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 at least 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>
<thead>
<tr>
<th>Reproductive Years</th>
<th>Average Age</th>
<th>Menstrual Cycles</th>
<th>Signs &amp; Symptoms</th>
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<td></td>
<td>First Period: 9-15 years</td>
<td>Variable</td>
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<td>16-30 years</td>
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<td>31-42 years</td>
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<td>Fertility progressively declines</td>
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<td>Menopausal Transition</td>
<td>Early Transition: 40s</td>
<td>Lengths of cycles vary increasingly</td>
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|                    | 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 chemoprevention in breast cancer patients (for example, raloxifene 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 is 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). Geld 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 & dizziness, joint pain, general aches and fatigue.

Headaches and Dizziness

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 2002, 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 visor, 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 2017)

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 dizziness 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
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 and 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-Agus 2009). Warren, Shu and Domínguez (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, progesterone). 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 gonadotropin 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 a 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 perimenopausal 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 follicles 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 al 1988) 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; Shidesler 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; Shidesler 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 al 1986). Sporadic and transient increases in estradiol concentrations, neither accompanied nor followed by elevations in progesterone, have been noted in some postmenopausal women (Metcalf et al 1982). 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, Tremolieres 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, along with 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 osteoporosis (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

Normal Bone

Osteoporotic Bone
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 Ikkram 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 Vuppurturi 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 Ikkram 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 responsivenocea 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 citruline. 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 as 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 porimonopausal 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 bloodstream; 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 cardiovascular, 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 menopause 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 case 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 de-angements 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 comorbid 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**

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 (Samba 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) \( >25\text{kg/m}^2 \) and obesity as BMI \( >30\text{kg/m}^2 \). 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 cardiovascular 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 abnormally 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 cardiovascular 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, Kolkata, 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 0106 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 the 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 (Wilc 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 et 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 Rhatia 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 1170 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 values 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 60 years 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 explain 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 multicentric 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 19.3 mg/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 reductase (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**

- High BMI: 13 – 84%
- HTN: 13 – 51%
- Hyperlipidemia: 30 – 85%
- MS: 25%
- IDA: 56%
- SCH: 13 – 38%
- T2DM: 15-21%
- Osteoporosis: 29 – 44%

* HTN: Hypertension, MS: Metabolic Syndrome, T2DM: Type 2 Diabetes Mellitus, SCH: Subclinical Hypothyroidism, 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 labeled 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 (Cannor 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 are to 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 Formulary 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 Formulary 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**

<table>
<thead>
<tr>
<th>Lipid Fraction</th>
<th>Single exercise session</th>
<th>Regular Exercise Participation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>Decreases of 7% to 69%; approximate mean change 20%</td>
<td>Decreases of 4% to 37% Approximate mean change 24%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>No change*</td>
<td>No change</td>
</tr>
<tr>
<td>LDL-C</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Small dense LDL-C particles</td>
<td>No change</td>
<td>Can increase LDL particle size usually with TAG lowering</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Increases of 4% to 18% Approximate mean change 10%</td>
<td>Increases of 4% to 18% Approximate mean change 8%</td>
</tr>
<tr>
<td>Chylomicron and VLDL-C</td>
<td>Usually lower</td>
<td>Usually lower</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Postprandial lipemia</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>apoA1</td>
<td>No change</td>
<td>Increased</td>
</tr>
<tr>
<td>apoB</td>
<td>Parallels LDL changes</td>
<td>Parallels LDL changes</td>
</tr>
<tr>
<td>apoE, apoE3, apoE4</td>
<td>Varied response based on age, homozygote/heterozygote phenotype</td>
<td>Varied response based on age, homozygote/heterozygote phenotype</td>
</tr>
<tr>
<td>LPL Activity</td>
<td>Delayed change (≥ 4 h)</td>
<td>Increased</td>
</tr>
<tr>
<td>HL Activity</td>
<td>No change</td>
<td>No change or reduced (may be reduced with weight loss)</td>
</tr>
<tr>
<td>LCAT Activity</td>
<td>Increased/no change</td>
<td>Increased/no change</td>
</tr>
<tr>
<td>CETP Activity</td>
<td>No change</td>
<td>No change/increased</td>
</tr>
</tbody>
</table>

*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 (i.e., 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 SFA, 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 cays. 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 invtro 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 guar gum at the level of 25g barley/day and 4% guar gum 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 (by 116.3mg/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 ‘*Sutrassthana*’ (meaning ‘Summary’)

![Verse](image)

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 *Pooidae* and tribe *Triticaceae*. 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 stigmasteryl 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, Irnak and Jonnala 2009, Dunford and Edwards 2010). Polyphenol tests revealed the presence of flavonoids, triterpenoids, anthranol, alkaldoids, 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 chemotherapy 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 enothelial 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 15g level 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 Cardiovascular Health & Cancer

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Duration</th>
<th>Subjects</th>
<th>Form</th>
<th>Dosage</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kothari et al 2011</td>
<td>14 days</td>
<td>Hypercholesterolemic rats</td>
<td>Juice</td>
<td>5ml/kg, 10ml/kg per day</td>
<td>TC ↓, TAG ↓, LDL ↓, VLDL ↓, FBS ↓</td>
</tr>
<tr>
<td>Das et al 2012</td>
<td>12 weeks</td>
<td>Hyperlipidemic rabbits</td>
<td>Ethanol extract</td>
<td>500mg/kg per day</td>
<td>TC ↓, TAG ↓, LDL ↓, MDA ↓, FBS ↓, HDL ↑</td>
</tr>
<tr>
<td>Shirude 2011</td>
<td>14 days</td>
<td>Hyperglycemic rats</td>
<td>Juice</td>
<td>100mg/kg per day</td>
<td>FBS ↓</td>
</tr>
<tr>
<td>Kothari et al 2008</td>
<td>21 days</td>
<td>Normolipidemic rats</td>
<td>Juice</td>
<td>5ml/kg, 10ml/kg per day</td>
<td>TC ↓, TAG ↓, LDL ↓, VLDL ↓, HDL ↑</td>
</tr>
</tbody>
</table>

#### Oxidative Stress

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Duration</th>
<th>Subjects</th>
<th>Form</th>
<th>Dosage</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pant et al 2013</td>
<td>1 lifecycle</td>
<td>Drosophila flies</td>
<td>Powdered</td>
<td>10mg per 10ml diet per day</td>
<td>SOD ↓, Catalase ↓</td>
</tr>
<tr>
<td>Shyam et al 2007</td>
<td>30 days</td>
<td>30 healthy adults</td>
<td>Powdered</td>
<td>1g</td>
<td>Vit C↑, MDA ↓, SOD ↑</td>
</tr>
</tbody>
</table>

#### Inflammation

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Duration</th>
<th>Subjects</th>
<th>Form</th>
<th>Dosage</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ben-Arye et al 2002</td>
<td>1 month</td>
<td>23 ulcerative colitis patients</td>
<td>Juice</td>
<td>100ml per day</td>
<td>↓ rectal bleeding, ↓ disease severity</td>
</tr>
</tbody>
</table>

#### Cancer

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Duration</th>
<th>Subjects</th>
<th>Form</th>
<th>Dosage</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bar Sela et al 2007</td>
<td>3 sessions of chemotherapy</td>
<td>60 breast cancer patients</td>
<td>Juice</td>
<td>16ml per day</td>
<td>Chemotherapy dosage ↓, myelotoxicity ↓</td>
</tr>
<tr>
<td>Wheat et al 2006</td>
<td>1 session of radio therapy</td>
<td>Breast cancer patients</td>
<td>Aqueous extract</td>
<td>25ml per day</td>
<td>Skin toxicity ↓</td>
</tr>
<tr>
<td>Dey et al 2006</td>
<td>6 months</td>
<td>400 cancer patients</td>
<td>Juice</td>
<td>30ml per day</td>
<td>Hb↑, Total Protein↑, Albumin↑</td>
</tr>
</tbody>
</table>
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 stakeholders. 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.