1| INTRODUCTION

The overall health and health quality of middle aged women has become a major public health concern around the world. As a result of increase in life expectancy, a woman spends a third of her life in menopause. The transition from reproductive to non-reproductive stage tends to occur over a period of few years. During this transition period, women undergo many changes along with its underlying hormonal changes which abruptly disturb their daily activities and overall health. The cessation of the cycles would be the menopause stage. This happens with the irregularity in the regular cycles.

During this transition period, women experience different symptoms, like physical disturbances (hot flashes) and psychological complaints (mood swings). Most of women in India, do not understand these symptoms and changes taking place in life, and spend their valuable life battling these problems and associated diseases.

As per WHO (1996), there were an estimated 467 million women in perimenopause state in 1990 and this number is expected to increase 1200 million by the year 2030. (Nayak *et al*., 2014)

The individual experience of the menopause transition varies widely. Important influential factors include the age at which menopause occurs, personal health and wellbeing, and each woman's environment and culture. Management options range from lifestyle assessment and intervention through to hormonal and non-hormonal pharmacotherapy, each of which has specific benefits and risks. (Davis *et al*., 2015)

The different terminology associated with reproductive life can be defined as follows:

Menopause (natural menopause) – the term natural menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Natural menopause is recognized to have occurred after 12 consecutive months of amenorrhea, for which there is no other obvious pathological or physiological cause. Menopause occurs with the final menstrual period (FMP) which is known with certainty only in retrospect a year or more after the event. An adequate biological marker for the event does not exist. (Source: WHO, 1996)

Perimenopause – the term perimenopause should include the period immediate prior to the menopause (when the endocrinological, biological, and clinical features of approaching menopause commence) and the first year after menopause. (Source: WHO, 1996)

Menopausal transition – the term menopausal transition should be reserved for that period of time before the FMP when variability in the menstrual cycle is usually increased. This term can be used synonymously with "premenopause" although this latter term can be confusing and preferably should be abandoned. (Source:WHO, 1996)

Premenopause – the term premenopause is often used ambiguously to refer to the one or two years immediately before menopause or to refer to the whole of the reproductive period prior to the menopause. The group recommended that the term be used consistently in the latter sense to encompass the entire reproductive period up to the FMP. (Source: WHO, 1996)

Post menopause – the term post menopause is defined as dating from the FMP, regardless of whether the menopause was induced or spontaneous. (Source: WHO, 1996)

Premature menopause – ideally, premature menopause should be defined as menopause that occurs at an age less than two standard deviations below the mean established for the reference population. In practice, in the absence of reliable estimates of the distribution of age at natural menopause in populations in developing countries, the age of 40 years is frequently used as an arbitrary cut-off point, below which menopause is said to be premature. (Source: WHO, 1996)

Induced menopause – the term induced menopause is defined as the cessation of menstruation which follows either surgical removal of both ovaries (with or without hysterectomy) or iatrogenic ablation of ovarian function (e.g. by chemotherapy or radiation). [Source: WHO, 1996]

Climacteric – The phase in the aging of women marking the transition from the reproductive phase to the non-reproductive state. This phase incorporates the perimenopause by extending for a longer variable period before and after the perimenopause. [Source: IMS, 2011]

Climacteric syndrome – the climacteric is sometimes, but not necessarily always, associated with symptomatology. When this occurs, it may be termed the "climacteric syndrome." [Source: IMS, 2011]

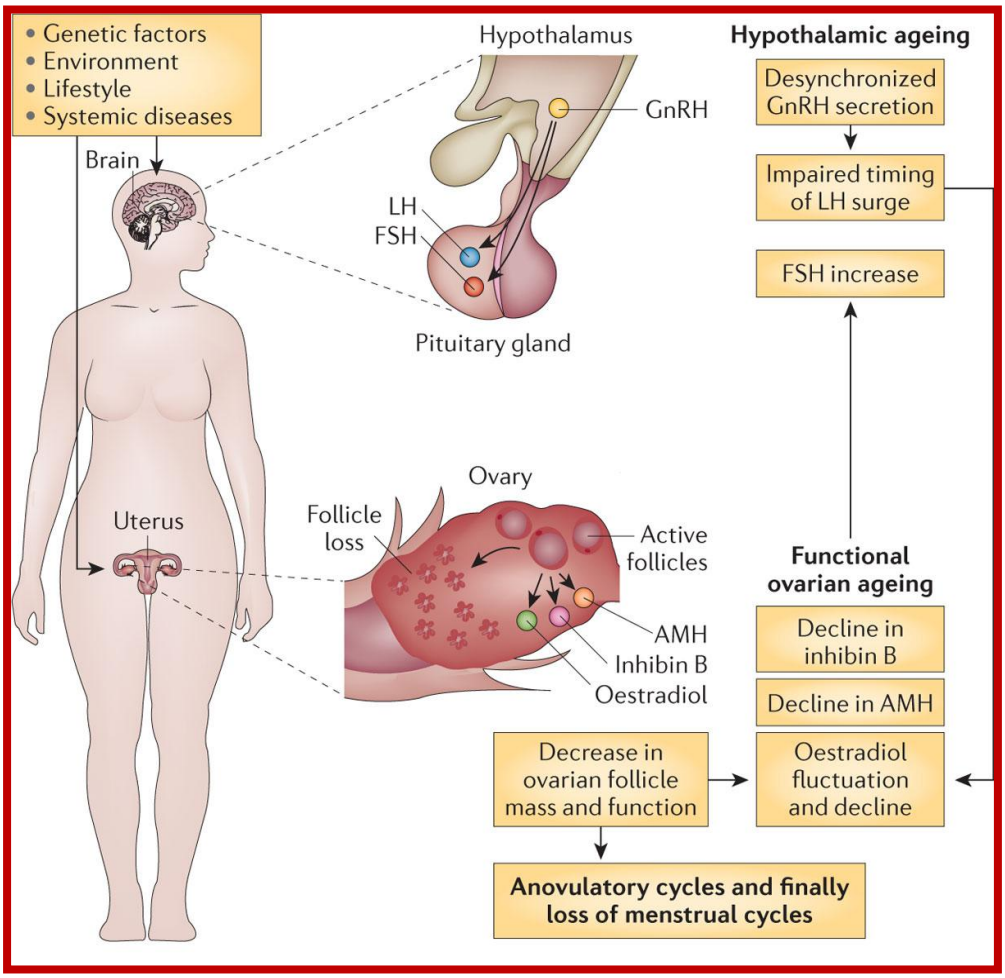
PHYSIOLOGICAL CHANGES DURING MENOPAUSAL TRANSITION

The functional lifespan of human ovaries is determined by a complex and yet largely unidentified set of genetic, hormonal and environmental factors (Figure 1.1). Women undergo menopause when follicles in their ovaries are exhausted. The clinical manifestations of perimenopause and menopause result from dynamic interactions between neuroendocrine changes and alterations in the reproductive endocrine axis that governs the function of the ovaries. However, changes due to aging in non reproductive endocrine system such as hypothalamus, pituitary, uterus, and ovary also contribute for observed manifestations. (Chervenak J and Santoro N, 2015)

Hypothalamic aging leads to de-synchronized gonadotropin-releasing hormone (GnRH) production and an impaired surge of luteinizing hormone (LH) release from the pituitary gland. These central nervous system changes, together with ovarian ageing, impair ovarian follicle maturation, hormone production (inhibin B, anti-Mullerian hormone (AMH) and estradiol) and ovulation. This leads to cycle irregularities and follicle-stimulating hormone (FSH) up regulation. (Davis *et al*., 2015)

During the perimenopause, accelerated folliculogenesis results in the shortening in follicular phase length and mean cycle length becomes highly variable. This irregularity of menstrual cycles is unpredictable at a later stage of perimenopause.

Mid cycle estrogen concentrations found to be normal or increased and levels of progesterone and androgens to be normal or decreased, independent of major changes in sex hormone-binding globulin in perimenopause.



(Source: Davis *et al*., Nature Reviews/Primer; Volume 1, 2015)

Figure 1.1: Menopausal loss of ovarian function

Menopausal symptoms

The classical symptoms of menopause include periodic sweating/hot flushes, depression, insomnia, impaired memory, lack of concentration, nervousness, bone and joint aches etc. (Figure 1.2)

Hot flashes:

Hot flash is one of the vasomotor symptoms, reported very commonly during menopausal transition. A hot flash is a sudden episode of vasodilation in the face and neck, which lasts 1-5 minutes and is accompanied by profuse sweating.

Women with narrower thermoneutral zone are reporting it more, such that subtle changes of core temperature elicit thermoregulatory mechanisms such as vasodilation, sweating or shivering. Declining levels of estrogens and inhibin B, as well as increasing FSH levels, justify only part of the disturbed thermoregulation, which is associated with changes in brain neurotransmitters and peripheral vascular reactivity. (Davis *et al*. 2015; Archer *et al*., 2011)

Heart discomfort:

Palpitation is frequently associated with anxiety and does not necessarily indicate a structural or functional abnormality of the heart, but it can be a symptom arising from an objectively rapid or irregular heartbeat. Palpitation can be intermittent and of variable frequency and duration, or continuous. Associated symptoms include dizziness, shortness of breath, sweating, headaches, and chest pain.

Sleep problems:

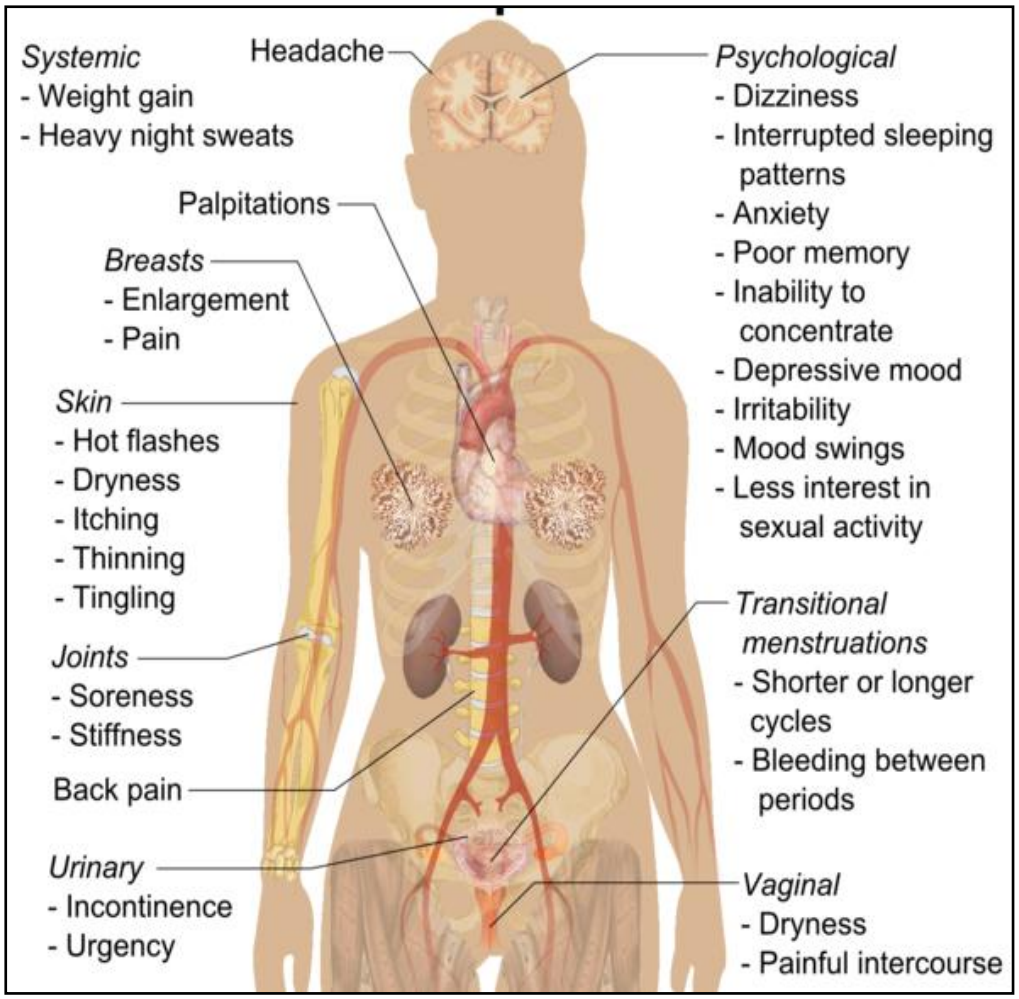
Sleep disturbances have been identified as an important symptom associated with the menopause transition and early menopause.

Mood swings/depression:

As a result of hormonal fluctuation during menopausal transition, women may experience feelings of depression and mood swings from extreme highs to severe lows in a short period of time.

Irritability:

Irritability is an excitation response to stimuli. The term is used for both the physiological reaction to stimuli and for the pathological, abnormal or excessive sensitivity to stimuli. It is usually used to refer to anger or frustration. Irritability may be demonstrated in behavioral responses to both physiological and behavioral stimuli including environmental, situational, sociological, and emotional stimuli.



(Source: https://en.wikipedia.org/wiki/Menopause)

Figure 1.2: Symptoms observed during menopausal transition

Anxiety:

Poor coping skills (e.g., rigidity/inflexible problem solving, denial, avoidance, impulsivity, extreme self-expectation, affective instability, and inability to focus on problems) are associated with anxiety. Anxiety is also linked and perpetuated by the person's own pessimistic outcome expectancy and how they cope with feedback negativity.Temperament (e.g., neuroticism) and attitudes (e.g. pessimism) have been found to be risk factors for anxiety. The emotional effects of anxiety may include "feelings of apprehension or dread, trouble concentrating, feeling tense or jumpy, anticipating the worst, irritability, restlessness, watching (and waiting) for signs (and occurrences) of danger, and, feeling like your

mind's gone blank as well as nightmares/bad dreams, obsessions about sensations and feeling like everything is scary.

Physical and mental exhaustion (Forgetfulness/memory loss):

Persistent feeling of weakness, tiredness, and lowered energy levels rather than just sleepiness or drowsiness during transitional period. Women may experience mental blocks, memory lapses or found difficulty in concentrating. This physical and mental exhaustion can have a drastic impact on daily life, work productivity and quality of life.

Sexual problems:

The most commonly reported symptoms from women are that, they become less interested in sex during menopause. This is caused by physical changes brought upon by reduced estrogen. These changes can include a delayed clitoral reaction time and slow or absent orgasmic response. Low sexual drive during menopause can also happen due to other menopausal symptoms like vaginal dryness or depression or by prescription drugs, including medication prescribed to treat menopausal symptoms.

Bladder problems:

Similar to changes observed in vaginal tissues due to lower level of estrogen, the lining of urethra also gets drier, thinner and less elastic. The surrounding pelvic muscle also becomes weak. Due to these women become more susceptible to urinary tract infection. Women may also experience the need to urinate more frequently or leakage of urine (urinary incontinence). The incontinence can result from a sudden urge to urinate or may occur during straining when coughing, laughing or shifting heavy objects.

Vaginal dryness or vaginal atrophy:

The decreased production of estrogen and progesterone can affect the thin layer of moisture that coats the vaginal walls. Women may experience itching around the vulva as well as stinging or burning. These vaginal changes may lead to increased risk of vaginal infections. The drier and less elastic tissues of vagina can make sexual intercourse painful for women (dyspareunia), which result into decreased interest in sex.

Changes in Breast:

Hormonal changes can lead to breast pain, soreness, tenderness in one or both the breasts. Breast pain might occur at different times or at different intensities in individual women.

Joints and muscular discomfort:

Joint pain is an unexplained soreness in muscles and joints, which is unrelated to the effects of fluctuating hormone levels on the immune system. Estrogen prevent inflammation in the joints, so low levels of estrogen during menopause can lead to increased instances of inflammation, and therefore increased joint pain. Low estrogen levels also raise cortisol, and high level of cortisol cause the muscles in the body to tighten and become fatigued.

Osteoporosis:

Estrogen is involved the process of calcium absorption into the bones. Thus after menopause the chances of a woman suffering from osteoporosis are higher. Reduced bone density can also make women susceptible to fractures.

Weight gain:

Women may also have weight gain to some extent during menopausal transition. The distribution of body fat may change, with body fat being accumulated more in the waist and abdominal area than n the hips and thighs.

Skin changes:

Collagen is responsible for keeping skin toned, fresh-looking and resilient. Rapid loss in collagen production during perimenopause results into itchy skin. Changes in skin texture, including wrinkles, may develop along with worsening of adult acne.

Since the body continues to produce small levels of the male hormone testosterone, some women may experience some hair growth on the chin, upper lip, chest or abdomen.

Hair Loss:

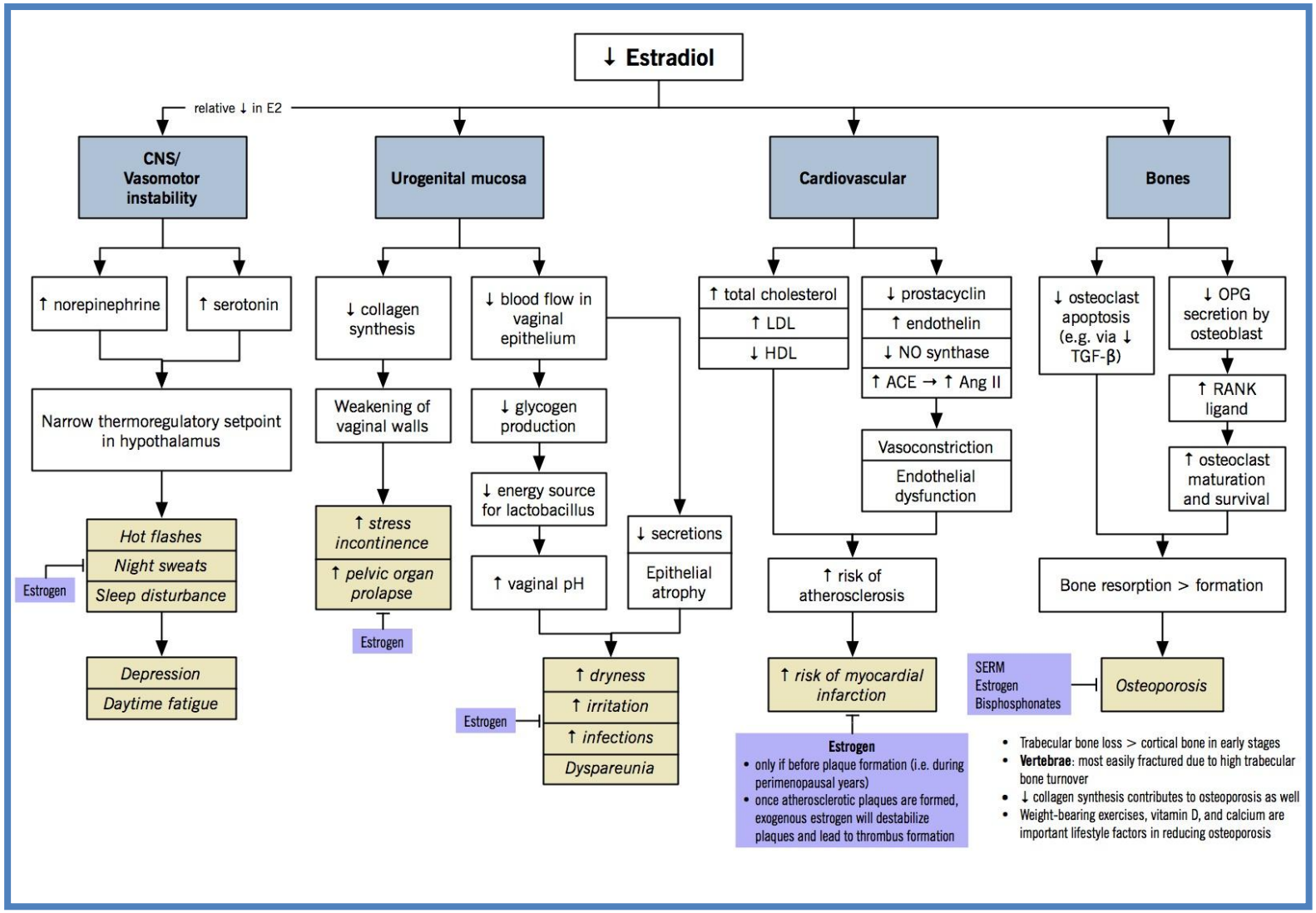
Reduced estrogen may also lead to hair loss or hair become brittle or dry.

The physiological mechanisms that trigger and control menopausal symptoms are not much explained and understood. The possible pathophysiology for such symptoms can be summarised as in Figure 1.3.

The perimenopausal period has distinct endocrine characteristics, with early perimenopause being a period of raised pituitary gonadotropin production: follicle stimulating hormone (FSH) and luteinizing hormone (LH) plus increased estradiol secretion. The later stages of perimenopause are characterized by high FSH levels and decreased estradiol production (Soules *et al*., 2001).

Reproductive status is often confirmed by the presence of elevated plasma gonadotropin, that is, high FSH levels in the context of low plasma estradiol levels (Rubinow, Roca, & Schmidt, 2007).

In terms of the mood symptoms accompanying perimenopause, estrogen seems to have the most significant role through its modulation of serotonin, a neurotransmitter responsible for mood regulation (Rubinow *et al*., 1998).



(Source: www. pathophys.org/menopause. Adaptedfrom:Lentz: Comprehensive Gynaecology, 6E; William textbook of Endocrinology, 12E)

Figure 1.3: Pathophysiology for menopausal symptoms

Estrogen has wide spread actions throughout the central nervous system (CNS) and modulates the transcription of many enzymes, as well as the receptor proteins for many neurotransmitters and neuropeptides (Ciocca & Roig, 1995). As a result, estrogen regulates a great deal of serotonin activity. For example, estrogen modulates the synthesis of serotonin (Cohen & Wise, 1988), serotonin reuptake, serotonin receptor transcription, and the response to serotonin stimulation (Matsuda, Nakano, Kanda, Iwata, & Baba, 1991).

In addition to its effects on serotonin receptors, estrogen has been found to augment noradrenergic transmission (NA), a neurotransmitter responsible for regulating sleep and arousal (Deecher, Andree, Sloan, & Schechter, 2008). Estrogen increases NA turnover, decreases NA reuptake, and decreases the number and sensitivity of dopamine (D2) receptors. The central serotonergic system, noradrenergic and the estrogenic system are prominently involved in the regulation of mood and behavioural states (Rubinow *et al*., 1998), and fluctuations in estrogen levels seen in perimenopause may therefore directly cause many of the observed physical and psychological symptoms through altered activation of the key areas of the CNS. (Duncan & Gibbs *et al*., 2015)

The Menopause Rating Scale (MRS)

Owing to lack of standardized scales to measure the severity of aging-symptoms and their impact on the health-related Quality of Life (HRQoL), the Menopause Rating Scale (MRS) was developed which is a standardized self-administrable questionnaire that can be filled by women. (Heinemann *et al*., 2003)

It consists of 11 symptoms which can be categorized into 3 domains:

- Psychological domain : symptoms – depression, irritability, anxiety, physical andmental exhaustion,

- Somato-vegetative domain; symptoms – hot flush/sweating, cardiac complaints, sleeping disorders, joint and muscle complaints and
- Urogenital domain: symptoms – sexual problems, urinary complaints, vaginal dryness.

Each sub scale (domain) can get 0 (no symptom) or upto 4 (very severe) scoring points, score increases point by point depending on the severity of the complaints. (Appendix III)

The composite scores for each of the domains (sub-scales) is based on adding up the scores of the symptoms of the respective domains.

Psychological sub-scale (P): 0 to 16 scoring points

Somato-vegetative sub-scale (S): 0 to 16 scoring points

Urogenital sub-scale (U): 0 to 12 scoring points

The composite score (total score –MRS) is the sum of the domain scores, which ranges between 0 (asymptomatic) to 44 (highest degree of complaints). (Heinemann *et al*., 2003)

The prevalence of vasomotor symptoms in premenopause and during the menopausal transition varies by ethnicity. It was reported that women with increased cortisol during the late menopausal transition have more severe vasomotor symptoms than those with normal cortisol. The severity of symptoms did not differ with respect to age, BMI (body mass index) or stress level. (Woods *et al*., 2006)

Menopause and thyroid

Thyroid plays a role in regulating overall body metabolism and influences the heart, brain, kidney and reproductive system, along with muscle strength and appetite.

Thyroid dysfunction is the common endocrine disorder in females. Normal reproductive behaviour and physiology is dependent on having

essentially normal levels of thyroid hormone. Thyroid hormones play an important role in normal reproductive function both through direct effects on the ovaries and indirectly by interacting with sex hormone binding proteins. (Poppe & Glinoer, 2003) Onset of thyroid dysfunction increases with age.It is estimated that 26% of premenopausal and menopausal women are diagnosed with thyroid disease. (Wartofsky *et al*., 2006) The estimated annual incidence of hyperthyroidism for women ranges from 0.36 to 0.47 per 1,000 women, and for men ranges from 0.087 to 0.101 per 1,000 men. In terms of hypothyroidism, the estimated incidence is 2.4 per 1,000 women each year. (Roos *et al*., 2007)

The weight gain in hypothyroidism may be due to the reduction in removal rate of triglycerides and cholesterol which is due to the decrease in the plasma post heparin lipolytic activity. From data obtained general reduction in body weight of hyperthyroid patients was observed. This reduction in weight may be due to the accelerated rate of degradation of most lipids out of proportion to synthesis, so body lipids depots consequently become depleted and levels of various plasma lipid components fall.

Thyroid dysfunction can lead to a variety of potentially serious symptoms, including fatigue, anxiety, palpitations, forgetfulness, weight fluctuations and high blood pressure etc. Most of them are associated with symptoms observed during perimenopause and post menopause phase.

According to the American Association of Endocrinologists (AACE), millions of women with menopausal-like symptoms, including those taking estrogen, may be suffering from undiagnosed thyroid disease.

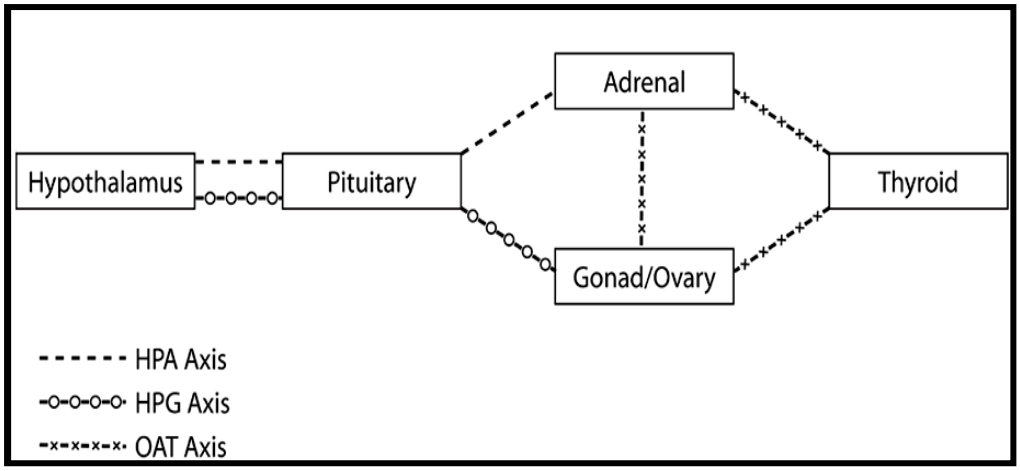


Figure 1.4: Common hormonal axis between adrenals, thyroid and ovaries – OAT axis (Ovarian Adrenal Thyroid axis)

Ovarian Adrenal Thyroid (OAT) axis ties all three organs i.e. the adrenals, thyroid and the ovaries.Any dysfunction of one organ will affect the other organs physiologically, clinically or sub-clinically. These three organs are therefore intimately co-dependent hormonally on each other for optimal function. (Figure 1.4) If the adrenal glands are weak, there is often concurrent thyroid malfunction and menstrual cycle irregularity. Similarly, an under-active thyroid often makes adrenal fatigue worse off. Those who suffer from ovarian hormonal imbalance such as estrogen dominance often have any pre-existing sub-clinical hypothyroidism exacerbated.

All three organs of this axis must be in a state of optimum balance for a woman to feel good. Like a three-legged stool, all three legs must be in perfect balance for the stool to be safe to Seaton. Imbalance of the OAT axis leads to a myriad of conditions that are annoying in their mildest form and incapacitating when severe. (Lam M, 2012)

HORMONE REPLACEMENT THERAPY

Hormone Replacement Therapy (HRT) is a medical treatment with a medication containing one or more female hormones, commonly estrogen plus progestin (synthetic progesterone), and sometimes testosterone. The purpose of HRT is to restore female hormones and allow the body again to function normally.

HRT is available as tablets, skin patches, gels, nasal spray or a vaginal ring. HRT is generally used to treat menopausal symptoms and to ease menopausal transition. Hormone levels usually start to change during perimenopause, the years just before menopause.HRT is strongly recommended to women who experience premature menopause.

Climacteric symptoms may compromise overall wellbeing and quality of life. HRT is highly effective for the relief of vasomotor symptoms, as well as other climacterics complaints. (Nasreen SZA, 2005) HRT conjugated equin estrogen 1.2gm/day given cyclically significantly improves hot flushes insomnia and vaginal dryness. Various psychological problems such as anxiety irritability and poor memory also improve. HRT improves cognitive function and prevents AD (asheimers disease). It is also associated with reduction of some colonic cancer. Most striking feature of HRT is it reduces fracture risk in women up to 74years of age and increase bone density in women up to 80 years of age. HRT has also been shown to improve bone density in established osteoporosis. (Nasreen SZA, 2005)

Though HRT confers several health benefits, the use and prescription of HRT decreased after the results of Women's Health Initiative Study in the USA and Million Women Study about the HRT were published during 2002 - 2004. These studies raised the concern over the safety of HRT, over possible increased risk of breast cancer, cardiovascular

diseases, ovarian cancer, endometrial cancer, venous thrombosis etc. (Dr Louise Newson, 2016)

Worldwide revolution for the improvement of patient safety is gaining momentum; hence, to overcome the side effects of HRT, other complementary and alternative therapies are often used. They include dietary and herbal supplement, acupuncture, massage therapy, yoga, meditation and laughing exercise, other life style changes. There are several natural products/foods/herbs have been studied for menopausal symptoms like Black cohosh, Phytoestrogen, Dong quai, Ginseng, red clover, Soy etc. and proved beneficial to treat menopausal transition. During the last decade, the use of herbal medicine has been increased. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of several health problems.

PHYTOESTROGENS

Phytoestrogens are plant-derived poly-phenolic compounds. Their name comes from the Greek *"Phyto (plant)"* and "*estrogen"*, the hormone which gives fertility to female mammals. The Greek word "estrus"- means "sexual desire" and "gene" is "to generate". It has been proposed that plants use phytoestrogens as part of their natural defence against the overpopulation of herbivore animals by controlling male fertility. (Mascie-Taylor CGN, Bentley, Gillian R, 2000)Herbivores would be at particular risk from plant chemicals that interfered with reproductive functioning because of their high exposure.

There are historical examples of such adverse reproductive effects; for example the breeding problems were encountered in sheep of Western Australia; where the syndrome, termed clover disease, was characterised by a cystic condition of the ovaries, irreversible endometriosis and a failure to conceive. This was caused by ingestion of subterranean clover (*Trifolium subterraneum*) which contains high levels of isoflavone phytoestrogens. Further investigation identified equol, formed in the digestive tract following bacterial metabolism of formononetin, as the ultimate oestrogenic compound responsible for this adverse effect. (Lindsay DR and Kelly RW, 1970)

They can be classified as flavonoids which include the isoflavones, prenylated flavonoids and coumestans; and non-flavonoids that include lignans. The different class of phytoestrogen and members in each class are summarized in Figure1.5.

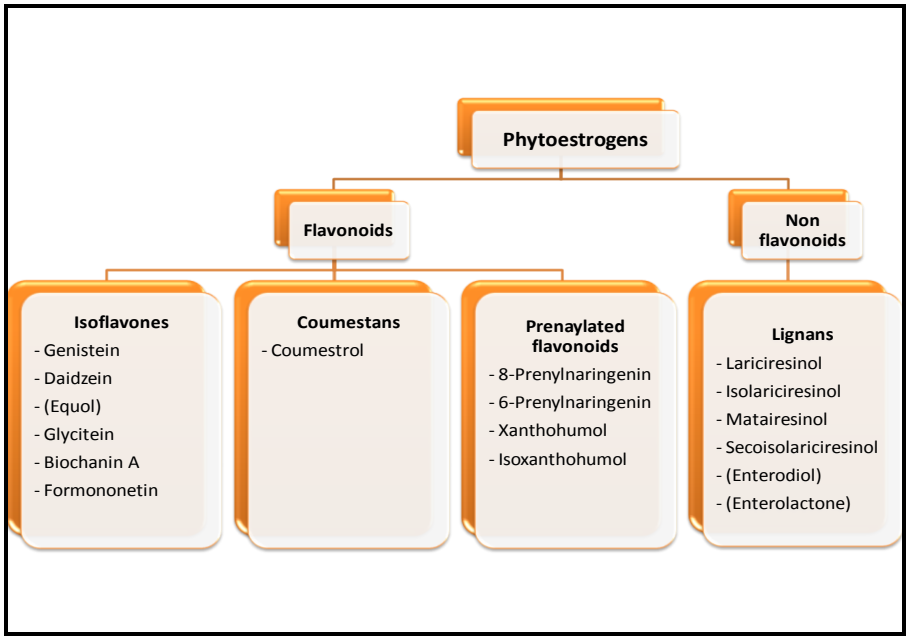


Figure1.5: Different classes of phytoestrogen and members in each class

(The compounds in bracket are not inherently present in plants but are oestrogenic products resulting from metabolism of members of that class of phytoestrogens. This cannot be considered as an exclusive list as other phytoestrogens may be identified as constituents of food in the future)

Chemical Structure

Phytoestrogens are non-steroidal plant derived compound, having similarity in the structure with human estradiol (17-B estradiol), capable of interacting with estrogen receptors by sitting in and blocking receptor sites against estrogen, showing both agonist and antagonist method of action. They are also called as "Dietary estrogens".

The structural similarities between the human estradiol and different classes of phytoestrogens are shown in Figure1.6.The key structural elements that enable phytoestrogens to bind with high affinity to estrogen receptors and display estradiol-like effects are

- The phenolic ring that is indispensible for binding to estrogen receptor
- The ring of isoflavones mimicking a ring of estrogens at the receptors binding site
- Low molecular weight similar to estrogens (MW=272)
- Distance between two hydroxyl groups at the isoflavones nucleus similar to that occurring in estradiol
- Optimal hydroxylation pattern

1. *Isoflavones: Structure and Properties*

Isoflavones are naturally found as biologically inactive glycoside conjugates containing glucose or carbohydrate moieties, where the glucose group is often estrified with an acetyl- or malonyl group to from acetyl- or malonylglucosides. The unconjugated (aglycone) form is the bioactive form.

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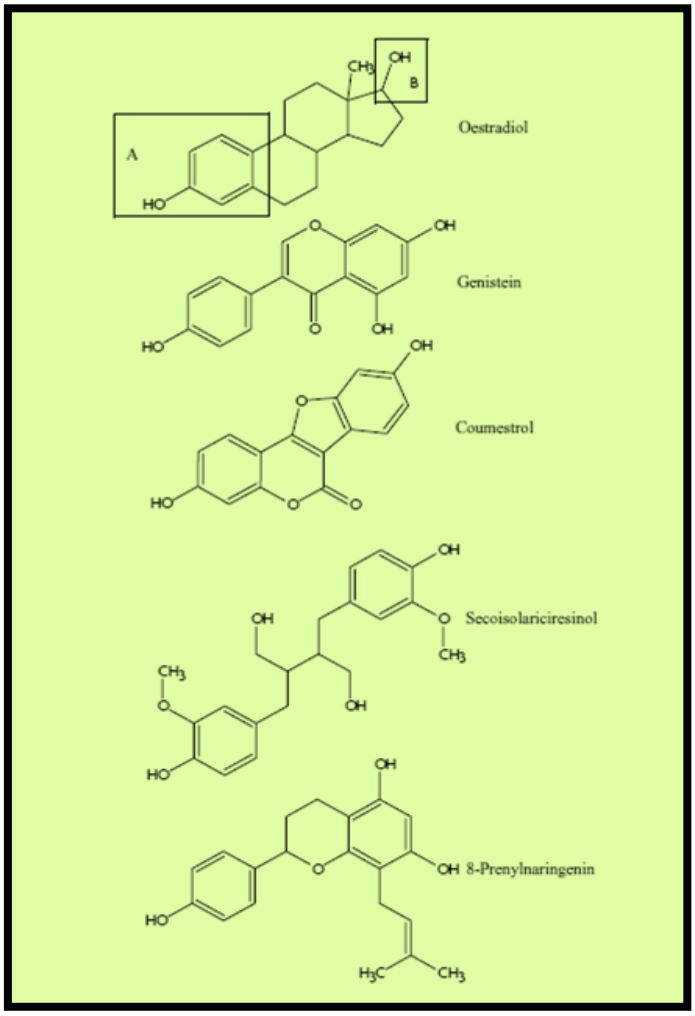
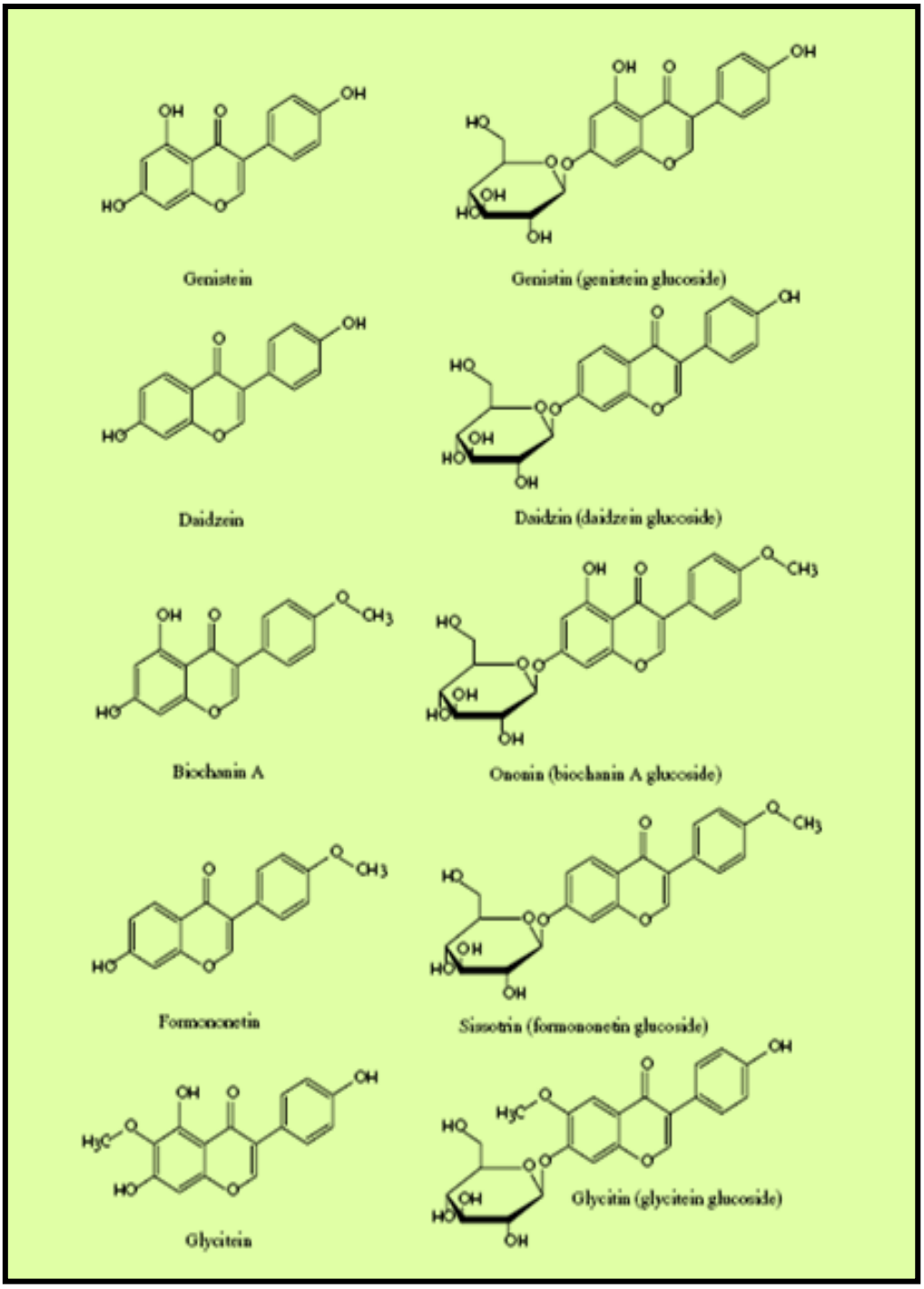


(Source: COT Report-Phytoestrogen and Health; 2003)

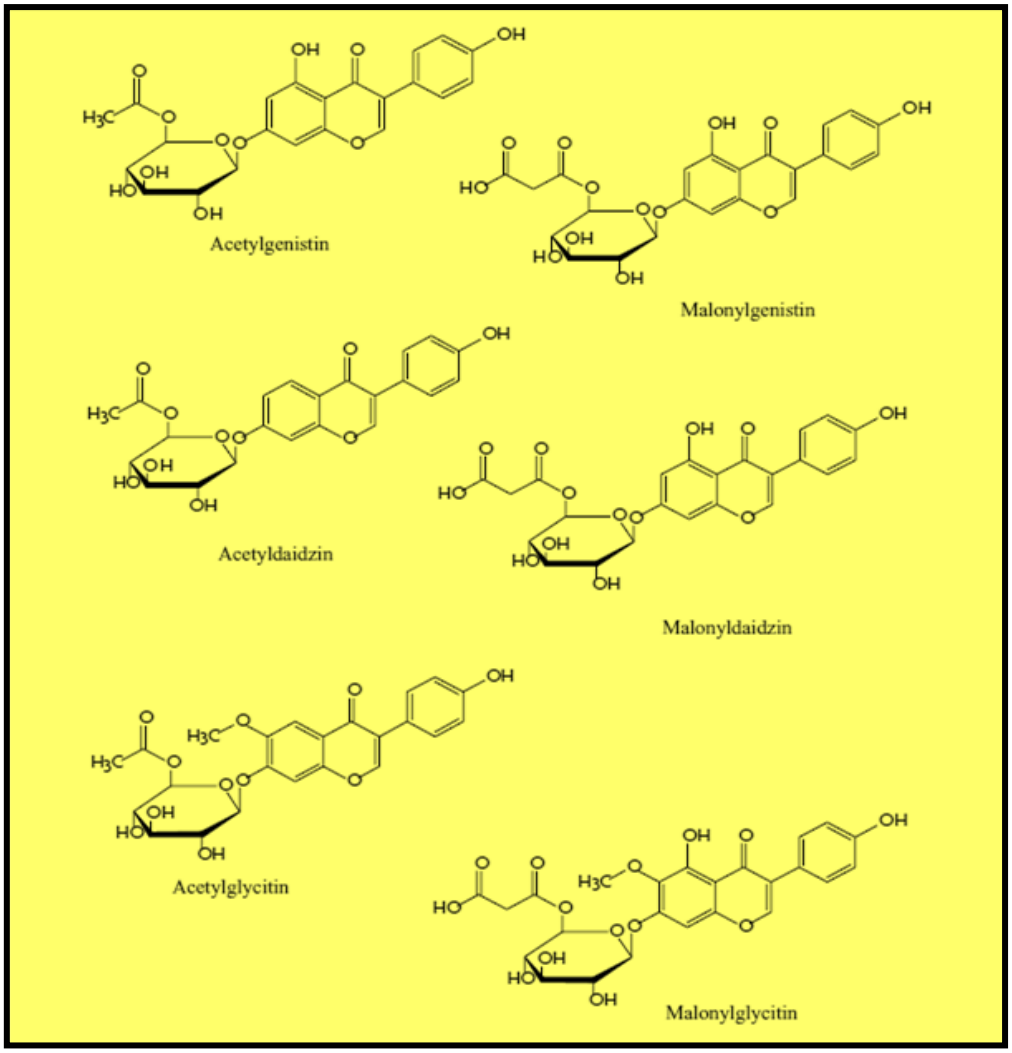
Figure 1.6: The structural similarities of phytoestrogens to estradiol

[All the strictures possess the phenolic (A) and hydroxyl (B) moieties outlined in boxes on the estradiol structure and the distances between the two groups in each compound are similar]



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.7: Chemical structures and names of the isoflavones most commonly found in food



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.7: Chemical structures and names of the isoflavones most commonly found in food (Contd.)

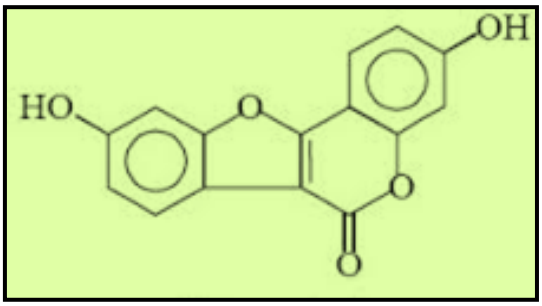
Isoflavones are low molecular weight hydrophobic compounds. Conjugation to glucose, glucuronide or sulfate groups increases water solubility. Acetylation or malonylation of glucose conjugates and methylation of the isoflavone moiety will alter water solubility.

Under acidic conditions, the glucones can be deconjugated to give aglucones. Whilst under acidic or basic conditions the acetyl- and malonyl groups can also be removed. In addition, malonyl groups can decarboxylate (lose CO_2) thus yielding acetyl groups. In the body, enzymes in the gut and liver can carry out these reactions during metabolism.

2. Coumestans

The Coumestrol is most commonly found and identified form of comestans in food. In compare to other phytoestrogen components, this is not well studied by researchers.

The structure of coumestrol is shown below.

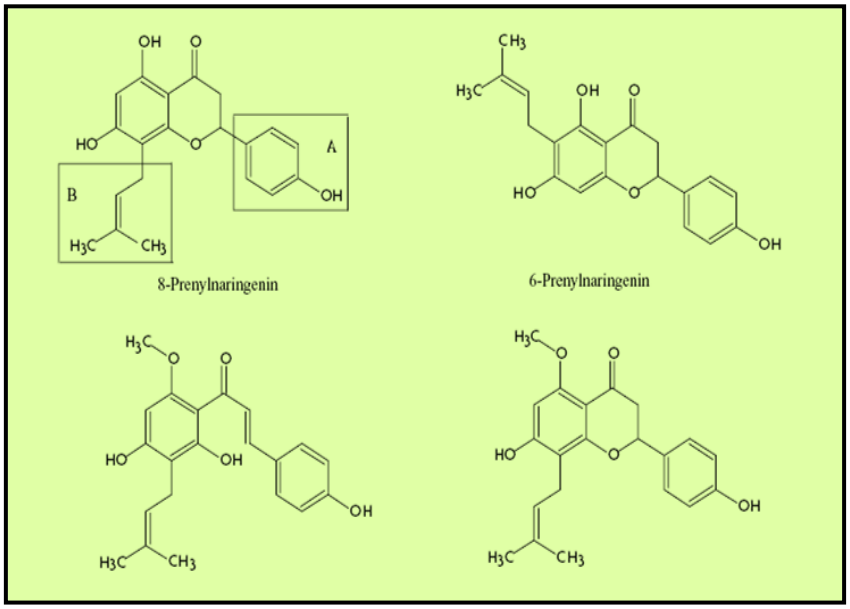


(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.8: Structure of coumestrol

3. Prenylated Flavonoids

The structure of these compounds is similar to the isoflavones, but substituted with a prenayl group (B) and the phenol ring (A) is oriented in a different direction (Figure 1.9). The presence of the prenyl group makes these less water soluble than isoflavones.



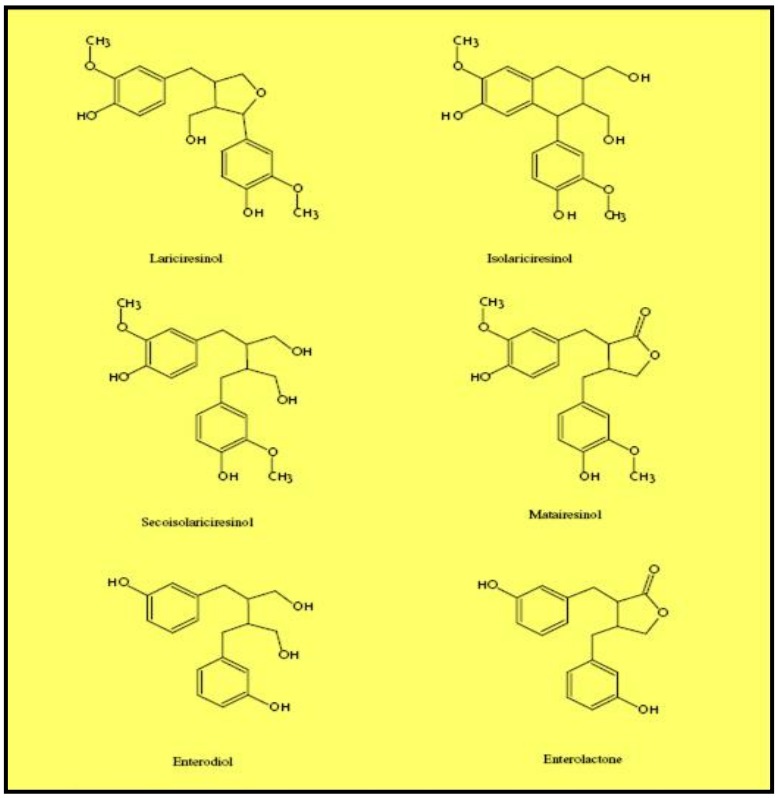
(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.9: The chemical structure of the prenylated flavonoids identified in food

4. Lignans

The lignans are the 2,3-substituted di-1,4-benzylbutane structure. The principal lignans identified in food are lariciresinol, isolariciresinol, matairesinol and secoisolariciresinol.

They are present as linked glucosides of differing chain length. For this reason, isolation of these compounds from plants and foods requires chemical treatment after which they are in the form of aglucones or glucosides.



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.10: Structures of Lignans most commonly found and identified in food

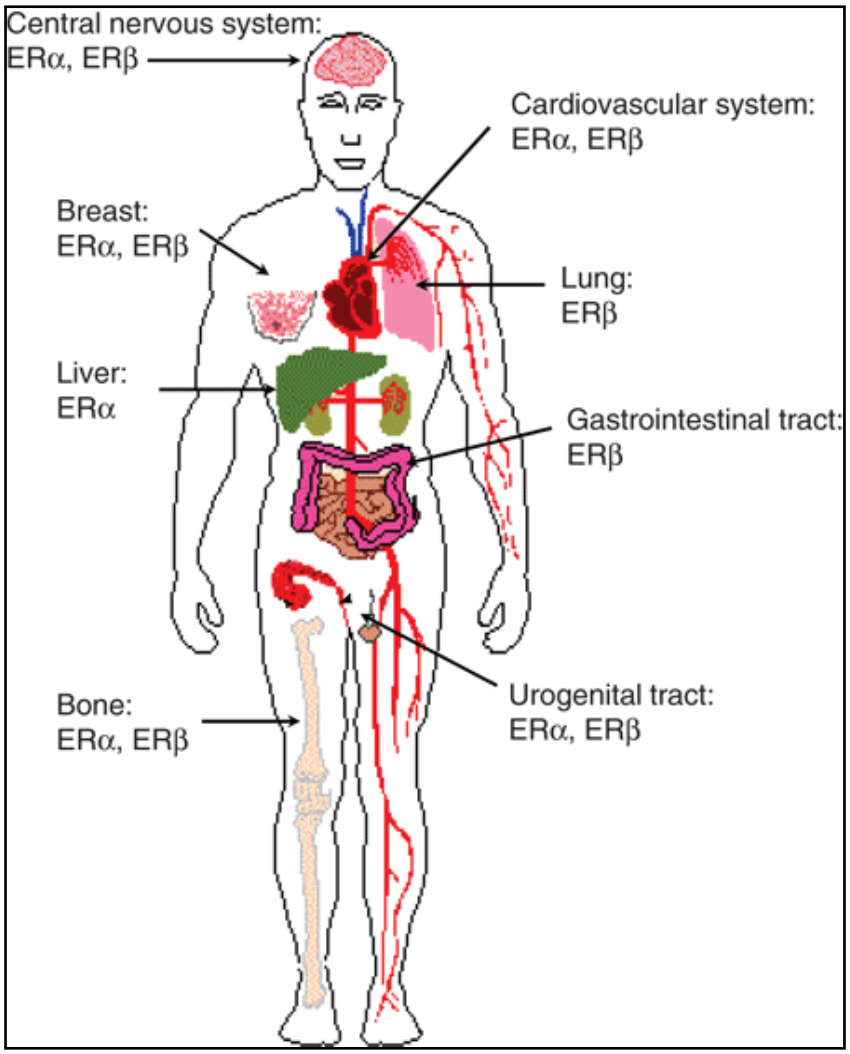
Lignans are thought not to be oestrogenic themselves, but are converted to the oestrogenic compounds enterolactone and enterodiol by the gut microflora. (Setchell KDR, Adlercruetz H, 1988)

Mechanism of Action

Phytoestrogens exert their effects primarily through binding to estrogen receptors (ER). (Turner JV, Agatonovic-Kustrin S, Glass BD, 2007)The binding affinity to ERs is determined by a planar ring system, two ring structures divided by two carbon atoms, and spacing between hydrophobic and hydrogen bond interaction. (Zhao E, Mu Q, 2011; Turner JV, Agatonovic-Kustrin S, Glass BD, 2007)There are two variants of the estrogen receptor, alpha (ER-α) and beta (ER-β) and many phytoestrogens display somewhat higher affinity for ER-β compared to ER-α. (Turner JV, Agatonovic-Kustrin S, Glass BD, 2007; Phytoestrogen – Wikipedia, 2014)

ERα is a major subtype in the tissues of breast,uterus, cervix, vagina and other reproductive organs. ERβ generally expressed in the ovary,prostate, testis, spleen, lung, hypothalamus and thymus. (Zhao E, Mu Q, 2011; Hall JM, Couse JF, Korach KS, 2001) (Figure 1.11)

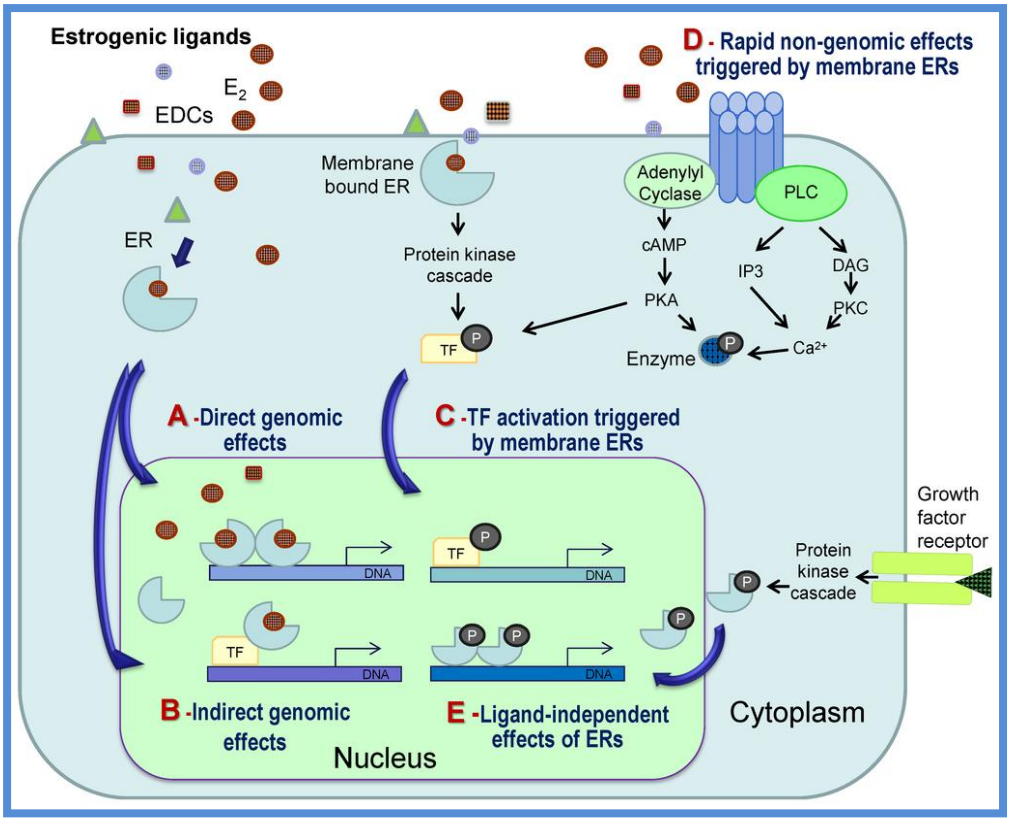
The ER-dependent and ER-independent effect of phytoestrogen indicate multiple mechanisms directing potential physiological and/or pharmacological actions in reproductive system and relevant diseases. (Zhao and Mu, 2011; Cassidy A, Faughnan M, 2000)



(Source:http://endocomprehensive.blogspot.in/2013/11/estrogen-receptors-importance-in.html)

Figure 1.11: Several sites for Estrogen receptors in the body

In the classical mode of action (**A**), an estrogenic ligand binds and activates intracellular estrogen receptors (in fish, ERα, ERβa or ERβb), which dimerize in the nucleus, bind to estrogen-response elements in the promoters of target genes and regulate their transcription, through the recruitment of a range of possible cell-specific co-regulators.



(Source: Laurentino *et al., OA Biotechnol.* **2012**, *1(2):* 4)

Figure 1.12: The possible mechanisms involved in the cellular actions of natural estrogens (e.g., 17β-estradiol, E2) and other estrogenic compounds

Alternative mechanisms of action involve (**B**) indirect regulation of gene expression by interaction of ligand-bound ERs with other transcription factors (TF). As shownin Figure 1.12 (**C**,**D**) estrogen actions initiated by binding to membrane receptors (ERs or G-protein coupled receptors, such as the GPER) and activation of protein kinase cascades or alterations in the levels of secondary messengers, resulting in (**C**) the activation of transcription factors that regulate gene expression or (**D**) rapid non-genomic effects, such as the activation of specific enzymes. While genomic actions can take hours to days, non-genomic effects occur in seconds or minutes.

In addition, ERs can be activated and regulate gene expression in a ligand-independent manner (**E**) through phosphorylation (P) in response to growth factor binding to their membrane receptors. Natural estrogens may compete with several EDCs (represented by different colors and shapes) for multiple receptors and pathways, resulting in a complex response that depends on the cellular context in terms of receptors and interacting proteins and, thus, may differ between tissues and circumstances.

Dietary phytoestrogens have received increasing attention for their effect on human health due to their structural and functional similarity to 17β-estradiol and, therefore, their ability to affect endocrine pathways. Several studies reported a beneficial effect on human health, for example, for cancer, cardiovascular disease, osteoporosis, menopausal symptoms, male infertility, and obesity and type-2 diabetes.

Metabolism of Phytoestrogens

Absorption, digestion, metabolism and excretion (ADME) have not been clearly reported in humans with respect to phytoestrogens. The research based information available on the isoflavones (daidzein and genistein) and to lesser extent on lignans (enterodiol and enterolactone), but no data reported on coumestans.

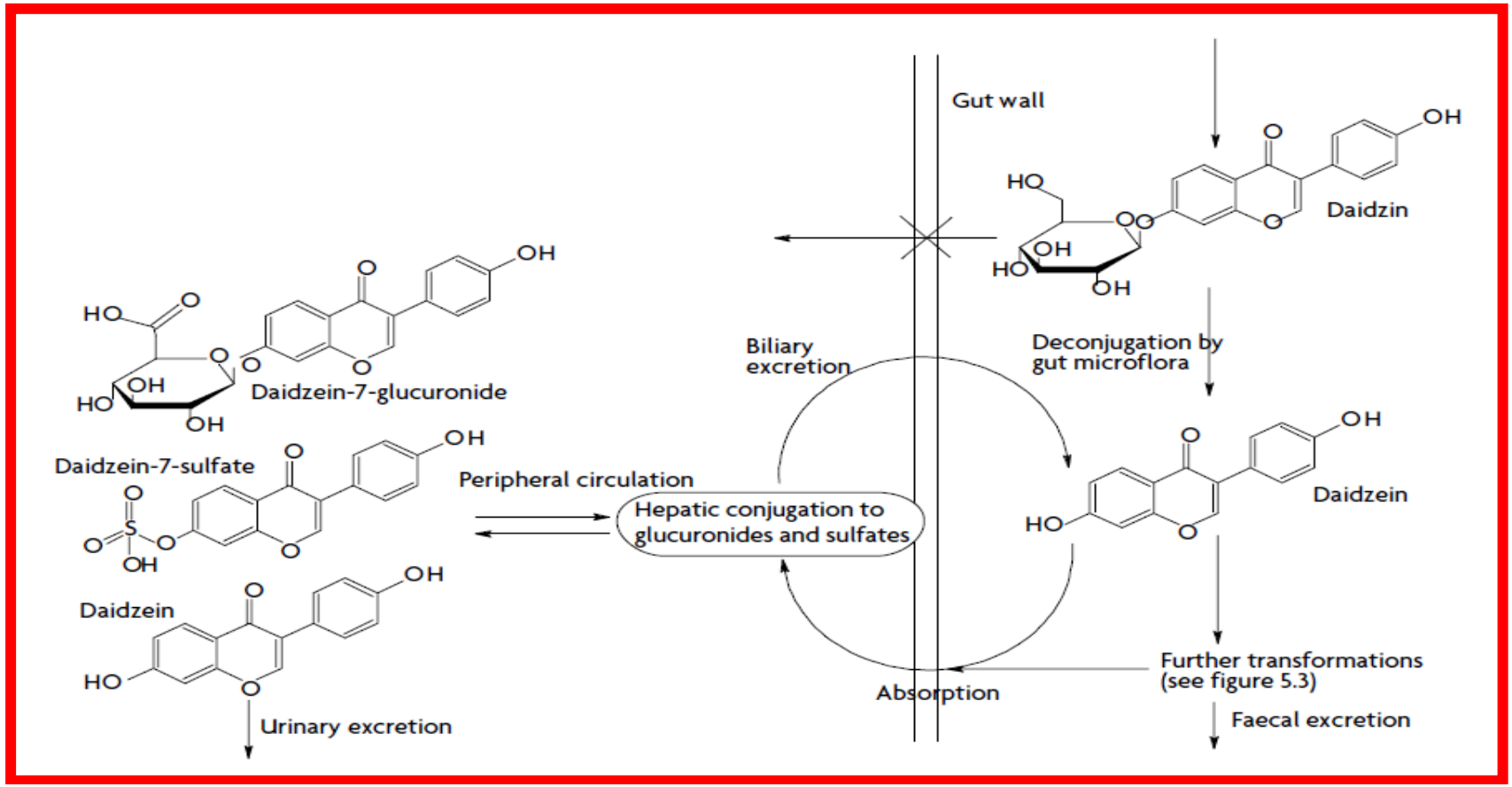
In food, isoflavones are generally present in their glucoside form. Isoflavone glucosides are not absorbed intact across the enterocyte of healthy adults and uptake requires hydrolysis of the isoflavone glucosides to their aglucone form. These aglucone forms have higher ***hydrophobicity and lower molecular weight***, which makes them more readily absorbed than parent glucosides.

Allred *et al* (2001) suggested that isoflavone glucosides can be converted to aglucones by enzymes in saliva. There is a controversy found for the acid hydrolysis of glucosides occurs in the stomach. (Piskula *et al*., 1999) Day *et al* (1998) reported that the liver and enterocytes of the human small intestine contain β-glucosidase enzyme capable of hydrolysing some naturally occurring flavones and isoflavones glucosides.

As shown in Figure 1.13, prior to the absorption form the gut, daidzin is converted to daidzein by gut micro floral enzymes. It is partially converted to glucuronide and sulphate conjugates by enzymes in the liver before entering the peripheral circulation. These conjugates can be excreted back into the gut from the liver via the bile duct (enterohepatic circulation) where they can be conjugated by gut micro floral enzymes. They may then be re-absorbed or further transformed in the gut and absorbed.

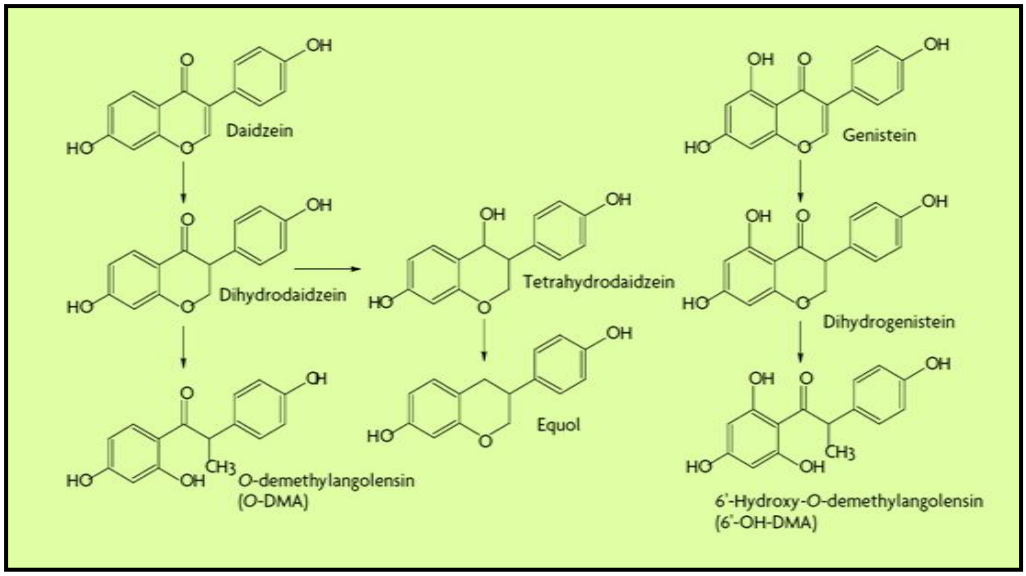
In humans, prior to absorption, the isoflavones may be further metabolized by the gut micro flora, with genistein being converted

to the hormonally inert *p*-ethyl-phenol and daidzein reduced to the estrogenically active isoflavone equol and the non-estrogenic *O*-demethylangolensin (*O*- DMA). (COT report-Phytoestrogen and Health, 2003)



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.13: Absorption of daidzein from the gut



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.14: Metabolism of daidzein and genistein based on human urinary metabolites

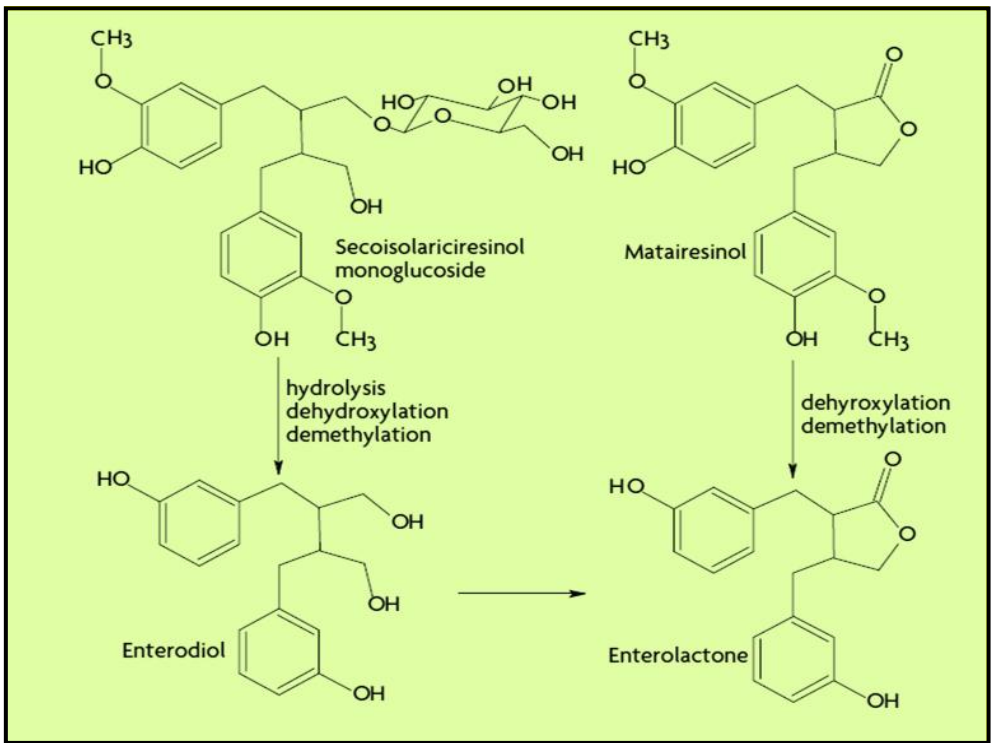
Lignans have been shown to undergo bacterial hydrolysis and metabolism. Colonic fermentations results in the removal of glucose residue, demethylation and dehydroxylation to the diphenol compounds, enterolactone and enterodiol, which are absorbed. Enterodiol may be further metabolized to enterolactone in the gut (Figure 1.15). (COT report-Phytoestrogen and Health, 2003)

Urine and bile are both important excretory routes for phytoestrogens. Several bacterial metabolites of isoflavones and lignans have been detected in urine and feces.

The majority of isoflavones are excreted in urine, either as parent compound or metabolites with only a small percentage of absorbed isoflavones appearing in the feces either as parent compounds or metabolites.

Conjugates excreted in the bile can undergo deconjugation by gut bacteria and undergo enterohepatic circulation (Figure 1.13). Elimination via the

feces is thus largely determined by the degree of enterohepatic circulation, which may result in prolonged exposure to these compounds.



(Source: COT Report-Phytoestrogen and Health; 2003)

Figure 1.15: Conversion of Lignans to enterolactone and enterodiol

Isoflavone and lignan phytoestrogens have been detected in a number of body fluids such as urine, plasma, faeces, prostatic fluid, semen, bile, saliva, breast milk, breast aspirate and cyst fluid. The major isoflavones and their metabolites detected in the blood and urine of humans and animals are daidzein, genistein, equol and *O*-DMA (COT report-Phytoestrogen and Health, 2003; Adlercreutz *et al*, 1995; Knight & Eden, 1996).

Lignans identified in human plasma and urine include enterolactone, enterodiol, lariciresinol and isolariciresinol (COT report-Phytoestrogen and Health, 2003; Jacob *et al*, 2002).

The peak concentrations of daidzein and genistein are achieved within 5-8 hours after ingestion. Plasma concentrations of genistein and daidzein

begin to rise within 2 hours of an ingested dose and can occur as early as 15 minutes after ingestion. It has been observed that a number of individuals exhibit more than one plasma peak, which probably reflects entero hepatic circulation of the isoflavones. The plasma half-lives for genistein and daidzein have been estimated at 5-8 hours. (COT report-Phytoestrogen and Health, 2003)

The data indicate considerable inter-individual variation in plasma and excretion profiles for daidzein and genistein and their metabolites.

The large inter-individual variation in phytoestrogen metabolism is likely to be largely a consequence of inter-individual differences in the gut microflora involved in metabolizing these compounds. Various physiological, pathological and environmental factors are likely to influence gut bacterial profile, including hygiene, antibiotic use, bowel disease, stress, gut motility, gastric pH, mucin secretion, bile secretion, diet and intestinal transit time. Gender, genetics and ethnicity may have a role in influencing gut bacterial profile although this remains to be clearly established (COT report-Phytoestrogen and Health, 2003; Kirijavainen & Gibson, 1999; Rowland*et al*, 1999).

Analysis of Phytoestrogens

Phytoestrogens and their metabolites are generally present in parts per billion to parts per million concentrations in plants, solid and liquid foodstuffs as well as in biological matrices such as plasma, serum, urine and faeces. Phytoestrogens must be isolated from the major constituents of these matrices before quantification. The type of matrix, phytoestrogen and the analytical method determine which isolation or extraction procedures are required. Extraction of phytoestrogens is usually not 100% efficient. The ratio of the internal standard to the analyte is measured during analysis to make this correction.

The quantification of phytoestrogens in foodstuffs as well as in pharmacological and toxicological studies is dependent on accurate and precise analytical methodology.

A general scheme of the steps involved in preparing a sample for analysis is shown in Figure 1.16.

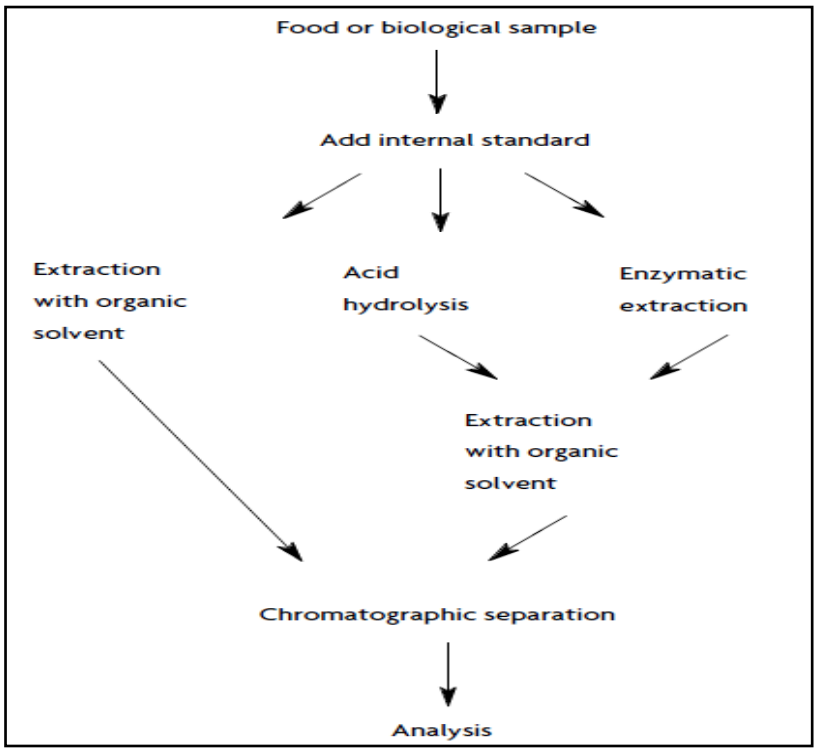


Figure 1.16: Sample preparation for phytoestrogen analysis

The most widely used techniques for measurement of phytoestrogens are:

- reversed phase high performance liquid chromatography with ultraviolet detection (HPLC-UV)
- gas chromatography with mass spectrometric detection (GC-MS)
- liquid chromatography with mass spectrometric detection (LC-MS)

The most appropriate analytical method is dependent on the type of biological matrix and compound to be analysed. (Hughes *et al*., 2003)

The range of detection limits reported varies widely for each method and between individual laboratories also, but generally the best reported sensitivity is as follows: immunoassay>HPLC–mass spectrometry=HPLC–multichannel electrochemical detection (coularray)>GC–single ion monitoring-mass spectrometry>HPLC–UV diode array>HPLC–single channel electrochemical detection. (Wilkinson AP, Wahala K, Williamson G, 2002)

Among the several studies on phytoestrogens, isoflavones (daidzein, genistein) have been most studied; very few and rare studies have done to evaluate the effect of lignan and coumestrol on human health. Rishi RK (2002) has also opined in his review that due to lack of funding and problems in analytical techniques this kind of research have got less attention. But in current scenario, study of effects of individual compounds in various clinical conditions is the need of the hour.

Based on dietary phytoestrogens, structure activity relationship studies should be carried out and more synthetic and semisynthetic compounds (like ipriflavone) should be evaluated. Genetic modification of soybean and other plants and improvement in food technology to enhance phytoestrogen production is inevitable.

Existing knowledge about phytoestrogens suggest that they have the power to reduce the deleterious effects, though its efficacy needs to be tested. Most of the identified phytoestrogen constituents consist of

sterols, coumestans and isoflavonoids. (Sharol Tilgner, Book: Herbal Medicine from the Heart of the Earth, 2003) Evidence for the beneficial effects of phytoestrogens is on the increase, but further studies are required to establish reference range for Indian population. In contrary, several databases have been generated for the phytoestrogenic components and amount in different diets/foods of western population.

The socio-economic changes are bringing nutrition transition; this in turn should benefit the health scenario of women - both urban and rural. For a woman hailing from the lower strata of society, it is better that she concentrates on having a good diet than opting for expensive treatments like HRT. Such studies on Indian women are not well documented. There is a need to assess the beneficial effects of these foods and its impact on menopause, if consumed on a daily basis (Davis S.2001).

Withthis hypothesis, a primitive study was conducted by Nair *et al*, (2007-2008) to confirm the effect of dietary phytoestrogen on menopausal problems. The results revealed 10.4% women were suffering from menopause and thyroid related symptoms (n=1000). As the fenugreek and sesame seeds are promising for their phytoestrogenic content (based on literature), the nutrition health education was given to women (n=104) to incorporate fenugreek and sesame seeds with other phytoestrogenic, iron and calcium rich foods on daily basis (45 days). The increase in consumption of phytoestrogen rich foods was ensured through the measurements of urinary isoflavone estimation and food frequency pattern of the subjects. The effect on menopausal symptoms was assessed by pre-tested questionnaire and FSH was also assessed to understand the effect of phytoestrogen at hormone level. After an intervention, there was a significant increase in urinary isoflavone excretion which reflects the improvement in phytoestrogen consumption amongst study population. There was a negative association observed

between FSH and urinary isoflavone excretion along with significant relief in menopausal symptoms after an intervention. Therefore, the study confirmed the beneficial effect of dietary phytoestrogen in ameliorating menopausal problems are of great value.

As the identification and quantification of phytoestrogenic components is not available for Indian foods/diet, what kind and amount of food should be consumed to have a significant improvement during menopausal transition is not being carried out till date.

To address the cause and concerns, based on primitive research by Nair *et al*., (2007-08) the present study was designed with the broad objective of **"Impact assessment of quantified phytoestrogens on MRS score and reproductive hormones amongst women in perimenopause phase"**

The specific objectives of the study were as follows:

1. To screen the women (n=1000) undergoing menopause related issues.
2. To map the prevalence of perimenopausal women.
3. To identify and quantify the phytoestrogenic compounds in selected foods.
4. To create awareness regarding consumption of phytoestrogen and importance of stretching and laughing exercise by providing Nutrition Health Education (NHE).
5. To compare the impact of different phytoestrogenic foods to understand its role and benefits to combat menopause.
    a) Effect on hormonal status
    b) Effect on menopause related symptoms