. The Journal of Clinical Endocrinology & Metabolism 1999, Vol. 84, No. 6; pg: 1790-1797.
243. Meherishi, Khandelwal, Swarankar, Kaur. Attitudes and practices of gynecologists in Jaipur toward management of menopause. J Midlife Health. 2010 Jul-Dec; 1(2): 74–78.
244. Mehta P and Patel N (Unpublished M.Sc. Dissertation). Effect of Soy Foods on Health & Nutritional Status of Institutionalized Elderly (2007). Department of Foods and Nutrition, Faculty of Home Science, M S University of Baroda, Vadodara, Gujarat.
245. Meldrum DR, Davidson BJ, Tataryn IV, Judd HL. Changes in circulating steroids with aging in postmenopausal women. Obstet Gynecol 1981; 57:624.
246. Mente A, Razak F, Blankenberg S, Vuksan V, Davis A, Miller R et al. Ethnic variation in adiponectin and leptin levels and their association with adiposity and

insulin resistance. Diabetes Care. 2010 Jul;33(7):1629-34. doi: 10.2337/dc09-1392. Epub 2010 Apr 22.

247. Mercuro G, Deidda M, Piras A, Dessalvi CC, Maffei S, Rosano GM. Gender determinants of cardiovascular risk factors and diseases. J Cardiovasc Med (Hagerstown). 2010 Mar; 11(3):207-20. doi: 10.2459/JCM.0b013e32833178ed.
248. Meshram I, Arlappa N, Balkrishna N et al. Prevalence of hypertension, its correlates and awareness among adult tribal population of Kerala state, India. J Postgrad Med. 2012 Oct;58(4):255-61. doi: 10.4103/0022-3859.105444.
249. Metcalf MG, Donald RA, Livesey JH. Pituitary-ovarian function before, during and after menopause: a longitudinal study. Clin Endocrinol 1982; 17:489.
250. Metzger B, Barnes D, Reed J. A comparison of pectin, polyphenols, and phytosterols, alone or in combination, to lovastatin for reduction of serum lipids in familial hypercholesterolemic swine. J Med Food. 2009 Aug; 12(4):854-60. doi: 10.1089/jmf.2008.0140.
251. Meulenbeld, G. A History of Indian Medical Literature (Groningen, 1999--2002), vol. IA, pp. 7-180.
252. Michalek A, Mahoney M, Calebaugh D. Hypothyroidism and diabetes mellitus in an American Indian population. J Fam Pract. 2000 Jul; 49(7):638-40.
253. Midha T, Nath B, Kumari R et al. Prevalence and determinants of obesity in the adult population of Kanpur district -- a population-based study. J Indian Med Assoc. 2011 Aug;109(8):538-42.
254. Mikola L. Acid Carboxypeptidases in Grains and Leaves of Wheat, *Triticum aestivum* L. Plant Physiology July 1986 vol. 81 no. 3 823-829.
255. Misso M, Murata Y, Boon W et al. Simpson ER. Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. Endocrinology 2003; 144:1474–1480.
256. Moller M, Radestad A, von Schoultz B et al 2013. Effect of estrogen and testosterone replacement therapy on cognitivefatigue. Gynecol Endocrinol. 2013 Feb;29(2):173-6. doi: 10.3109/09513590.2012.730568.

257. Mukhopadhyay S, Basak J, Kar M, Mandal S, Mukhopadhyay A:The Role Of Iron Chelation Activity Of Wheat Grass Juice In Patients With Myelodysplastic Syndrome. J. Clin. Oncology 2009, 7012-7014.
258. Murugesan D, Arunachalam T, Ramamurthy V, Subramanian S. Association of polymorphisms in leptin receptor gene with obesity and type 2 diabetes in the local population of Coimbatore. Indian J Hum Genet. 2010 May-Aug; 16(2): 72–77. doi: 10.4103/0971-6866.69350
259. Mushayandebvu T, Adel TE, Gimpel T, et al. Evidence for diminished midcycle androgen production in older reproductive aged women. Fertil Steril 1996;65:721.
260. Nair and Chauhan. Prevalence of Menopausal hypothyroidism and assessment of the impact of multiple food based approaches to combat the situation among women of urban Vadodara 2006. Masters Degree thesis, Department of Foods and Nutrition, Faculty of Family and Community Sciences, The M.S. University of Baroda, Vadodara.
261. Nambiar V, Guin P, Parnami S, Daniel M. Impact Of Antioxidants From Drumstick Leaves On The Lipid Profile Of Hyperlipidemics. Journal of Herbal Medicine and Toxicology 4 (1) 165-172 (2010).
262. Nazir A, Papita R, Anbalagan VP, Anjaan RM, Deepa M, Mohan V. Prevalence of diabetes in Asian Indians based on glycated hemoglobin and fasting and 2-H post-load (75-g) plasma glucose (CURES-120). Diabetes Technol Ther. 2012 Aug; 14(8):665-8. doi: 10.1089/dia.2012.0059. Epub 2012 Jul 23.
263. Nilsson S, Makela S, Treuter E et al. Mechanisms of estrogen action. Physiological Reviews 2001; 81:1535–1565.
264. Oh K, Jung K, Choi J et al. Headaches in middle-aged women during menopausal transition: aheadache clinic-based study. Eur Neurol. 2012; 68(2):79-83. doi: 10.1159/000336838.
265. Olaolorum F, Lawoyin T. Experience of menopausal symptoms by women in an urban community in Ibadan, Nigeria. Menopause. 2009 Jul-Aug;16(4):822-30. doi: 10.1097/gme.0b013e318198d6e7.

266. Oliveira-Paula GH, Lacchini R, Coeli-Lacchini FB, Junior HM, Tanus-Santos JE. Inducible nitric oxide synthase haplotype associated with hypertension and responsiveness to antihypertensive drug therapy. Gene. 2012 Dec 22. pii: S0378-1119(12)01587-9. doi: 10.1016/j.gene.2012.12.059. [Epub ahead of print].

267. Pan A, Yu D, Denmark-Wahnefried W et al. Meta-analysis of the effects of flaxseed interventions on blood lipids. Am J Clin Nutr. 2009 August; 90(2): 288–297. doi: 10.3945/ajcn.2009.27469.

268. Pandey R, Gupta R, Misra A, Misra P, Singh V, Agrawal A et al. Determinants of urban-rural differences in cardiovascular risk factors in middle-aged women in India: A cross-sectional study. Int J Cardiol. 2011 Aug 29.

269. Pandey S, Srinivas M, Agashe S, Joshi J, Galvankar P, Prakasam C et al. Menopause and metabolic syndrome: A study of 498 urban women from western India. J Midlife Health. 2010 Jul-Dec; 1(2): 63–69. doi: 10.4103/0976-7800.76214.

270. Panotopoulos G, Ruiz J, Raison J et al . Menopause, fat and lean distribution in obese women. Maturitas 25 (1996) pg:II- 19.

271. Pant D, Dave M, Tiwari A. Wheatgrass (*Triticum aestivum L.*) Supplementation Promotes Longevity in Drosophila melanogaster. Annals of plant sciences, 2013, 02 (1), 49-54.

272. Panza JA, Quyyumi AA, Callahan TS, et al. Effect of antihypertensive treatment on endothelium-dependent vascular relaxation in patients with essential hypertension. J Am Coll Cardiol 1993; 21:11451151.

273. Pastore l, Carter R, HUlka B et al. Self-reported urogenital symptoms in postmenopausal women: Women's Health Initiative. Maturitas. 2004 Dec 10;49(4):292-303.

274. Paul T, Thomas N, Seshadri M, Oommen R, Jose A, Mahendri N. Prevalence of osteoporosis in ambulatory postmenopausal women from a semiurban region in Southern India: relationship to calcium nutrition and vitamin D status. Endocr Pract. 2008 Sep;14(6):665-71.

275. Pearce E. Thyroid dysfunction in perimenopausal and postmenopausal women. Menopause Int. 2007 Mar; 13(1):8-13.

276. Pellicer A, Simon C, Remohi J. Effects of aging on the female reproductive system. Hum Reprod 1995;10:7783.
277. Petersen K, Dufour S, Feng J et al. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. Proceedings of the National Academy of Sciences of the United States of America 2006, vol. 103 no. 48, pg: 18273-18277.
278. Petersen K, Dufour S, Feng J, Befroy D, Dziura J, Dalla Man C et al. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. Proceedings of the National Academy of Sciences 2006 vol 103 (48): 18273-18277.
279. Petkeviciene J, Smallinskiene A, Luksiene D, Jureniene K, Ramazauskiene V, Klumbiene J et al. Associations between Apolipoprotein E Genotype, Diet, Body Mass Index, and Serum Lipids in Lithuanian Adult Population. PLoS One. 2012; 7(7): e41525. doi: 10.1371/journal.pone.0041525.
280. Pines A, Fisman EZ, Drory Y, et al. Menopause-induced changes in doppler-derived parameters of aortic flow in healthy women. Am J Cardiol 1992; 69:11041106.
281. Pines A, Fisman EZ, Levo Y, et al. The effects of hormone replacement therapy in normal postmenopausal women: Measurements of doppler-derived parameters of aortic flow. Am J Obstet Gynecol 1991;164:806812.
282. Pinto S, Virdis A, Ghiadoni L, Bernini G, et al. Endogenous estrogen and acetylcholine- induced vasodilation in normotensive women. Hypertension 1997; 29(2):268273.
283. Pittler M, Thompson C, Ernst E. Artichoke leaf extract for treating hypercholesterolaemia. Cochrane Database Syst Rev. 2002;(3):CD003335.
284. Plehn JF, Davis BR, Sacks FM, Rouleau JL, Pfeffer MA, Bernstein V, et al. Reduction of stroke incidence after myocardial infarction with pravastatin: the Cholesterol and Recurrent Events (CARE) study. The CARE Investigators. Circulation. 1999; 99:216–23.
285. Polderman KR, Stenhouwer DA, van Kamp GJ, et al. Influence of sex hormones on plasma endothelin levels. Ann Intern Med 1993;118:429432.

286. Prasad D, Kabir Z, Das B. Prevalence and risk factors for metabolic syndrome in Asian Indians: A community study from urban Eastern India. J Cardiovasc Dis Res v.3 (3); Jul-Sep 2012.
287. Prasad D, Kabir Z, Dash A et al. Prevalence and risk factors for metabolic syndrome in Asian Indians: A community study from urban Eastern India. J Cardiovasc Dis Res. 2012 Jul-Sep; 3(3): 204–211. doi: 10.4103/0975-3583.98895.
288. Prasad D, Kabir Z, Dash A et al. Prevalence and risk factors for diabetes and impaired glucose tolerance in Asian Indians: a community survey from urban Eastern India. Diabetes Metab Syndr. 2012 Apr-Jun;6(2):96-101. doi: 10.1016/j.dsx.2012.05.016.
289. Prasad DS, Kabir Z, Dash AK, Das BC. Prevalence and risk factors for diabetes and impaired glucose tolerance in Asian Indians: a community survey from urban Eastern India. Diabetes Metab Syndr. 2012 Apr-Jun;6(2):96-101. doi: 10.1016/j.dsx.2012.05.016. Epub 2012 Jun 19.
290. Procope BJ. Studies on urinary excretion, biological effects and origin of estrogens in postmenopausal women. Acta Endocrinol 1968;95(suppl):135.
291. Promensil USA 2009. Available from the URL: http://www.promensilusa.com/Content/StageSymptoms.aspx.
292. Proudher AJ, Ahmed AIH, Crook D, et al. Hormone replacement therapy and serum angiotensin-converting-enzyme activity in postmenopausal women. Lancet 1995; 346:899.
293. Radha V, Vimaleswaran KS, Ayyappa KA, Mohan**.** Association of lipoprotein lipase gene polymorphisms with obesity and type 2 diabetes in an Asian Indian population. Int J Obes (Lond). 2007 Jun;31(6):913-8. Epub 2007 Feb 13.
294. Rahman S, Rubiah S, Mun V. Assessment of menopausal symptoms using modified Menopause Rating Scale (MRS) among middle age women in Kuching, Sarawak, Malaysia. Asia Pacific Family Medicine 2010, 9:5.
295. Rahman S, Salehin F, Iqbal A. Menopausal symptoms assessment among middle age women in Kushtia, Bangladesh. BMC Research Notes 2011, 4:188.

296. Rai V, Iyer U, Mani UV. Effect of Tulasi (Ocimum sanctum) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. Plant Foods Hum Nutr. 1997; 50(1):9-16.
297. Rampal S, Mahadeva S, Guallar E, Bulqiba A, Mohamed R, Rahmat R. Ethnic differences in the prevalence of metabolic syndrome: results from a multi-ethnic population-based survey in Malaysia. PLoS One. 2012;7(9):e46365. doi: 10.1371/journal.pone.0046365.
298. Ravikumar P, Bhansali A, Ravikiran M, Bhansali S, Walia R, Shanmugasundar G, Thakur JS, Kumar Bhadada S, Dutta P: Prevalence and Risk factors of diabetes in a community-based study in North India: the Chandigarh Urban Diabetes Study (CUDS). Diabetes Metab 2011, 37:216–221.
299. Ravikumar P, Bhansali A, Ravikiran M, Bhansali S, Walia R, Shanmugasundar G, Thakur JS, Kumar Bhadada S, Dutta P: Prevalence and risk factors of diabetes in a community-based study in North India: the Chandigarh Urban Diabetes Study (CUDS). Diabetes Metab 2011, 37:216–221.
300. Ray A, Biswas U, Mukherjee A et al. Assessment of iodine and non-iodine deficiency hypothyroidism in women of reproductive ages in the sub-Himalayan plains of West Bengal. Indian J Physiol Pharmacol. 2009 Oct-Dec; 53(4):359-64.
301. Reubinoff BE, Wurtman J, Rojansky N, Adler D, Stein P, Schenjer JG, et al. Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: a prospective study. Fertil Steril 1995;64:963-8.
302. Reyes FI, Winter JSD, Faiman C. Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol and progesterone levels. Am J Obstet Gynecol 1977; 129:557564.
303. Riccardi G, Giacco R, Rivellesse A. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr. 2004 Aug;23(4):447-56.
304. Riemens SC, Sluiter WJ, Dullaart RP. Enhanced escape of non-esterified fatty acids from tissue uptake: its role in impaired insulin-induced lowering of total rate

of appearance in obesity and type II diabetes mellitus. Diabetologia (2000) 43:416–426.

305. Rifici VA, Khachadurian AK. The inhibition of low-density lipoprotein oxidation by 17-b estradiol. Metabolism 1992;41(10):11101114.
306. Rosales E, Iannone M, Groppa M et al. Nitric oxide inhibits nitrate reductase activity in wheat leaves. Plant Physiol Biochem. 2011 Feb;49(2):124-30. doi: 10.1016/j.plaphy.2010.10.009.
307. Roselli M, Imthurn B, Keller PJ. Circulating nitric oxide levels in postmenopausal women substituted with 17-b estradiol and norethisterone acetate: A two-year follow-up study. Hypertension 1995; 25:848853.
308. Rossmanith W, Szilagyi A, Scherbaum W. Episodic thyrotropin (TSH) and prolactin (PRL) secretion during aging in postmenopausal women. Horm Metab Res 1992; 24:185.
309. Rush E, Plank L, Chandu V, Laulu M, Simmons D, Swinburn B, Yajnik C. Body size, body composition, and fat distribution: a comparison of young New Zealand men of European, Pacific Island, and Asian Indian ethnicities. N Z Med J. 2004 Dec 17; 117 (1207):U1203 15608799.
310. S. R. Davis , C. Castelo-Branco, P. Chedraui, M. A. Lumsden, R. E. Nappi, D. Shah and P. Villaseca as the Writing Group of the International Menopause Society for World Menopause Day 2012. Understanding weight gain at menopause. Climacteric 2012; 15:419–429. DOI: 10.3109/13697137.2012.707385 Accepted 25-06-2012.
311. Sack MN, Rader DJ, Cannon RO. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. Lancet 1994;343:269270.
312. Sadeghi M, Khalili M, Pourmoghaddas M, Talaei M. The correlation between blood pressure and hot flashes in menopausal women. ARYA Atherosclerosis Journal 2012, 8(1): 32-35.
313. Samaan SA, Crawford MH. Estrogen and cardiovascular function after menopause. J Am Coll Cardiol 1995; 26:14031410.

314. Samuels R, Mani UV, Iyer U, Nayak U. Hypocholesterolemic effect of spirulina in patients with hyperlipidemic nephrotic syndrome. J Med Food. 2002 Summer; 5(2):91-6.
315. Santen RJ, Leszcynski D, Tilson-Mallet N, et al. Enzymatic control of estrogen production in breast cancer: relative significance of aromatase versus sulfatase pathways. Ann NY Acad Sci 1986; 464:126.
316. Sarrel PM. Blood flow. In: Lobo RA, ed. Treatment of the Postmenopausal Woman. Raven, New York, 1994,pp.251262.
317. Sawant A, Mankeshwar R, Shah S, Raghavan R, Dhongde G, Raje H et al. Prevalence of Metabolic Syndrome in Urban India. Cholesterol, vol. 2011, Article ID 920983, 7 pages, 2011. doi:10.1155/2011/920983.
318. Sawin C, Castelli W, Hershman J. The aging thyroid: thyroid deficiency in the Framingham study. Arch Intern Med 1985;145:1386.
319. Seifer DB, Gardiner AC, Lambert-Messerlian G, Schneyer AL. Differential secretion of dimeric inhibin in cultured luteinized granulosa cells as a function of ovarian reserve. J Clin Endocrinol Metab 1996;81:736739.
320. Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Berk CA. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. Fertil Steril 1997;67:110114.
321. Self Nutrition Data 2012. Available from the URL: http://nutritiondata.self.com/facts/custom/900675/2.
322. Sharma D, Vatsa M, Lakshmy R et al. Study of cardiovascular risk factors among tertiary hospital employees and their families. Indian Heart J. 2012 Jul-Aug;64(4):356-63. doi: 10.1016/j.ihj.2012.06.001.
323. Sharma S, Tandon V, Mahajan A. Understanding menopausal symptoms in urban women. Vol. 9 No. 1, January-March 2007.
324. Shatrughna V, Kulkarni B, Kumar PA, Rani K, Balkrishna N. Bone status of Indian women from a low-income group and its relationship to the nutritional status. Osteoporos Int. 2005 Dec;16(12):1827-35.
325. Shaw LJ, Bairey Merz CN, Pepine CJ, Reis SE, Bittner V, Kelsey SF, Olson M, Johnson BD, Mankad S, Sharaf BL, Rogers WJ, Wessel TR, Arant CB, Pohost

GM, Lerman A, Quyyumi AA, Sopko G (2006). Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part I: gender differences in traditional and novel risk factors, symptom evaluation, and genderoptimized diagnostic strategies. *J Am Coll Cardiol*, 47(3 Suppl): S4-2.

326. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N* Engl J Med. 1995; 333:1301–07.
327. Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. J Clin Invest 1975; 55:699706.
328. Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab 1976; 42:629636.
329. Shideler SE, DeVane GW, Kaira PS, et al. Ovarian-pituitary hormone interactions during the perimenopause. Maturitas 1989;11:331.
330. Sia SK, Chiou HL, Chen SC, Tsai CF, Yang SF, Ueng KC. Distribution and phenotypic expression of mineralocorticoid receptor and CYP11B2 T-344C polymorphisms in a Taiwanese hypertensive population. Mol Biol Rep. 2012 Dec 29.
331. Silander K, Alanne M, Kristiansson K et al. Gender Differences in Genetic Risk Profiles for Cardiovascular Disease. PLoS ONE 3(10): e3615. doi:10.1371/journal.pone.0003615.
332. Simmons S. Growth, development and physiology. Chapter 3. In: EG Heyne, ed. Wheat and wheat improvement, Edition 2. ASA Inc, CSSA, Inc and SSS of America Inc, Madison Wisconsin, USA.
333. Singh RB, Pella D, Mechirova V, Kartikey K, Demeester F, Tomar R et al. Prevalence of obesity, physical inactivity and undernutrition, a triple burden of diseases during transition in a developing economy. The Five City Study Group. Acta Cardiol 2007;62:119-27.

334. Singh V, Sahu M, Yadav S et al. Incidence of obesity among the pre-menopausal and post-menopausal working women of Raipur district (Chhattisgarh State). World Journal of Science and Technology 2012, 2(6):83-86.

335. Sinha S, Misra P, Kant S, Krishnan A, Nongkynrih B, Vikram N. Prevalence of metabolic syndrome and its selected determinants among urban adult women in South Delhi, India. Postgrad Med J. 2012 Oct 30.

336. Smith SC Jr, Blair SN, Bonow RO, et al. AHA/ACC guidelines for preventing heart attack and death in patients with atherosclerotic cardiovascular disease: 2001 update: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. J Am Coll Cardiol. 2001; 38: 1581–1583.

337. Sniderman A, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. International Journal of Epidemiology 2007; 36:220–225. doi:10.1093/ije/dyl245.

338. Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, Woods N. Stages of Reproductive Aging Workshop (STRAW). Journal of Women's Health Gender-Based Medicine 2001, 10:843–848.

339. Srivastav K. Extracts from two frequently consumed spices--cumin (Cuminum cyminum) and turmeric (Curcuma longa)--inhibit platelet aggregation and alter eicosanoid biosynthesis in human blood platelets. Prostaglandins Leukot Essent Fatty Acids. 1989 Jul; 37(1):57-64.

340. Stearns V, Ulmer L, Lopez J et al. Hot flushes. The Lancet, vol 360 (9348); 7th December 2002, pg: 1851-1861.

341. Stevens CD (2010). Labeled Immunoassays (chapter 10), in Section II: Basic Immunological Procedures, in Clinical immunology and Serology: A Laboratory Perspective, 3rd Edition. FA Davis Company, Philadelphia, USA.

342. Stramba-Badiale M, Fox KM, Priori SG, Collins P, Daly C, Graham I, Jonsson B, Schenck-Gustafsson K, Tendera M (2006). Cardiovascular diseases in women: a

statement from the policy conference of the European Society of Cardiology. *Eur Heart J*, 27(8): 994- 1005.

343. Sultan T, Butt M, Ahmad R et al. Supplementation of Powdered Black Cumin (Nigella sativa) Seeds Reduces the Risk of Hypercholesterolemia. Functional Foods in Health and Disease 2011, 1(12):516-524.
344. Svendsen O, Hassager C and Christiansen C. Age- and Menopause-Associated Variations in Body Composition and Fat Distribution in Healthy Women as Measured by Dual-Energy X-Ray Absorptiometry. Metabolism, Vol 44, No 3 (March), 1995: pp 369-373.
345. Taddei S, Virdis A, Ghiadoni L, et al. Menopause is associated with endothelial dysfunction in women. Hypertension 1996; 28:576582.
346. Terauchi M, Hiramitsu S, Akiyoshi M et al. Associations among depression, anxiety and somatic symptoms in peri- and postmenopausal women. J Obstet Gynaecol Res. 2013 Feb 4. doi: 10.1111/j.1447-0756.2012.02064.x.
347. The World Health Organization Western Pacific Region, The International Association for the Study of Obesity, and The International Obesity Task Force. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney: Health Communications Australia Pty Limited, 2000.
348. Thomas Addison Unit Endocrinology Modules 2008. Available from the URL: www.addison.ac.uk.
349. Thompson PD, Buchner D, Pina IL, et al. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). Circulation. 2003; 107: 3109–3116.
350. Thurston RC, Sutton-Tyrrell K, Everson-Rose S, Hess R, Powell L, Matthews K. Hot flashes and carotid intima media thickness among midlife women. Menopause. 2011 Jan 14.
351. Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, Matthews KA. Hot flashes and subclinical cardiovascular disease: Findings from the Study of

Women's Health Across the Nation Heart Study. Circulation. 2008; 118:1234–1240.

352. Toda K, Takeda K, Akira S et al. Alternations in hepatic expression of fatty-acid metabolizing enzymes in ArKO mice and their reversal by the treatment with 17beta-estradiol or a peroxisome proliferator. J Steroid Biochem Mol Biol 2001; 79:11–17.
353. Tremollieres FA, Poulilles JM, Ribot CA. Relative influence of age and menopause on total and regional body composition changes in postmenopausal women. Am J Obstet Gynecol 1996; 175:1594-600.
354. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003 Jun 26; 348(26):2599-608.
355. Trinder P, 1969. Glucose estimation by glucose oxidase method using phenol and 4- aminophenazone. Ann Clin Biochem 1969;6:24-31.
356. Tulloch A, Hoffman L. Leaf wax of *Triticum aestivum.* Phytochemistry, Volume 12, Issue 9, September 1973, Pages 2217–2223.
357. Udipi S, Karandikar S, Mukherjee r, Agarwal S, Dhudhre P. Variations in fat and fatty acid intakes of adult males from three regions of India. Indian J Public Health. 2006 Jul-Sep;50(3):179-86.
358. Unger JB, Meeks GR. Hysterectomy after endometrial ablation. Am J Obstet Gynecol 1996; 175:1432.
359. United Nations, (1988). 'Global trends and prospects of ageing population structures'. In Economic and Social Implication of Population Ageing. United Nations Publications.
360. Unni J, Garg R, Pawar R. Bone mineral density in women above 40 years. Journal of Midlife Health 2010, vol 1, issue 1, pg 19-22.
361. van Beresteyn EC, van t Hof MA, De Waard H. Contributions of ovarian failure and aging to blood pressure in normotensive perimenopausal women: a mixed longitudinal study. Am J Epidemiol. 1989; 129:947–955.

362. Van der Voort D, Geusens P, Dinant G. R isk factors for osteoporosis related to their outcome: fractures. Osteoporos Int 2001 ; 12 : 630 – 8.
363. Venugopal S, Iyer U. Management of diabetic dyslipidemia with subatmospheric dehydrated barley grass powder. Int J Green Pharm 2010; 4:251-6.
364. Vinas J, Borras C. Women live longer than men: understanding molecular mechanisms offers opportunities to intervene by using estrogenic compounds. Antioxidants and Redox Signaling, vol. 13, no. 3, pp. 269–278, 2010.
365. Vitale C, Mendelsohn ME, Rosano GM. Gender differences in the cardiovascular effect of sex hormones. Nat Rev Cardiol. 2009 Aug; 6(8):532-42. doi: 10.1038/nrcardio.2009.105. Epub 2009 Jun 30.
366. Vlassof C. Gender Differences in Determinants and Consequences of Health and Illness. J Health Popul Nutr 2007 Mar; 25 (1):47-61.
367. Wahrenberg H, Hertel K, Leijonhufvud BM, Persson LG, Toft E, Arner P: Use of waist circumference to predict insulin resistance: retrospective study. BMJ 2005, 330:1363-1364.
368. Waidyasekera H, Wijewardena K, Lindmark G. Menopausal symptoms and quality of life during the menopausal transition in Sri Lankan women. Menopause. 2009 Jan-Feb; 16(1):164-70. doi: 10.1097/gme.0b013e31817a8abd.
369. Wannamethee S, Papacosta O, Whincup P, Carson C, Thomas M, Lawlor D et al. Assessing prediction of diabetes in older adults using different adiposity measures: a 7 year prospective study in 6,923 older men and women. Diabetologia (2010) 53:890–898. DOI 10.1007/s00125-010-1670-7.
370. Warren M, Shu A and DominguezJ, 2004. Chapter 11: Menopause and hormone replacement therapy – in Female Reproductive Endocrinology. Editor Robert W Rebar.
371. Wheat J, Currie J: Herbal medicine for cancer patients: An evidence based review. The Internet Journal of Alternative Medicine 2008, 5: 28-30
372. WHO 2007. Women's Health Fact Sheet. Available from the URL: http://www.who.int/mediacentre/factsheets/fs334/en/.
373. WHO 2011. Non communicable diseases Fact Sheet. Available from the URL: http://www.who.int/mediacentre/factsheets/fs355/en/index.html.

374. WHO 2012. Cardiovascular Diseases (CVDs) Fact Sheet. Available from the URL: http://www.who.int/mediacentre/factsheets/fs317/en/.
375. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363: 157–63.
376. WHO Reports, 2002. APA Press Release, Public Affairs Office, Pam Willenz, 336-5707.
377. WHO Technical Report Series. Research on the Menopause in the 1990's. Report of a WHO Scientific Group. 1994: Geneva, Switzerland.
378. Wider B, Pittler M, Thompson-Coon J et al. Artichoke leaf extract for treating hypercholesterolaemia. Cochrane Database Syst Rev. 2009 Oct 7;(4):CD003335.
379. Wild S, Gojka R, Green A et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047–1053, 2004.
380. Williams G. Why do some women have menopause symptoms and others do not. Available from the URL: http://www.examiner.com/article/why-do-some-women-have-menopause-symptoms-and-others-do-not. June 15, 2012.
381. Williams JK, Adams MR, Klopfenstein S. Estrogen modulates responses of atherosclerotic coronary arteries. Circulation 1990; 81:16801687.
382. Williams W, Kriegsfeld L. Circadian Control of Neuroendocrine Circuits Regulating Female Reproductive Function. Front Endocrinol (Lausanne). 2012; 3: 60. doi: 10.3389/fendo.2012.00060.
383. Wilson T, Singh A, Vorsa N et al. Human glycemic response and phenolic content of unsweetened cranberry juice.. J Med Food 11, 46–54.
384. Witzum JL. Drugs used in the treatment of hyperlipoproteinemias. In: Hardman JG, Limbird LE, et al., eds. Goodman and Gilman's The pharmacological basis of therapeutics. 9th ed. New York, N.Y.: McGraw-Hill, 1996:875–97.
385. Wolever T, Gibbs A, Brand-Miller J et al. Bioactive oat β-glucan reduces LDL cholesterol in Caucasians and non-Caucasians. Nutr J. 2011 Nov 25;10:130. doi: 10.1186/1475-2891-10-130.

386. Wood MJ, Cox JL. Hormone Replacement Therapy to prevent cardiovascular disease: what studies show, how to advise patients. Postgraduate Medicine. 2000; 108: 59-60, 63-66, 69-72.

387. World Health Organization 2007. Report of the WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level.

388. World Health Organization 2012. World Health Statistics report 2012. Available from the URL: http://www.who.int/gho/publications/world_health_statistics/2012/en/index.html.

389. World Health Organization. Available from the URL Reference: www.who.int/healthinfo/statistics/bod_irondeficiencyanaemia.pdf

390. Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA 1995;273(3): 199207.

391. Yasui T, Uemura H, Tomita J, et al. Association of interleukin-8 with hot flashes in premenopausal, perimenopausal, and postmenopausal women and bilateral oophorectomized women. J Clin Endocrinol Metab. 2006; 91:4805–4808.

392. Yeh Y, Lin R, Yeh S et al. Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. In: Food Factors for Cancer Prevention (Ohigashi, H., Osawa, T., Terao, J., Watanabe, S. & Toshikawa, T., eds.), 1997; pp. 226–230. Springer, Tokyo, Japan.

393. Yeh Y, Liu L. Cholesterol-Lowering Effect of Garlic Extracts and Organosulfur Compounds: Human and Animal Studies. J. Nutr. 131: 989S–993S, 2001.

394. Zaman FA, Pal R, Zaman GS, Swati IA, Kayyum A: Glucose indices, frank and undetected diabetes in relation to hypertension and anthropometry in a South Indian rural population. Indian J Public Health 2011, 55:34–37.

395. Zanchetti A, Facchetti R, Cesana G et al. Menopause-related blood pressure increase and its relationship to age and body mass index: the SIMONA epidemiological study. J Hypertens 2005; 23(12): 2269-76.

396. Zeiher AM, Drexler H, Wollschlager H, et al. Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991; 83:391401.

APPENDIX I

V.M.C. ZONAL AND WARD OUTLINE OF BARODA CITY

Administrative Zone	Wards Included	Areas Covered
North Zone	5	Urmi school till GSFC over bridge, SSG hospital, Corporation building
	7	Fatehgunj, Railway station, Pandya hotel, Navayard, whole of Sama
	8	Karelibaug to Harni to Dandiya bazaar
	13	From Chhani, including Narmada Canal till Zenith Tins on NH8
West Zone	6	Gotri
	10	Frm Station, Alkapuri, RC Racecourse, Gorwa, till Sindhrot
	11	Area between Racecourse and OP Road and Gotri
East Zone	1	Market, Jubille Baug, Navabazaar, Mandvi, Nyay Mandir till Market Char raasta
	2	Fatehpura char raasta, Bhootdi-jhaanpa, manek park, airport, till highway, darjipura, airforce station till back to Fatehpura. Includes Warasiya
	9	Panigate, Ajwa road, leftside Mahavir chowk, highway till ward 2 boundary, leprosy hospital till Mahesh complex. Includes Ayurvedic college, panigate bus depot and Sardar industrial estate
South Zone	3	Pratapnagar, Dabhoi road, Danteshwar till highway till Mahesh complex (on Waghodia road)
	4	Rajmahal road, lalbaug crossing, manjalpur village till GIDC (Alwa naka) till GIDC last road (road no. 13), pratapnagat overbridge, ONGC, Baroda Diary,

		Bhavans School, Tarsali towards NH, Makarpura till Dabhoi road
	12	GIDC, Makarpura Jambua Highway Tarsali where ward 4 boundary ends. Then from behing Tarsali village till Lalbaug crossing. Included Kalali village and Atladra industrial area.

APPENDIX II

CONSENT LETTER FOR FORMATIVE RESEARCH PART A

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 – GUJARAT. INDIA

Phone: 0265 795526

Tele. ⟩ 0265-795522 [Ext.33]

Dated: /1/2012

I, _____, give my consent to be included as a subject in the study being carried out by Prof. Uma Iyer, and Ms. Nitya Elayath to investigate the burden of cardiometabolic risk factors in pre, peri and post menopausal women in a free-living population.

I understand that the study requires the participants to undergo a blood test to measure various parameters for which, the participants would be required to provide 10ml blood sample. Only disposable needles and syringes will be used for drawing blood, which will be done by a trained and authorized technician.

I have been explained to my satisfaction the purpose of this clinical trial and I am also aware of my right to opt out of the study any time.

_____

Signature

Name:

APPENDIX III

CONSENT LETTER FOR FORMATIVE RESEARCH PART B

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 – GUJARAT. INDIA

Phone: 0265 795526

Tele. ⟩ 0265-795522 [Ext.33]

Dated: /1/2012

I, _____, give my consent to be included as a subject in the study being carried out by Prof. Uma Iyer, and Ms. Nitya Elayath to investigate the burden of cardiometabolic risk factors in pre, peri and post menopausal women in health check up section of Jivraj Mehta Smarak Health Foundation, Ahmedabad.

I understand that the study requires the participants to undergo a blood test to measure various parameters for which, the participants would be required to provide 10ml blood sample. Only disposable needles and syringes will be used for drawing blood, which will be done by a trained and authorized technician. I have been explained to my satisfaction the purpose of this clinical trial and I am also aware of my right to opt out of the study any time.

_____

Signature

Name:

APPENDIX IV

CONSENT LETTER FOR WHEATGRASS SUPPLEMENTATION STUDY

DEPARTMENT OF FOODS AND NUTRITION
FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA 390 002 – GUJARAT. INDIA

Phone: 0265 795526
Tele. } 0265-795522 [Ext.33]

Dated: /1/2012

I, _____, give my consent to be included as a subject in the study being carried out by Prof. Uma Iyer and Ms. Nitya Elayath in the M. S. University to investigate the benefits of Wheatgrass powder in the management of primary hyperlipidemia. I understand that the study requires the participants to consume 4 wheatgrass capsules everyday for a period of 10 weeks and the period of consumption of capsules will be preceded and followed by a blood test to measure various parameters for which, the participants would be required to provide 10ml blood sample. Only disposable needles and syringes will be used for drawing blood, which will be done by a trained and authorized technician. The participants are also required not to take any cholesterol medication during the study.

I have been explained to my satisfaction the purpose of this clinical trial and I am also aware of my right to opt out of the study any time.

Signature ---------- ----------

Name: ---------- Prof Uma Iyer

APPENDIX V

RECIPE: KHAKHRA

Procedure

1. Mix all the ingredients except oil.
2. Add half the quantity of oil, and make into medium soft dough using adequate water.
3. Add 0.5g (/1g/ 1.5g) of barley grass powder into it.
4. Make into a ball and roll into a very thing chapatti.
5. Cook on a flat pan using the remaining oil. Care is to be taken to continuously apply pressure when cooking to avoid and air spaces developing or any puffing.

Nutritive Value Information:

Ingredients	Amt	Energy (Kcal)	CHO (g)	Protein (g)	Fat (g)	Iron (mg)	Ca (mg)
Wheat flour	15	51	10.4	1.82	0.26	0.74	7.2
Cumin seeds	0.25	1	-	0.05	-	0.03	2.7
Red chilli powder	0.25	-	0.08	0.04	-	-	-
Omum	0.06	1	-	-	-	-	-
Turmeric	0.06	-	-	-	-	0.04	-
Asafoetida	0.07	-	0.05	-	-	0.03	-
Salt	0.66	-	-	-	-	-	-
Oil	1.5	14	-	-	1.5	-	-
TOTAL		**67**	**10.53**	**1.91**	**1.76**	**0.84**	**9.9**

RECIPE: THEPLA

Procedure

1. Weigh all the ingredients and mix them in a bowl.
2. Add 0.5g (/1g/ 1.5g) of barley grass powder into it.
3. Add only half the quantity of oil i.e. 2.5g into the dry mixture.
4. Add water slowly and make into medium soft dough.
5. Make it into a ball and roll it flat and little thicker than chapatti.
6. Cook on both sides using the remaining quantity of oil on a flat pan.

Nutritive Value Information:

Ingredients	Amt	Energy (Kcal)	CHO (g)	Protein (g)	Fat (g)	Iron (mg)	Ca (mg)
Wheat flour	20	69	14.2	2.36	0.3	1.06	8.2
Cumin seeds	0.5	2	-	0.1	0.08	0.06	5.4
Omum	0.3	1	-	0.05	0.07	-	4.6
Red chilli powder	0.36	-	-	-	-	-	-
Asafoetida	0.07	-	-	-	-	-	0.5
Salt	0.89	-	-	-	-	-	-
Oil	5	45	-	-	5	-	-
TOTAL		**117**	**14.2**	**2.51**	**0.45**	**1.12**	**18.7**

RECIPE: MUTHIYA

Procedure

1. Clean and weigh all the ingredients.
2. Chop the clean the fenugreek leaves.
3. Separately dry roast rice, bengal gram dal and red gram dal lightly in a deep pan.
4. Coarsely grind them and mix all the ingredients except Gingelly seeds and oil.
5. Add 0.5g (/1g/ 1.5g) of barley grass powder into it.
6. Use water to shape the mixture into cylindrical rolls and steam them in a steamer or pressure cooker.
7. Cut into small pieces of about 5.5cm length.
8. Heat oil in a pan add gingelly seeds to flutter, put off the flame and add the muthiya pieces to coat with the tempering.

Nutritive Value Information:

Ingredients	Amt	Energy (Kcal)	CHO (g)	Protein (g)	Fat (g)	Iron (mg)	Ca (mg)
Rice	50	173	39.1	3.4	0.25	0.35	5
Bengal gram dal	25	92	15	5.2	1.4	2.38	14.5
Red gram dal	25	84	14.4	5.58	0.43	0.68	18.25
Fenugreek leaves	30	15	1.8	1.32	0.27	0.58	118.5
Curds	2.5	3	0.1	0.12	0.16	-	5.3
Sugar	2.5	10	2.5	-	-	-	-
Gingelly seeds	2.5	14	0.6	0.46	1.08	0.23	36.25
Salt		-	-	-	-	-	-
Oil	15	135	-	-	15	-	-
TOTAL		**526**	**73.5**	**16.08**	**18.59**	**4.22**	**197.8**

APPENDIX VI

SENSORY EVALUATION OF WHEATGRASS INCORPORATED RECIPES

(COMPOSITE RATING TEST)

Name: Date:

Age:

Phone Number:

Time: Time of previous meal:

Taste the samples and check how much you like or dislike each one.

For each of the following characteristics given in the table below, please indicate the score of the product on a scale of 1 to 10.

Characteristics	Product Code		
	A	B	C
Aroma			
Appearance			
Color			
Flavor/Taste			
Consistency			
Aftertaste			
Overall Acceptability			

Comments:

_____

_____

Signature

APPENDIX VII

FORMATIVE RESEARCH CUM WHEATGRASS TRIAL QUESTIONNAIRE

[A] Background Information

1. Name
2. Regionality:
3. Date of Birth
4. Age
5. Sex:
6. Religion: a) Hindu b) Islam c) Christian z) Other
7. Address & Contact No:
8. Education
9. Occupation
10. Type of Family: a) Nuclear b) Joint c) Extended
11. Family Size:
12. Total monthly family income
13. Menopausal status
14. Age of onset of menopause

15. Do you / did you experience the following symptoms?

Sr No	Symptom	Severity				
		Not present	Mild	Moderate	Severe	Very Severe
	Vasomotor					
1	Hot Flashes					
2	Night Sweats					
	Somatic					
3	Headaches					
4	Muscle/ joint pains					
5	Numbness/ tingling in parts of body					
6	Feeling dizzy/ faint					
7	Pressure/ tightness in body/head					
8	Heart beating strongly					

9	Lack of energy/ feeling tired					
	Psychological					
10	Feeling tense/nervous					
11	Excitable					
12	Concentration difficulties					
13	Depressed/unhappy					
14	Irritable					
15	Loss of interest in things					
16	Crying spells					
17	Difficulty in sleeping					
	Urogenital					
18	Dryness of vagina/ pain during sex					
19	Urine incontinence					
20	Pain during urination					
21	Any changes in voice					

[B] Family History of NCDs

	CHD/MI	Hypertension	Diabetes	Dyslipidemia	Asthma	Cancer
Siblings						
Parents						
Both						
None						

[C] Anthropometry

Weight:	Waist Circumference:
Height:	Hip Circumference:
Body Mass Index:	Waist Hip Ratio:

[D] Medical History

1. Any major illnesses:

Disease	Since	Medications
Diabetes		
Hyperlipidemia		
High BP		
CHD		
Thyroid		
Asthma		
Cancer		
Orthopedic complaints		
Other		

[E] Biochemical Indicators

Hb:	VLDL:
FBS:	Apo A
Insulin:	Apo B
Lipid Profile	***Thyroid Hormones***
TC:	TSH
TAG:	FT4
HDL:	***Inflammation***
LDL:	hs- CRP

F] Physical Activity

2. Sleeping Pattern: a) Peaceful b) Disturbed
3. Average hours of sleep:
4. Occupation:
5. How many hours do you spend sitting at work?
6. How much time do you spend walking at work?
7. Does your work involve any moderate activity?
8. How do you usually travel to work, shopping, run errands, etc?

a) Drive/take a bus b) walk c) bicycle

9. How much time do you spend sitting while travelling to work/shopping?
10. How many hours do you spend **sitting** in a usual day?
11. Physical Activity other than travel and work:

Activity	Yes			No physical activity
	Type	Duration	Frequency (Daily, weekly, etc)	
Light activity (e.g. walking at leisurely pace)				
Moderate activity (e.g. carrying light loads, bicycling at a regular pace, brisk walking)				
Intense Activity (e.g. heavy lifting, digging, aerobics, running, fast bicycling, swimming, outdoor sports)				

[G] Dietary Intake

1. Frequency of consumption of:

	Yes		No
	Amount	Frequency	
GLV			
Fruits			
Salads			
Eggs			
Sea food			
Other non-vegetarian foods			
Biscuits/Bakery/Confectionery Items			
Wafers, fried snacks			
Frequency of eating out (including parties)			

2. Number of coffee/tea per day
3. Quantity of sugar per cup of coffee/tea:
4. Type of cooking oil used
5. Amount of oil used per year in the household:
6. Daily eating pattern

Time	Meal	Food eaten	Amount consumed