Chapter 2

Review of Literature

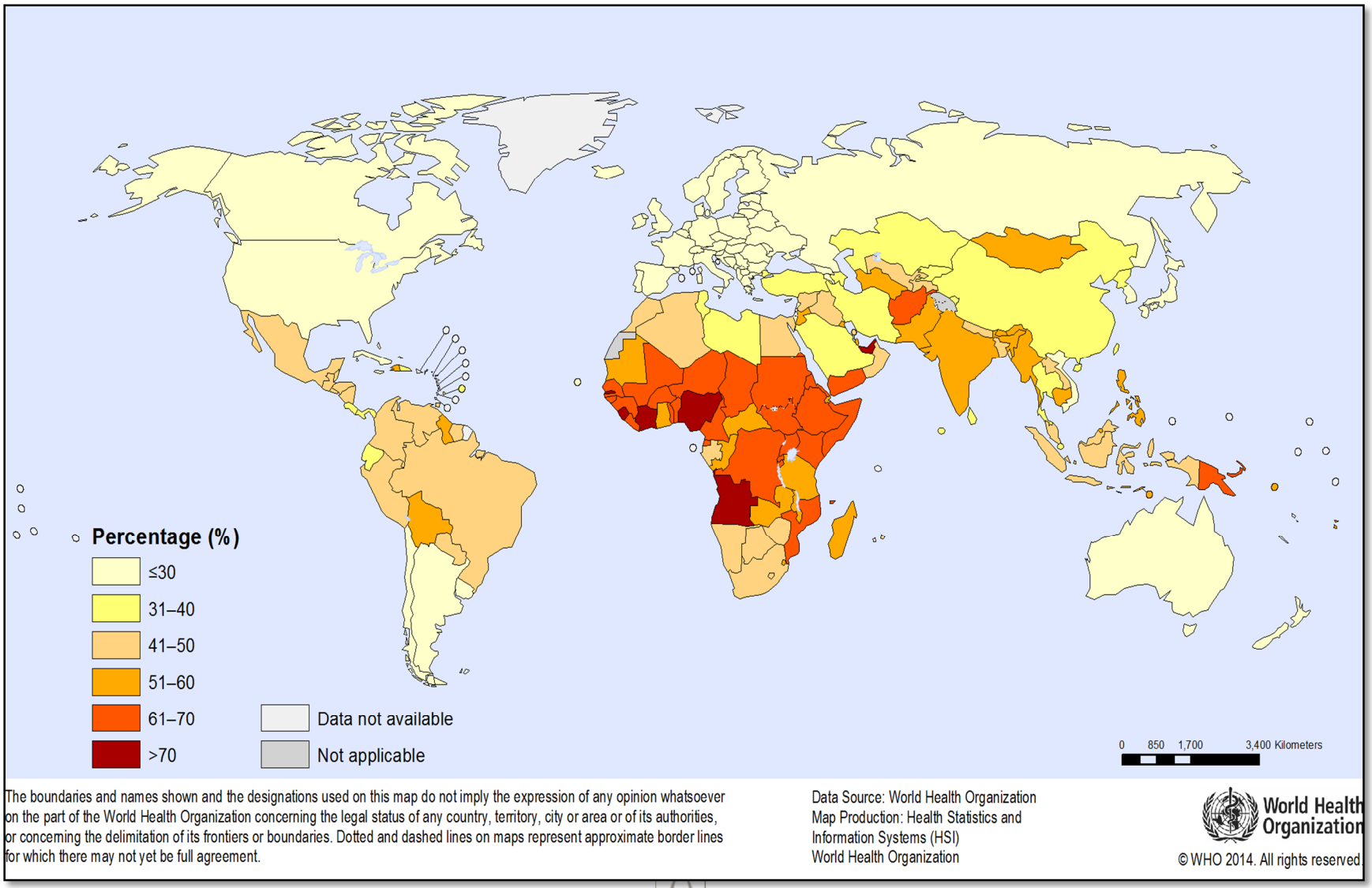
NON-COMMUNICABLE DISEASES AND WOMEN'S HEALTH

Women face various physiological changes and health related issues throughout their life span. However health care services are less accessible to them due to gender discrimination, economic, social, cultural, and legal barriers (US, Global health initiative, 2010). The average life expectancy rate and health life expectancy rate among south-east Asian (SEAR) females is 71 years, which is lower than the global female (73.8 years) population. Healthy life expectancy, which is an indicator of overall health of the population, is 63 years among SEAR females (World health Statistics, 2016). In the adolescence and reproductive age women pregnancy and child berth complications and HIV are the major cause of death in developing countries (WHO, 2009).

As the age progresses the burden of morbidity shifts to non-communicable diseases. The pprobability of dying from any of the four main NCDs between ages 30 and 70 is very high (26.1%) among Indians (NCD,2014). Cardiovascular diseases and diabetes are the leading causes of mortality among aged women (over 60 years) followed by other non-communicable diseases (WHO, 2009). According to WHO Global Observatory Data (2014), death rate of women due to non-communicable diseases is 586 per 1,00,000 populations and NCDs denotes about 51-60% of death among women in India (Figure 2.1). Among NCDs, highest death occur due to cardiovascular diseases (33.3%) followed by cancer (13%) and diabetes (3.1%) around the world (NCD Alliance, 2011).

While non-communicable diseases are on a rise worldwide, Indian women are at greater risk for developing these diseases. Indians genetically have relatively lower BMI and lean body mass with higher abdominal obesity and body fat "the thin fat type" (Joshi, 2012).

FIGURE 2.1: PERCENTAGE OF DEATHS DUE TO NON-COMMUNICABLE DISEASES OCCURRING UNDER AGE OF 70 (FEMALES), 2014



This genetic makeup of Indians, the so called "Asian Indian Phenotype" makes Asian Indians more prone to diabetes and premature coronary artery disease (Mohan et al, 2007). On one hand the non-modifiable genetic factors are not favorable for Indian population, the environmental changes in the recent years have added to the misery. In the developing countries like India urbanization, economic development and globalization has led to tremendous changes in lifestyle and dietary practices which are deleterious for health (Shetty, 2002).

The common modifiable risk factors of NCDs are physical inactivity, unhealthy diet, tobacco and alcohol consumption. Smoking is the cause of death of 1 out of 20 women in India (World Bank, 2011). According to Global Adult Tobacco Survey (2009-10) 20.3% of Indian women (15+ years) consume tobacco with higher prevalence in rural than urban setting. The prevalence of alcohol consumption among Indian women is 10.6% with 0.4% having alcohol dependence (WHO, 2014). Around 4% and 6% of female mortality in lower middle income group countries can be attributed to low fruit and vegetable intake and physical inactivity respectively (WHO, 2004). Worldwide 8% death of women (20+ years) have been attributed to sodium consumption of >2g/day. Unhealthy diet and lifestyle practices can bring about metabolic changes in the body. Metabolic risk factors like high blood pressure, cholesterol, glucose levels and overweight/obesity can lead to 18%, 5% 7% and 6% of total death among women in lower middle income group countries (WHO, 2004).

Thus a vicious combination of the predisposed genetic factors, lifestyle and dietary negligence and gender discrimination poses a high risk of developing non-communicable diseases among Indian women. In India the population above 65 is expected to rise from 4.4% (2000) to 7.6% (2025), leading to increased burden of non-communicable diseases due to aging (World Bank, 2011). Menopause is a condition, unique for females, with extensive physiological and psychological changes in combination with advancement of age. It is emerging as a risk factor for various non-communicable diseases in women. A

key portion of the current review revolves around menopause and its association with NCDs.

MENOPAUSE: PHYSIOLOGY, ENDOCRINOLOGY AND SYMPTOMS

WHO (1990) has defined the term menopause (natural menopause) as "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." The term menopausal transition refers to the time before the final menstrual period when variability in the menstrual cycle is usually increased. The time period immediately prior to the menopause and the first year after initiation of menopausal transition is called perimenopause (WHO, 1990).

Table 2.1 (Kaur et al, 2016) describes the stages of normal reproductive aging in women recommended by reproductive aging workshop (STRAW, 2001). According to STRAW, three stages i.e. early (-5), peak (-4) and late reproductive (-3) stages cover reproductive age of the females during which menstruation cycle remains regular. Menopause transition covers early (-2) and late (-1) stages, with variability in menstruation cycle from 7 days to >60 days. '0' stage denotes to final menstruation period after which post menopause starts. Post menopause is also divided into 2 phases i.e. early (+1) and late (+2) which lasts for 4 years and until demise respectively. Peri-menopause is a term which includes menopausal transition and one year post final menstruation period.

Follicle loss in a woman's body is initiated at the fetal stage only and continues throughout childhood, adolescence and reproductive age at varying rates. The depletion rate is highest at 7^{th} month of fetal stage and at birth which further shows a linear loss till menopause transition. (Block, 1952; Baker, 1963). As women enter into the menopause transition, follicle loss is accelerated and ovaries are completely depleted of follicles at menopause (Richardson et al, 1987).

There is a vivid change in various hormones like follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, inhibin B and anti mullerian hormone (AMH) during menopause transition (Su and Freeman, 2009). FSH is a glycoprotein hormone released by anterior pituitary gland (Kumar et al, 1997). It regulates the development and selection of ovarian follicles, oocyte maturation and ovulatory process (Ulloa-Aguirre, 1998). During menopause transition, level of FSH increases which is an indicator of decreased follicle supply. In early transition phase the rise is more of intermittent however it gets sustained in the late transition stage (Randolph et al, 2011). Pituitary hormone also releases luteinizing hormone (LH) which increases during ovulation.

Estrogen is one of the major steroidal hormones related to the reproduction. There are three forms of estrogen produced by ovary: Estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the most powerful estrogen in non-pregnant women of reproductive age which is produced by overies during menstruation cycle (Elder and Thacker, 2013). It stimulates follicular growth and maturation. There is a decrease in estradiol levels with progression of menopause (Burger et al, 1995).

Secretion of progesterone hormone which is secreted by corpus luteum after ovulation is also inhibited during menopause transition due to anovulation. No production of progesterone leads to irregular bleeding as estrogen may lead to thickening of endometrium (Elder and Thacker, 2013).

Another hormone associated with menopause transition is inhibin which inhibits FSH secretion. There are two type of inhibin: A and B. Inhibin A starts decreasing 4 years prior to menopause (Overlie et al, 2005). Inhibin B is produced by the granulosa cells of the growing follicle and the levels are decreased with shrinkage of the follicle cohort (Danforth et al, 1998, Seifer et al, 1997). Similar to inhibin, antimullerian hormone or mullerian inhibiting substance (AMH/ MIS) is secreted by early antral follicles reflecting that the follicle reserve also decreases with menopause transition (Santoro and Randolph, 2011).

Negative and positive feedback loop plays a major role in regulating menstruation cycle among women. FSH released by pituitary glands stimulates follicle and these follicles produce estrogen (Hillier et al, 1981). Estrogen inhibits FHS production through negative feedback effect on hypothalamus gonadotropin releasing hormones. Simultaneously it stimulates luteinizing hormone for ovulation through positive feedback mechanism (Yen et al, 1972; Liu and Yen, 1983). LH hormone triggers formation of corpus luteum which releases progesterone hormone to facilitate pregnancy (Weiss et al, 2004). Progesterone in result also suppresses LH production through negative feedback on hypothalamus.

Blagosklonny (2010) proposed a quasi-programmed nature of menopause. The hypothalamus is extremely sensitive to estrogens before puberty therefore low levels of estrogen also keeps FSH levels suppressed (Winter and Faiman, 1973). At puberty due to increased resistance of hypothalamus low levels of estrogen fails to suppress FSH (Foster and Ryan, 1979). And the menstruation cycle goes on through the feedback loop. This resistance of hypothalamus keeps on increasing with age leading to increased FSH levels (Sherman et al, 1976). As depicted in figure 2.2 high FHS levels overstimulate ovary and eventually lead to depletion of follicles (Blagosklonny, 2010).

According to Blagosklonny (2010) menopause is not caused by any specific program. It is a quasi-program, caused by ever-increasing resistance of hypothalamus to estrogen (and other hormones) which was initiated during puberty to root menstruation. It means both initiation and termination of the menstrual cycle is caused by increase in resistance of hypothalamus to estrogen. The program which started the reproductive process in women only ended the same.

Sherman et al (1976) indicated that irregular cycles during menopausal transition may be due to hormonal changes denoting imbalanced maturation of follicles anovulation and altered feedback mechanism. There are various factors which may affect the timing of natural menopause. Socio demographic (education, employment, race/ethnicity),, menstrual and reproductive history (parity and OC use), body composition, family, genetic

and early childhood factors, physical activity and diet, smoking, socio economic status are the major factors associated with the age of menopause of women (Gold, 2011).

MENOPAUSAL SYMPTOMS

Due to deficiency of estrogen various symptoms can manifest during menopausal transition. Various organs in our body have estrogen receptors therefore estrogen plays a regulatory function of these organs. The length of menopausal symptoms can be initiated even 6 years before the final menstrual period and continue many years post menopause (Butler and Santoro, 2011; Santoro and Randolph, 2011; McKinlay et al, 1992).

Women in the menopausal transition experience various vasomotor (hot flushes and night sweats), urogenital (vaginal symptoms, urinary incontinence), somatic (fatigue, headache, joint pains) and psychological symptoms (trouble sleeping, depression, anxiety, labile mood) (Grady, 2006). Though, after adjusting for age and other confounders only vasomotor symptoms, vaginal symptoms, and trouble sleeping are found to be strongly associated with the menopausal transition (Dennerstein et al, 2000).

Vasomotor symptoms

Hot flashes and night sweats are two major vasomotor symptoms experienced by women. Hot flashes is the most frequently reported (85% of women) symptom by women with varying frequency, intensity and duration (Santoro et al, 2015). Estrogen deficiency appears to be the major reason behind hot flashes. However, the exact mechanism behind the same is still unclear. An abrupt estrogen withdrawal is observed to result into rapid onset of hot flashes. (Casper and Yen, 1985; Sturdee, 2008). A US based multicentric cohort resulted that high FHS levels were significantly associated with hot flashes after adjustment for levels of estradiol and other hormones, indicating FHS as a major causal factor for hot flushes (Casper and Yen, 1985).

Hot flushes may be caused due to abnormal thermoregulation by the anterior hypothalamus which regulates perspiration and vasodilation. Body is programmed to maintain a homeostatic temperature range called as thermoregulatory zone (Casper and Yen, 1985). Women with a narrow thermoregulatory zone can experience hot flashes more frequently (Freedman and Krell, 1999) as elevated body temperatures or low sweating thresholds may trigger the hot flashes (Freedman, 2001). Another hypothesis associated with hot flashes is reduced serotonin levels. Decreased estrogen levels may reduce serotonin levels and thus upregulate serotonin receptor in the hypothalamus which may lead to hyperthermia (Kligman and Younus, 2010; Berendsan, 2000). Interaction of estrogen with neurotransmitters, such as norepinephrine are also thought to cause hot flashes (Casper & Yen, 1985).

Urogenital symptoms

Menopause may lead to decreased vaginal blood flow and secretions, hyalinization of collagen, fragmentation of elastin, and proliferation of vaginal connective tissue. Acidity of vaginal fluid which prevents reproductive organs from various bacterial infections gets reduced, leading to higher chances of infections among women. (Grady, 2006). Estrogen deficiency have been hypothesized as the causal factor however according to Leiblum et al, (1983), these symptoms have been associated with lower serum levels of androgens.

The prevalence of urogenital symptoms has been reported as 27-60% by some studies, of which vaginal dryness and dyspareunia being most prevalent (Santoro and Komi, 2009; Pastore et al, 2004). As urethra and bladder contain various estrogen receptors, deficiency of estrogen may lead to higher risk of urinary tract infections (Santoro et al, 2015).

Somatic symptoms

Common somatic symptoms associated with menopause are headaches, dizziness, joint pain and fatigue. Fluctuation in estrogen and progesterone hormones may lead to headaches. Estrogen has vasodilatory effect whereas progesterone acts as vasoconstrictor (Oh et al, 2012; Lucchesi et al 2013). Symptoms of migraine headaches like nausea, vomiting and sensitivity to light and noise, abdominal pain may appear during menopause. (Karli et al 2012). Dizziness can be directly or indirectly related to menopause and can pose serious health concern (Terauchi et al 2013). Fatigue denotes lack of energy, mood changes, and decreased work performance (Greenblum et al 2013). Sleep disturbances among menopausal women may also lead to fatigue (Moller et al 2013, Greenblum et al 2013). Sleep quality is generally decreased with age, and menopause along with aging can bring rapid deterioration in sleep quality. Sleep quality can also be affected by chronic poor sleep habits and mood disorders (Santoro et al, 2015). Other menopausal symptoms like night sweats, anxiety and depression (Lucchesi et al 2012) can also cause fatigue.

Psychological symptoms

There are various hormones and neurotransmitters which are associated with mood and emotion control like serotonin norepinephrine, dopamine, and melatonin. Estrogen deficiency can alter the functioning of these neurotransmitters (McEwen, 1999; McEwen, 2001; Glazer et al, 2002; Heikkinen et al 2002). Depression, anxiety and mood disorders are two major psychological symptoms of menopause. Menopause transition can lead to 3-fold risk for the development of depression than pre menopause (Freeman et al, 2006). 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). Hormonal changes can many a time lead to anxiety among women. They tend to worry, feel down or more anxious than usual during perimenopause. (Bauld and Brown 2009).

TABLE 2.1: STAGES OF REPRODUCTIVE AGING WORKSHOP +10 STAGING SYSTEM FOR REPRODUCTIVE AGING IN WOMEN

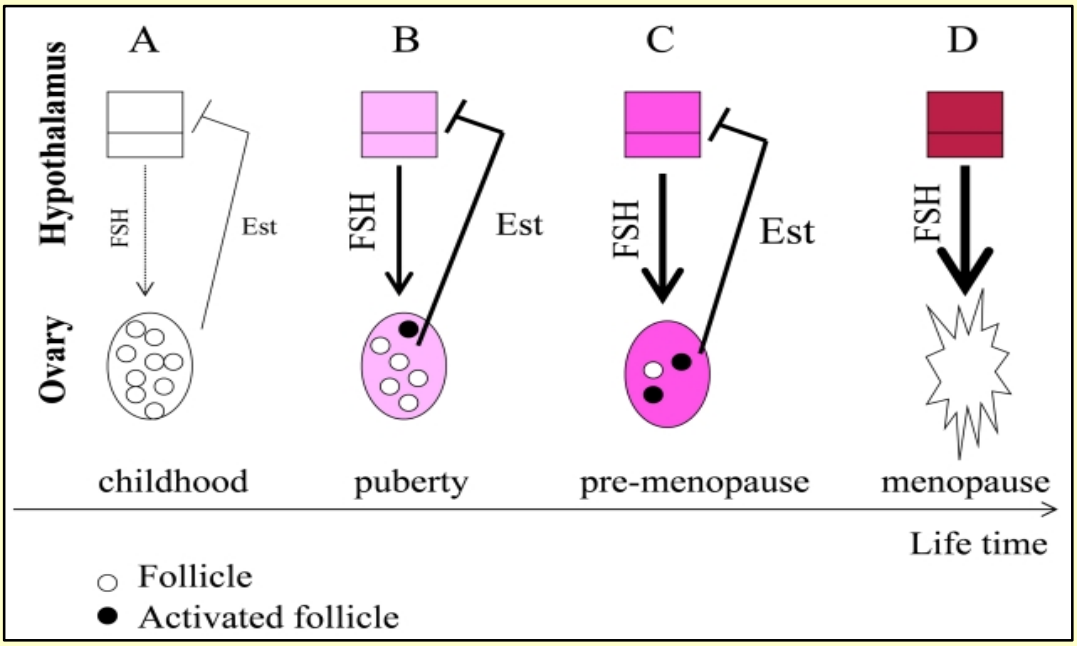
Menarche ▼ FMP (0) ▼

Stages	**-5**	**-4**	**-3b**	**-3a**	**-2**	**-1**	**+1a**	**+1b**	**+1c**	**+2**
Terminology	Reproductive				Menopausal transition		Post menopause			
	Early	Peak	Late		Early	Late*	Early*			Late
					Peri-menopause					
Duration of Stage	Variable				Variable		2 years (1+1)		3-6 years	Remaining lifespan
PRINCIPAL CRITERIA										
Menstrual cycles	Variable to regular	Regular	Regular	Subtle changes in flow length	Variable length persistent ≥7 days difference from consecutive cycle	Interval of amenorrhea ≥ 60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B	Low Low		Variable Low Low	↑ Variable Low Low	↑>25IU/L** Low Low		↑ Variable Low Low	Stabilizes Very Low Very Low		
Antral follicle count	Low		Low	Low	Low		Very Low	Very Low		
DESCRIPTIVE STATISTICS										
Symptoms					Vasomotor symptoms likely		Vasomotor symptoms most likely			Increasing symptoms of urogenital atrophy

*Blood draw on cycle days 2-5 ↑- Elevated **Approximate expected levels based on assays using current international pituitary standard

Source: Harlow et al, 2012

FIGURE 2.2: PROCESS FROM PROGRAMMED PUBERTY TO QUASI-PROGRAMMED MENOPAUSE



(**A**) In girls, the hypothalamus is extremely sensitive to estrogens and even low levels of estrogens inhibit FSH.

(**B**) The onset of menstrual cycle. While the hypothalamus is becoming resistant to estrogens, FSH stimulates the ovaries and estrogen production. Progressive activation of follicles from the dormant pool serves as the source of fertilizable ova.

(**C**) Pre-menopause. While the hypothalamus is becoming progressively resistant to estrogens, FSH progressively over-stimulates the ovaries.

(**D**) The ovaries fail. Menopause occurs when the primordial follicle pool is exhausted. Estrogen levels drop. The feedback between hypothalamus and the ovaries is disrupted.

Note: For simplicity, only the FSH-estrogen feedback loop is shown. FSH stimulates follicles and production of estrogens (Est). Estrogens inhibit FSH production (negative feedback).

Source: Blagosklonny (2010)

MENOPAUSE: ROLE IN PHYSIOLOGICAL AND METABOLIC ALTERATIONS

BODY COMPOSITION, OBESITY AND MENOPAUSE

Menopause brings about various hormonal changes. These hormones especially estrogen plays a crucial role in maintenance of body composition through two major mechanisms maintenance of body fat distribution and regulation of energy metabolism. During menopause estrogen production is hampered whereas androgen production remains constant (Wich and Carnes, 1995; Burger, 1994; Svendsen et al, 1993). High free testosterone levels and low sex hormone binding globulin can lead to body fat distribution and abdominal obesity in both pre and post-menopausal women (Evans et al, 1983; Kaye et al, 1991; Davis and Burger, 1996). A study performed by Rebuffe-Scrive et al (1986) resulted that after menopause the lipoprotein lipase (LPL) activity shows down in femoral adipose tissues while abdominal LPL activity remains the same causing higher abdominal fat accumulation. LPL activity is regulated by estrogen levels as evident by study performed by Lindberg et al (1990) and Price et al (1998). Lindberg et al (1990) indicated that estrogen alone or a combination of estrogen and progesterone therapy in post-menopausal women can increase femoral LPL activity. Transdermal estradiol treatment was found to be effective in decreasing adipose tissue LPL activity through regulation of gene expression as documented by Price et al (1998). Estrogen also plays role in maintaining energy balance through regulatory effect on energy intake and energy expenditure in hypothalamus (Figure 2.3). Estrogen receptor α supresses food intake through action on ARC POMC neurons whereas stimulates physical activity, energy expenditure and regulates body fat distribution through VMN SF1 neurons (Mauvais-Jarvis et al, 2013).

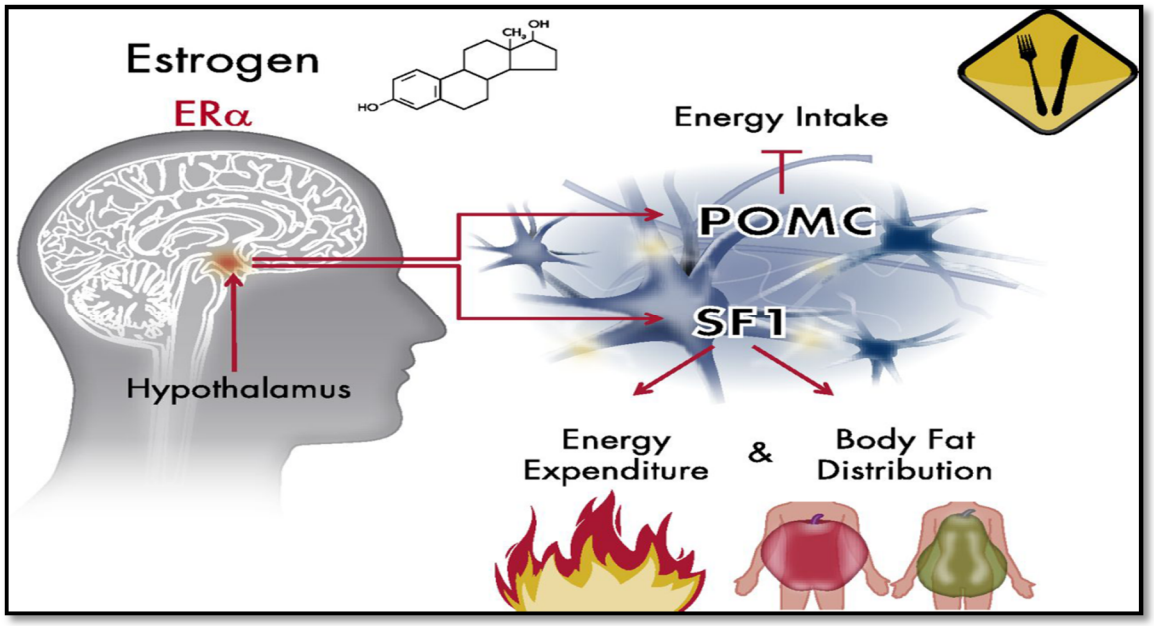
Menopause may lead to altered energy metabolism according to Poehlman et al (1995). The study showed that postmenopausal women had significantly lower resting metabolic rate (100kcal/day) than their premenopausal counterparts. Decreased physical activity along with low resting metabolic rate (RMR) can lead to positive energy balance and increased fat mass in post-menopausal women. (127 kcal/d) (Poehlman et

al, 1995). Decreased fat oxidation is also hypothesized to cause increased body fat mass among post-menopausal women. Fat oxidation unlike CHO and protein metabolism is not finely tuned with changes in intake. Therefore increased intake or decreased oxidation after menopause can lead to increased fat content of body (Calles-Escandon and Poehlman, 1997; Flatt 1987; Calles-Escandon et al, 1994). The underlying cause behind the reduced fat oxidation is speculated to be estrogen deficiency. Tchernof et al (2000), through a 6 year cohort demonstrated that respiratory quotient was increased in post-menopausal women in comparison to pre-menopausal ones suggesting that after menopause the body may rely more on carbohydrate as primary fuel source than fats. The authors hypothesized that changes in circulatory estrogen or androgen levels may lead to altered energy expenditure and substrate utilization (Figure 2.4) however the direct mechanism for the same is still unclear.

Fat and lean mass distribution was explored by Panotopoulos et al (1996) among European pre, peri and post-menopausal obese women using DEXA. The authors concluded that after adjustment for age and total fat, post-menopausal obese women had higher percentage of fat mass in the trunk area whereas premenopausal women had high percentage of fat and lean mass in the thigh and leg regions. Body fat percent was found to be significantly associated with menopause irrespective of age in a study performed by Svendsen et al (1995) on 407 Denish women. Similar results were observed in a cross sectional study (Douchi et al, 2002) of 566 pre and post-menopausal Japanese women and an eight year cohort during menopausal transition of 8 women performed by Franklin et al (2009) showed significantly higher fat mass in post-menopausal women.

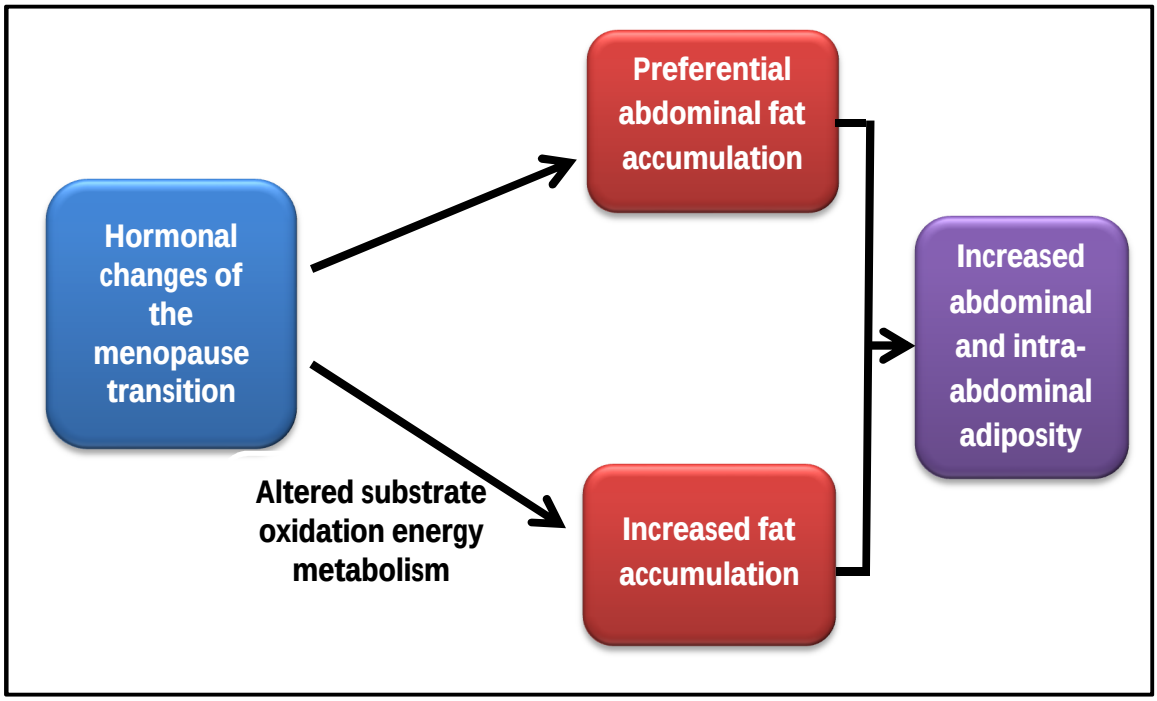
Obesity during menopause can affect bone health, menopausal symptoms and sexual health also. There is an ongoing debate weather obesity can impact bone health positively or negatively. Obese women are thought to be at lower risk of developing osteoporosis and fracture (Van Der Voort et al, 2001). However a recent longitudinal

FIGURE 2.3: SUMMARY OF HYPOTHALAMIC ESTROGEN ACTIONS REGULATING ENERGY BALANCE



Source: Mauvais-Jarvis et al, 2013

FIGURE 2.4: POTENTIAL MECHANISMS EXPLAINING MENOPAUSE RELATED INCREASES IN ABDOMINAL AND INTRA-ABDOMINAL ADIPOSITY



Source: Tchernof et al, 2000

study (n=60,393) including 10 countries revealed that the risk of incident ankle and upper leg fractures was significantly higher in obese postmenopausal women. Overweight among women can lead to various psychological symptoms like low self-esteem; mood swings etc. (Shah, 2009). A combination of obesity and these mood disorders can cause sexual dysfunctioning among post-menopausal women. However exact mechanism of interaction for the same is still not clear. Further post-menopausal women with abdominal obesity tend to suffer more from sexual problems in comparison to women with generalized obesity (Llaneza et al, 2007). According to the third Princeton Consensus Conference, treatment of the metabolic syndrome/obesity helps in reducing sexual dysfunction (Miner et al, 2012).

Various epidemiological studies have shown the association of menopause with obesity. A Brazilian study (Gravena et al, 2013) including 456 postmenopausal women ranging from 45-69 years revealed that 72.6% of the women were overweight (BMI ≥25) and 63.6% had abdominal obesity (WC≥88 cm). Dasgupta et al (2012) studied the relationship of obesity and menopause among Indian females (n= 169 pre-menopause, n=147 post-menopause) from Southern region. The results showed that post-menopausal women had significantly higher mean levels of weight ($p<0.001$), HC ($p<0.001$), WHR ($p<0.01$), BMI ($p<0.01$) and body fat percentage ($p<0.01$). The results were significant even after adjustment for age and BMI indicating the independent association of menopause and obesity.

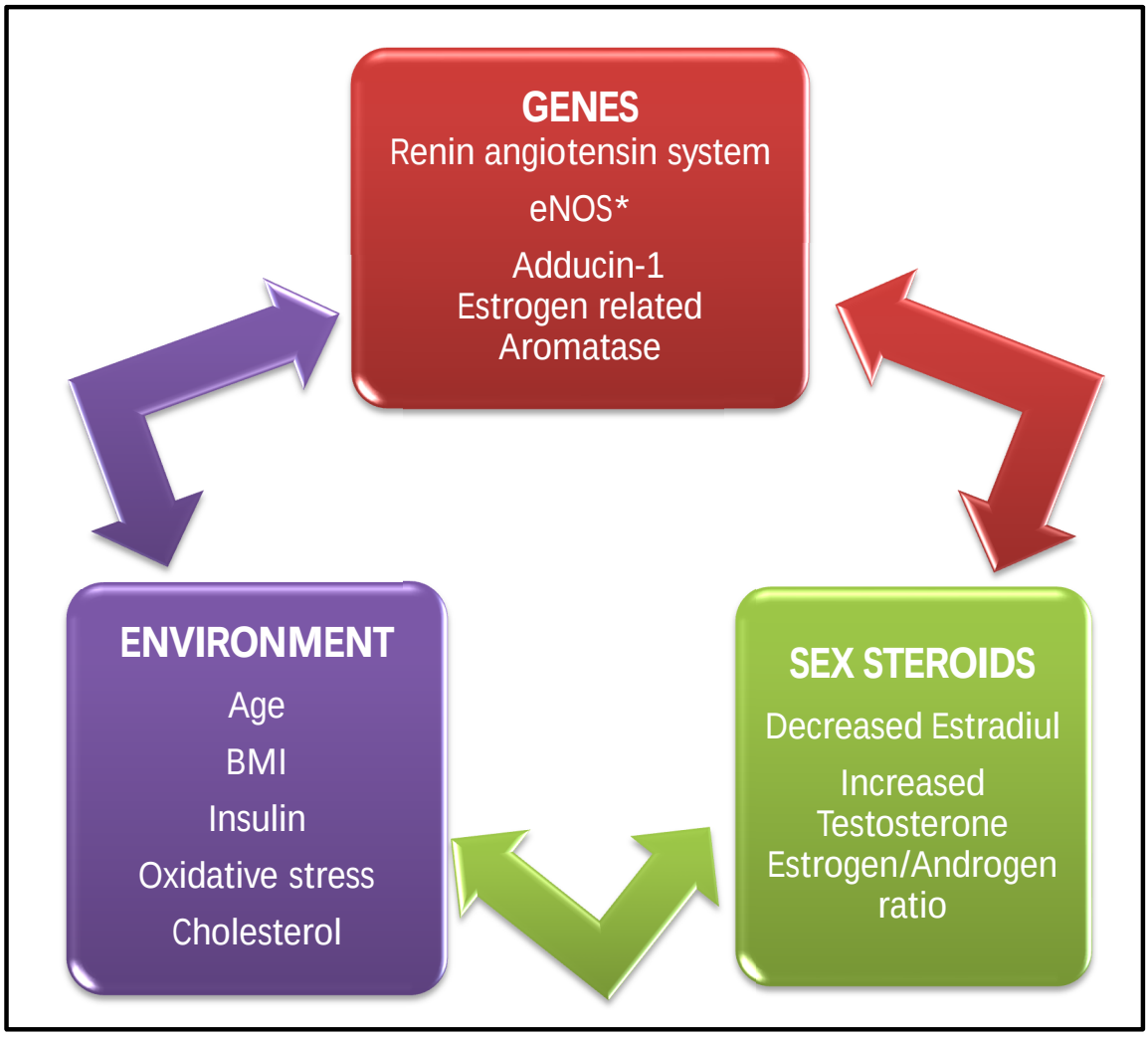
Khokhar et al (2010) also found higher prevalence of obesity (n=595) among post-menopausal women of North India (prevalence of BMI 70.30% and 75.09% , WC 75.15% and 89.05%, WHR 74.54% and 87.92% in pre and post-menopausal women respectively). A study performed by Elayath and Iyer (2013) in two major cities of west India Indicated that there was a significantly increase ($p<0.01$) in the prevalence of obesity across menopausal status of the women (n=399). About 53%, 62% and 75% of pre, peri and post-menopausal women were found to be obese in the study. Prevalence

of WC ($p<0.001$) and WSR ($p<0.001$) was also found to be significantly higher in post-menopausal women.

BLOOD PRESSURE AND MENOPAUSE

Menopause has been closely linked to high blood pressure among women. Rise in systolic blood pressure is observed higher in post-menopausal women in comparison to men (Staessen et al, 2001). Early age at menopause (age<45) was found to be independently associated with increase in pulse pressure (8.4, 95% CI 7.0–9.8) due to arterial stiffness than later menopause in a study performed by Luoto et al (2002). The major reason behind the increased blood pressure in post menopause is proposed due to low oestrogen levels which can lead to upregulation of the RAS and increase in plasma-renin activity (Schunkert et al, 1997). Salt sensitivity (renal hemodynamic response to salt) among post-menopausal women has also found to be higher which can be a potential factor for increased blood pressure (Pechere-Bertschi and Burnier, 2004). Salt sensitivity of systolic blood pressure in post-menopausal women not receiving HRT is thought to be related with increased levels of the NO synthase antagonist, asymmetrical dimethyl-L-arginine which leads to reduced bioavailability of NO (Scuteri et al, 2003). Estrogen and its metabolites scavenge free radicals and decrease ROS. Low estrogen levels thus causes increased oxidative stress which contributes to higher blood pressure. Coylewright et al (2008) explained the interrelationship of genetic, environmental factors and levels of sex hormones and their implications on development of hypertension (Figure 2.5). Decreased estradiol, increased testosterone levels leads to altered estrogen/androgen ratio along with other genetic and environmental factor can cause hypertension among post-menopausal women. Sympathetic activation leads to increased renin release and increase in angiotensin II (Ang II) which is a vaso-constrictor. Endothelial dysfunction along with reduction in NO and increase in endothelin may all lead to increased oxidative stress. All these changes contribute to increase in renal vasoconstriction resulting in hypertension (Figure 2.6). Furthermore it is postulated that chronic anxiety and depression may lead to increased

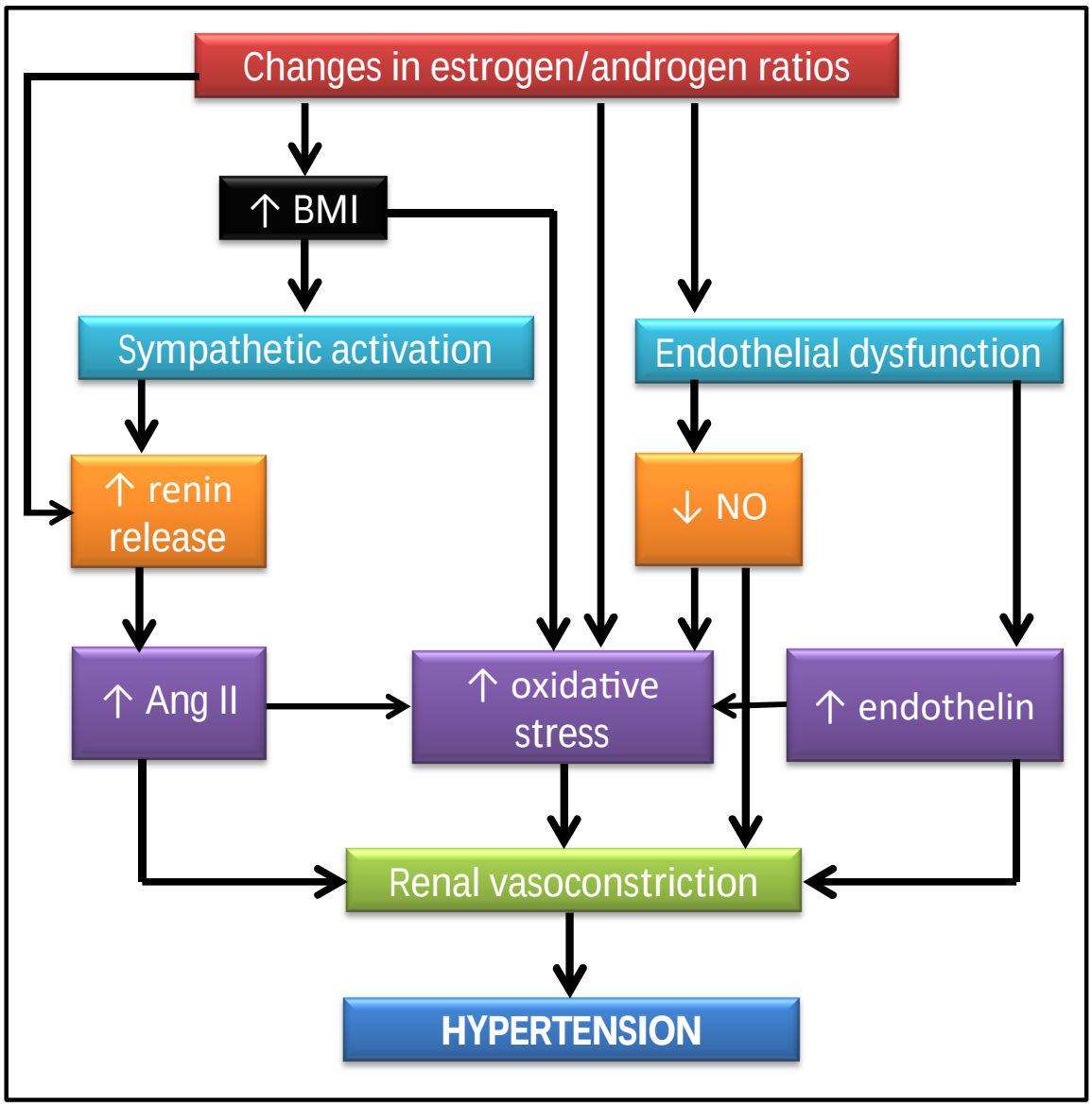
FIGURE 2.5: FACTORS CONTRIBUTING TO HYPERTENSION IN POSTMENOPAUSAL WOMEN



*eNOS- NO synthase.

Source: Coylewright et al, 2008

FIGURE 2.6: SEX HORMONES AND POSTMENOPAUSAL WOMEN HYPERTENSION



Source: Coylewright et al, 2008

blood pressure among post-menopausal however the area needs to be explored further (Yanes and Reckelhoff, 2011). The epidemiological data from different studies indicated high prevalence of hypertension among post-menopausal women. Prevalence of pre hypertension and hypertension among post-menopausal women was found to be 37% and 39.6% respectively in a population based study performed by Gupta et al (2014). Karnataka based Indian study on 216 women showed that post-menopausal women had significantly high systolic ($p<0.01$) and diastolic blood pressure ($p<0.05$) independent of age and BMI. Pandey et al (2010) indicated similar results in a study which included 498 women. The postmenopausal women were found to have significantly higher levels of SBP ($p<0.001$) after adjusting for age. Prevalence of hypertension among women from Ahmedabad and Vadodara was found to be 28%, 38% and 53% respectively in pre, peri and post-menopausal with a significant ($p<0.001$) increase with progression towards menopause.

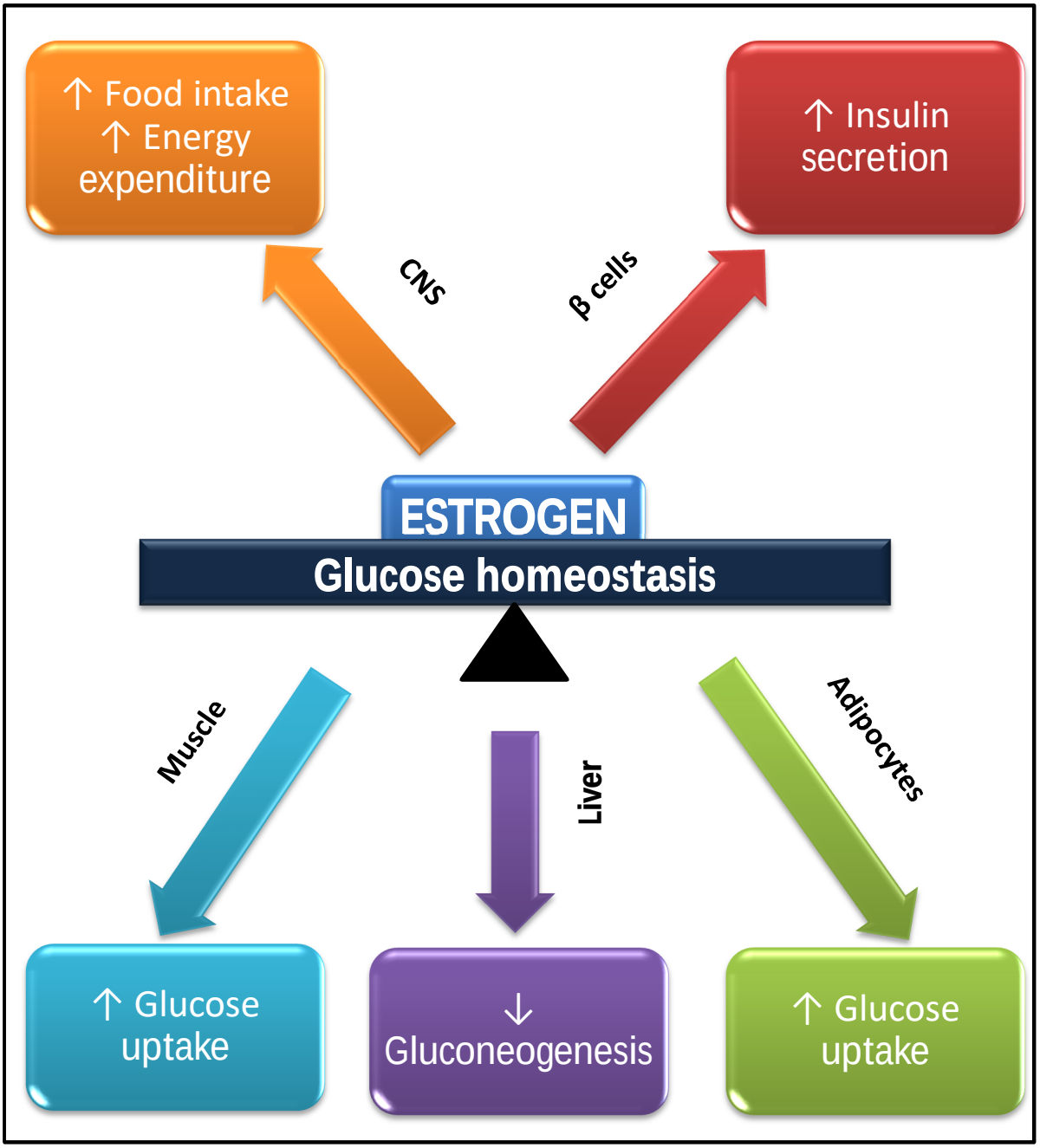
GLUCOSE METABOLISM AND MENOPAUSE

Menopause has a significant impact on insulin sensitivity, insulin resistance and development of type 2 diabetes mellitus. Estrogen helps in maintaining glucose metabolism (Figure 2.7) in tissues mainly liver, skeletal muscle, adipose tissue, pancreatic β cells, and CNS (Faulds et al, 2012) and its deficiency can lead to impaired glucose homeostasis in the body (Figure 2.8). Insulin sensitivity among post-menopausal or hystectomized women decreases along with other metabolic changes related to lipid metabolism and inflammation (Sites et al, 2002). Menopause can also impact the expression and translocation of GLUT 4 transporter. GLUT4 is a rate-limiting step in the insulin-induced glucose uptake in skeletal muscles (Bjornholm and Zierath, 2005). Estrogen treatment has found to improve glucose homeostasis through increase in muscle GLUT4 content in aging female rats (Moreno et al., 2010). Estrogen receptors participate in many of the steps of insulin-induced and insulin-independent glucose uptake. ERα and ERβ play key role in the physiology and pathology of the pancreas and important for proliferation, differentiation, and survival of β cells as well as regulation of

insulin synthesis and release. (Barros and Gustafsson, 2011). Estrogen influences the islet size but is important in determining as well as insulin release from the β cells (Godsland, 2005). It increases insulin secretion and reduced glucose plasma levels through classical transcriptional activation (Alonso-Magdalena et al, 2006). Cell apoptosis can lead to development of type 1 and 2 diabetes. Estrogen receptor α (ERα) is thought to protect β cells against apoptosis (Le May et al, 2006). ERα also protects liver from hypercholesterolemia (Lemieux et al, 2005), inflammation (Evans et al, 2002) and regulates glucose and lipid metabolism in liver ((Bryzgalova et al, 2006). Estrogen regulates the visceral fat diposition, leptin sensitivity, expression of insulin receptors in adipocytes, and lipogenic activity of lipoprotein lipase (Clegg et al, 2006). Therefore estrogen deficiency post menopause can lead to increased visceral fat mass decreased lipid utilization and insulin resistance (Wohlers and Spangenburg, 2010).

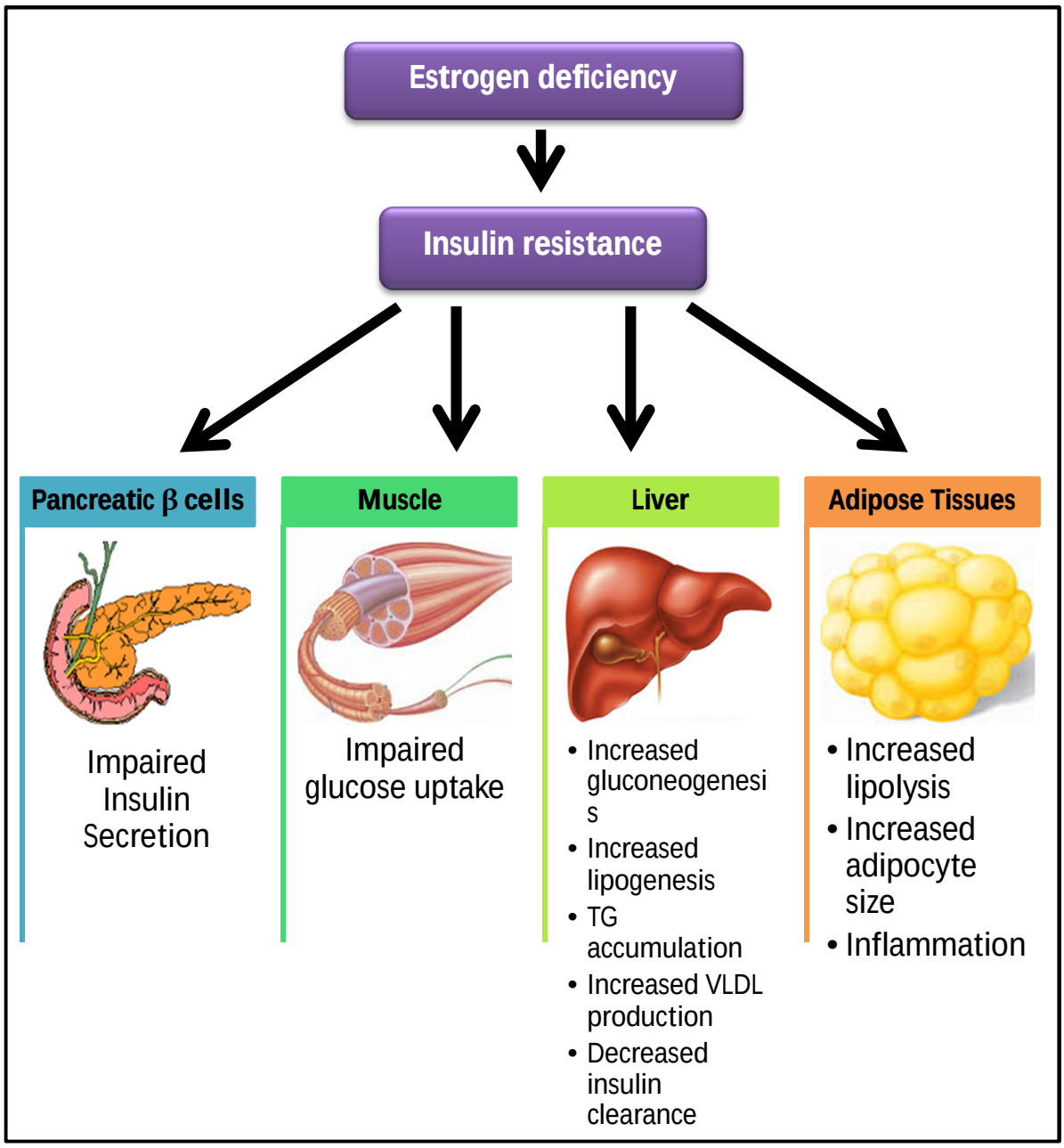
Epidemiological data also indicates the beneficial role of estrogen in prevention of insulin resistance and type II diabetes. A longitudinal study on 637 healthy non obese post-menopausal women was performed by Rossi et al (2004). Out of total women 21.4% took transdermal 17-β-estradiol and 78.6% never took hormones during their postmenopausal period. In the “hormones nonusers” group, diabetes developed in 10% whereas in the “hormones users” group, diabetes developed in 4.16% of the women. In another case control study on women not using any hormonal therapy (359 with newly diagnosed type 2 diabetes and 359 controls) higher plasma levels of sex hormone–binding globulin were prospectively associated with a lower risk of type 2 diabetes (Ding et al, 2009). Post-menopausal women from north India were having significantly higher levels of fasting blood glucose independent of age compared to pre-menopausal women in a study performed by Pandey et al (2010). Prevalence of diabetes was found to be significantly associated ($p<0.01$) with menopausal status in a study performed by Elayath and Iyer (2013). Dasgupta et al (2010) studied the effect of age v/s menopause on various metabolic aberrations among women and showed that post-menopausal women were found to have significantly higher mean values of fasting and post prandial

FIGURE 2.7: MODEL SHOWING ESTROGENIC CONTROL OF GLUCOSE HOMEOSTASIS BY REGULATORY ACTIONS IN CNS, B CELLS, MUSCLES, LIVER, AND ADIPOCYTES



Source: Faulds et al, 2012

FIGURE 2.8: OVERVIEW OF INSULIN RESISTANCE INDUCED BY ESTROGEN DEFICIENCY, AND SUBSEQUENT DISTURBANCES IN METABOLIC TISSUES



Source: Faulds et al, 2012

blood glucose levels. However this association was lost after adjusting the data for age and BMI.

LIPID METABOLISM AND MENOPAUSE

Estrogen levels play a significant role in the lipid metabolism. Estrogen reduces fatty acid and triglyceride synthesis and lipogenesis thus leading to suppression of white adipose tissue. The mechanism involves down-regulation of lipoprotein lipase and down-regulation of acetyl-coenzyme A carboxylate and fatty acid synthase which causes increased catecholamine-stimulated lipolysis and increased lipid-oxidative pathways in muscle (D'Eon et al, 2005). Estrogen hormonal therapy can suppress the genes related to various lipogenic enzymes like sterol regulatory element-binding protein 1c (SREBP-1c), stearoyl-CoA desaturase, fatty acid desaturase, and peroxisome proliferator-activated receptor (PPAR)-γ (Lundholm et al, 2008; Price et al, 1998). Lipid homeostasis and metabolism is regulated by a prominent gene Lipin 1 (LPIN1) (Reue and Dwyer, 2009). Upregulation of LPIN1 expression can lead to obesity (Miranda et al, 2007; van Harmelen et al, 2007). This LPIN1 gene is down-regulated by estrogen (Gonzalez et al, 2012).Estrogen inhibits lipogenesis and adipogenesis (Cooke and Naaz, 2004) through action in peripheral tissues. However its mechanism is direct action on white adipose tissues or indirect effect through central nervous system is still unclear. Estrogen receptor β (ERβ) exerts antilipogenic and antiadipogenic effects in adipocytes. Deficiency of ERβ increases PPARγ signaling and escalates WAT accumulation in female mice during high fat feeding (Foryst-Ludwig et al, 2008).

The epidemiological data also indicate higher lipid aberrations among post-menopausal women. A 7 year cohort on women health performed by Derby et al (2009) resulted that lipid abberations among women occurred during the late stages of menopause. TC, total LDL-C, TG, and lipoprotein (a) peaked during late peri and early post-menopause. (An Indian study (n=120) comparing the lipid profile of pre and post-menopausal women indicated that serum levels of TC, TG and LDL-C were significantly higher and HDL-C levels were significantly lower in post-menopausal women as compared to pre-

menopausal women of similar BMI (Bade et al, 2014). Similar results were observed in two recent studies on pre and post-menopausal women performed by Tiwari and Nagar (2015) and Kanwar et al (2014). Dasgupta et al (2012) studied the independent effect of menopause on lipid profile irrespective of age and BMI. Authors concluded that TC ($p<0.001$), LDL-C ($p<0.001$) and TG ($p<0.001$) levels were significantly higher in post-menopausal women and the significant difference was maintained even after adjustment for age and BMI. HDL-C levels were also significantly higher in post-menopausal women though the significance was lost after adjustment for BMI and age. The prevalence of significantly high TC ($p<0.001$), LDL-C ($p<0.05$) levels and low HDL-C ($p<0.05$) was found in the peri and post-menopausal women of Vadodara and Ahmedabad in comparison to pre-menopausal ones in a study performed by Elayath and Iyer (2013).

INFLAMMATION AND MENOPAUSE

Estrogens levels also affect the inflammatory status of the body. Hystectomy can lead to increased tissue inflammation (TNFα, iNOS, and CD11c) in perigonadal and inguinal fat. Absence of estrogen can elevate the T-cell marker CD3 and the Th1 cytokine interferon-γ in perigonadal fat of ovariectomized female mice (Rogers et al, 2009). Epidemiological data show that circulatory proinflammatory cytokines are elevated in women after natural menopause or surgical removal of ovaries (Pfeilschifter et al, 2002).

Macrophages are important component of innate and adaptive immunity. They also play significant role in regulating metablic processes and insulin sensitivity. (Ribas et al, 2010; Barros et al, 2006). A study performed by Ribas et al (2010) showed that altered plasma adipokine and cytokine levels, glucose intolerance, insulin resistance, and increased adipose tissue mass were observed in animals with a hematopoietic or myeloid-specific deletion of *Esr1*. In isolated macrophages, ERα is necessary for repression of inflammation, maintenance of oxidative metabolism, IL-4-mediated induction of alternative activation, full phagocytic capacity in response to

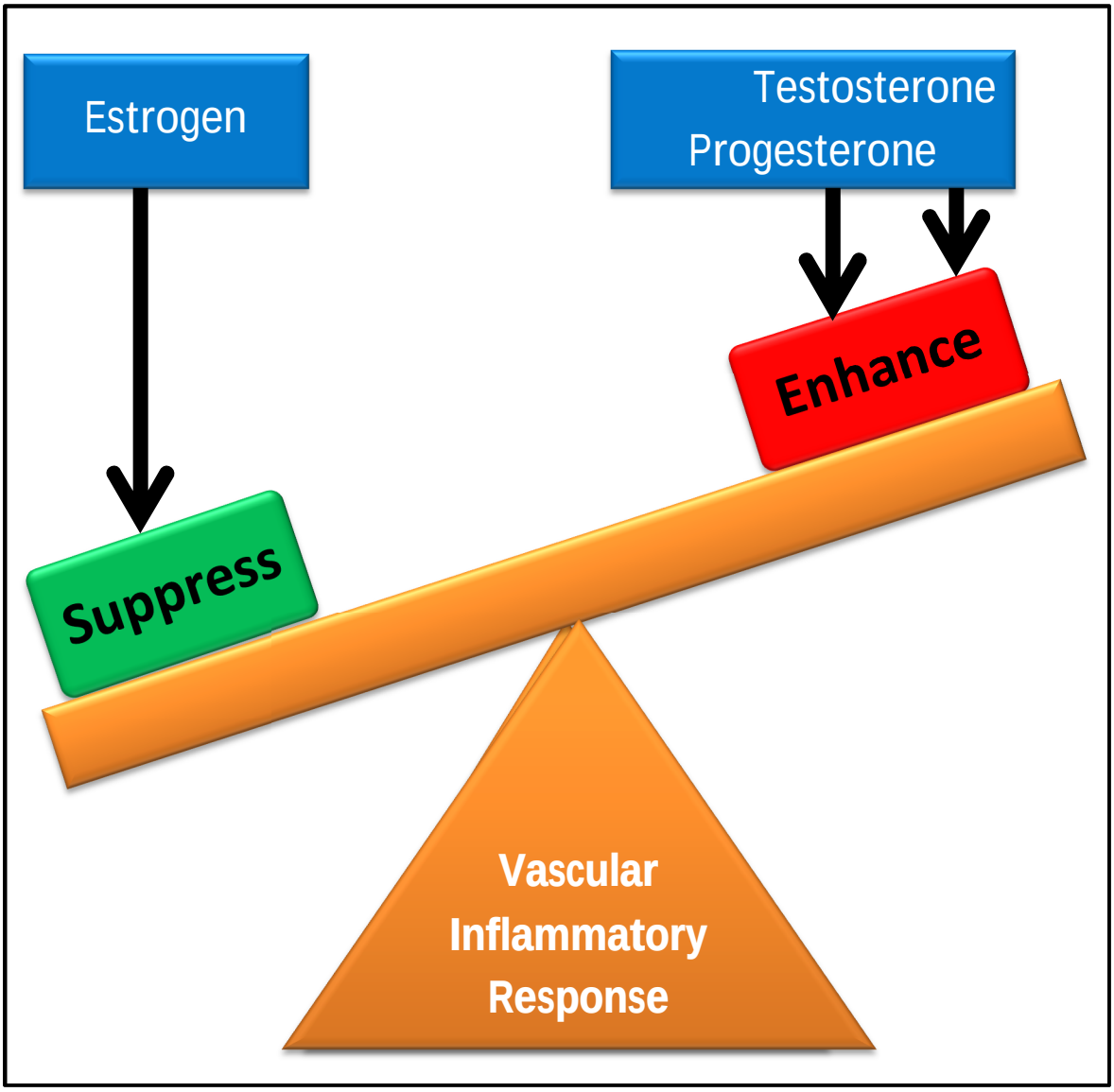
lipopolysaccharide, and oxidized LDL-induced expression of apolipoprotein E and ATP-binding cassette transporter (Perry et al, 2010).

Inflammatory processes in cerebral blood vessels can also be affected by estrogen levels (Figure 2.9). Adhesion of leukocytes in pial venules was decreased after treatment with estrogen in a study performed by Santizo and Pelligrino (1999). Leukocyte adhesion was found to be more in ovariectomized rats without estrogen in normal condition as well as during forebrain ischemia in comparison to estrogen-treated ovariectomized group or intact females (Santizo and Pelligrino, 1999; Santizo et al, 2000). The underlying mechanism has been proposed as increase in estrogen mediated eNOS. (Santizo al, 2002). Expression of adhesion molecules by cerebral microvascular endothelial cells is also suppressed by estrogen (Dietrich, 2004; Galea et al, 2002).

Other mechanisms which postulate the anti-inflammatory effect of estrogen in cerebral vessels are: suppression of COX-2 (which produces the inflammatory mediator PGE_2) (Ospina et al, 2002; Razmara et al, 2005; Sunday et al, 2004); suppression of Inducible NOS (iNOS) (Palmon et al, 1998) and suppression of endothelial NF-κB pathway that coordinates expression of a number of vascular inflammatory mediators (Galea et al, 2002; Ospina et al, 2002). 17β-estradiol is the only form of estrogen to exert beneficial impact on cerebrovascular inflammatory processes, in vivo as well as in vitro (Galea et al, 2002; Ospina et al, 2002). Testosterone and progesterone in contrast can increase the inflammatory process in cerebral blood vessels (Ramzara et al, 2005; Sunday et al, 2006).

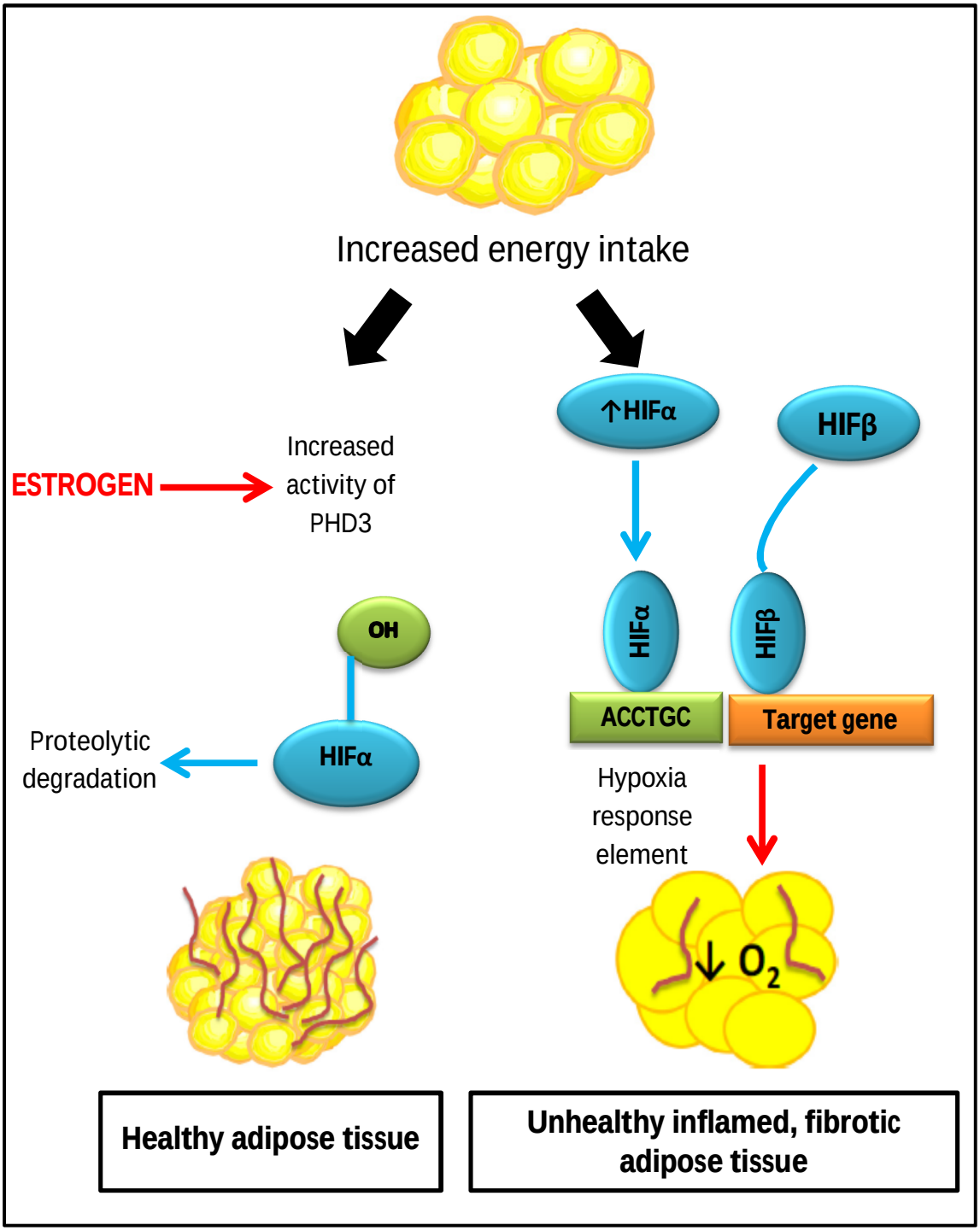
Palmer and Clogg (2015) have demonstrated the anti-inflammatory role of estrogen in adipose tissues (Figure 2.10). Chronic excessive energy intake can lead to hypoxia of visceral adipose tissues. Hypoxia occurs due to increased adipocyte size and tissue mass and this further lead to inflammed and fibrotic adipose tissues. HIF-1 up-regulates the inflammatory mediators like IL-6, NF-kB and TNFα and markers of fibrosis like Col-6. ERα helps in decreasing adipose tissue inflammation through increased activity of prolyl

FIGURE 2.9: OPPOSING EFFECTS OF SEX STEROID HORMONES ON CEREBROVASCULAR INFLAMMATION



Source: Krause et al, 2006

FIGURE 2.10: ANTI-INFLAMMATORY EFFECT OF ESTROGEN IN ADIPOSE TISSUES



Source: Palmer and Clegg, 2015

hydroxylase domain enzyme (PHD3) which hydrolyzes HIF-1 leading to its degradation. Thus estrogen helps in reducing inflammation and fibrosis in adipose tissues of the females. Various inflammatory markers have been studied in different epidemiological studies to establish the association between menopause and inflammation. Tumor necrosis factor (TNF-α) was found to be higher in postmenopausal (n=45) in comparison to premenopausal (n=44) women (4.81 ± 1.99 vs. 3.54 ± 0.85 pg/mL) in a study performed by Sites et al (2001). In this study C reactive protein (CRP) levels were associated with cardio metabolic risk in post-menopausal women. Wasir et al (2007) indicated that prevalence of at risk CRP levels was quite high (44.7%) among postmenopausal women of urban slums in north India. Dasgupta et al (2012) compared the metabolic aberrations between pre and post-menopausal women (n=216) of Karnataka and reported that CRP levels were significantly higher in post-menopausal females than their pre menopause counterparts. However this association between menopause and inflammation was lost after adjusting for age and BMI.

THYROID FUNCTIONS AND MENOPAUSE

Subclinical hypothyroidism (TSH > 4mU/l; normal free T4 [0.9 to 1.9 ng/dL]), is an emerging comorbidity of non-communicable diseases. According to Pearce et al (2007) untreated hypothyroidism can significantly increase the risk of chronic diseases as well as osteoporosis among post-menopausal women. A cross sectional study performed by Park et al (2009) on 2205 Korea euthyroid post-menopausal women indicated that TSH levels were a strong predictor for development of metabolic syndrome among the women. The prevalence of metabolic syndrome increased with gradual increase in TSH quartile. Many a times the symptoms of thyroid disorders are difficult to differentiate from menopausal symptoms (Schindler, 2003). Badaway et al (2007) also reported similar trend indicating that symptoms of thyroid dysfunction can lead to enhanced menopausal symptoms. Treatment of thyroid dysfunctions along with estrogen replacement therapy markedly improved menopausal symptoms in this study. Similarly Hernandez-Valencia et al (2008), reported a significant decrease in the frequency and

severity of menopausal symptoms ($p<0.05$) with levothyroxine treatment in postmenopausal women with subclinical thyroidism. In contrast to the above review multi ethnic SWAN study on 3242 mid aged women revealed that fearfulness was the only menopausal symptom associated with subclinical hypothyroidism. Sexual hormones like FSH, SHBG, dehydroepiandrosterone sulphate (DHEA-S), testosterone, and oestradiol were also not associated with TSH levels (Sowers et al, 2003).

Though there is not much literature available on association between menopausal status and thyroid dysfuncioning, some studies on Indian population have reported high prevalence of hypothyroidism among Indian females than males. Prevalence of hypothyroidism among 892 Indians settled in America was explored by Michalek et al (2000). The results revealed that more females (5%) were suffering from hypothyroidism than males (0.2%). A population based study in Cochin (Menon et al, 2009) on 971 adult subjects observed that the prevalence of hypothyroidism was higher in women (11.4%) in comparison to men (6.2%). Another cross sectional study on thyroid function including 4409 adults from Delhi consuming iodized salt was performed by Marwaha et al (2012). In line with the previous trends, prevalence of hypothyroidism was 21.4% in women compared to 15.9% in men. Elayath and Iyer (2013) did not find any significant difference in prevalence of subclinical hypothyroidism among pre (23%), peri (18%) and post-menopausal (28%) women from western region of India.

NUTRITIONAL ANAEMIA AND MENOPAUSE

According to Kotecha (2011) “The term 'nutritional anemia' encompasses all pathological conditions in which the blood hemoglobin concentration drops to an abnormally low level, due to a deficiency in one or several nutrients. The main nutrients involved in the synthesis of hemoglobin are iron, folic acid, and vitamin B_{12}”. In the recent years nutritional anemia especially deficiency of folate and B12 is coming up as important risk factor for development of chronic diseases (Santilli et al, 2016).

In a departmental study performed by Elayath and Iyer (2013) at Vadodara and Ahmedabad, prevalence of iron deficiency anemia was found to be similar across all the

menopausal stages i.e. 50%, 51% and 47% in pre, peri and post-menopausal women respectively. Prevalence of iron deficiency anemia in women of reproductive age has been widely studied. An Observational Cohort of the Women's Health Initiative(WHI-OS) including 93,676 postmenopausal women (50 to 79 years) indicated that dietary deficiencies of multiple nutrients were associated with a 21% greater risk of persistent anemia (OR-1.21, 95% CI:1.05–1.41). Use of supplements was not associated with lower prevalence of anemia among women. The authors recommended a more comprehensive approach to combat nutritional anemia among post-menopausal women with major focus on improving nutrient density and quality of diet rather than relying only on supplements (Thomson et al, 2011). There is lack of data on comparing prevalence of nutritional anemia (iron, B12 and folate deficiency) between menopausal status of Indian population. Prevalence of iron, vitamin B12 and folate among mid age females need to be studied due to interlink of menopause, chronic diseases and nutritional anemia.

LIVER FUNCTIONS AND MENOPAUSE

Menopause can head to altered hepatic health of a woman. Estrogen inhibit the development of fibrosis through action on stellate cells which transform into myofibroblast-like cells, proliferate and express α smooth muscle actin (α-SMA) (Ramadori et al, 1990). α-SMA synthesize type I collagen, type III collagen, type IV collagen, laminin, fibronectin and proteoglycans which are fibrotic in nature and estrogen therapy was found to reduce their production in a rat model (Yasuda et al, 1999). Estrogen can also help in maintaining hepatic health through protection of mitochondrial structure and functions. Estrogen receptors (ERs) and estrogen binding proteins are located within the mitochondria of liver (Chen et al, 2005). Estrogen modulates the transcription of mitochondrial DNA (Chen et al, 2004) and ERβ1 provides protection against mitochondrial membrane depolarization (Flynn et al, 2008).

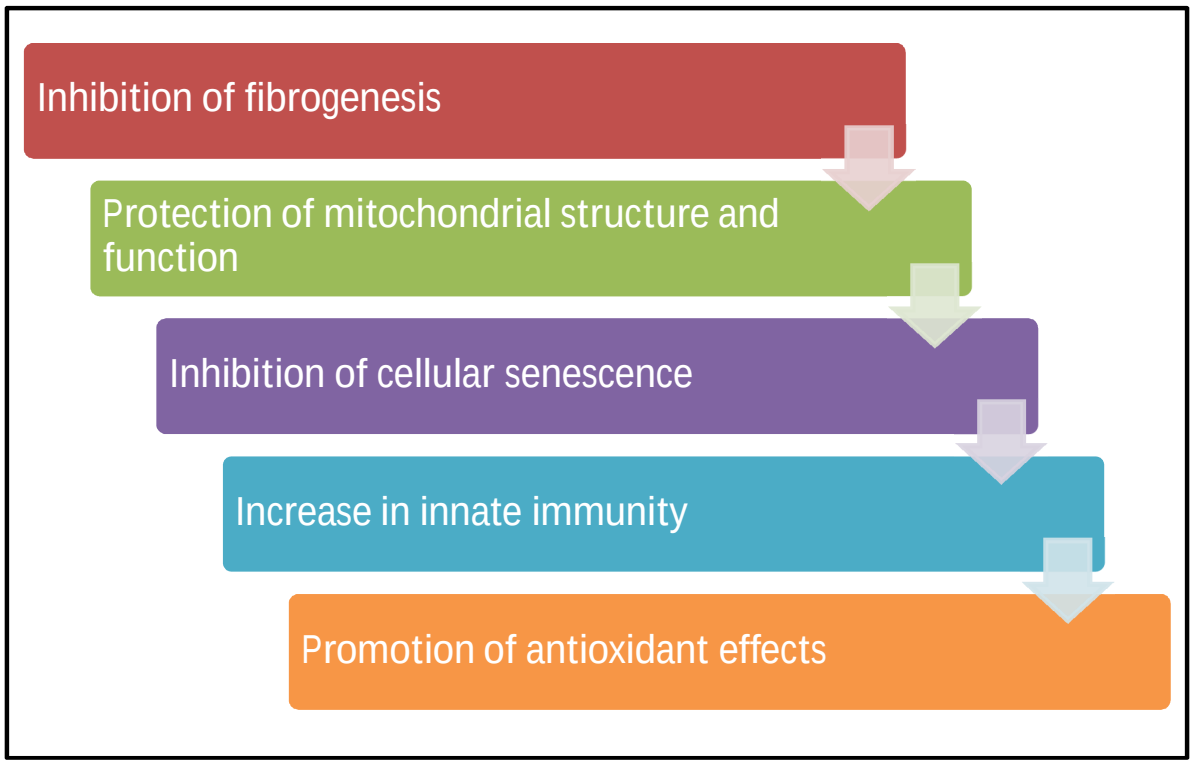
Other benefits of estrogen (Figure 2.11) for maintaining liver health are: inhibition of cellular senescence, increase in innate immunity and promotion of antioxidant effect

(Brady, 2015). Epidemiological data on liver diseases and menopause also indicate the inter-relationship of estrogen levels and hepatic health. Hepatitis C virus affected post-menopausal women were found to have higher rates of fibrosis progression than pre-menopausal ones in a study performed by Di-Martino et al (2004). The authors showed that hormonal therapy had a beneficial impact on recovery from the infection. High necro-inflammatory activity and higher rates of steatosis in liver cells have been observed in postmenopausal women compared to premenopausal women due to higher levels of TNF-α and IL-6 (Villa et al, 2011; Pfeilschifter et al, 2002). Recent studies suggest that during non-alcoholic fatty liver disease (NAFLD) the severity of hepatic fibrosis is higher in postmenopausal women and men in comparison to pre-menopausal women (Yang et al, 2014; Yoneda et al, 2014). Kumari et al (2010) compared the liver functions (n=80) of pre and post-menopausal women and found that the serum albumin and calcium level were significantly lower in post-menopausal women whereas the levels of total bilirubin and direct bilirubin were significantly higher in post-menopausal women as compared to pre-menopausal women ($p<0.01$). Estrogen deficiency during menopause leads to onset of bone loss and risk of developing osteoporosis. Serum alkaline phosphatase (a liver function biomarker) was found as an independent indicator for prediction of fracture in a prospective study on 512 post-menopausal women (Ross et al, 2000). High levels of serum alkaline phosphatase and urine hydroxyproline indicate the onset of bone loss during menopause (Crilly et al, 1980). Bhattari et al (2014) found significantly higher levels of alkeline phosphatase ($p<0.05$) and normal levels of serum calcium in post-menopausal women compared to pre-menopausal counterparts.

RENAL FUNCTIONS AND MENOPAUSE

The effect of menopause on the renal functioning has not been explored by many researchers. 17β-Estradiol replacement therapy has been found to be effective in improving renal functions associated with diabetic nephropathy in female animal model

FIGURE 2.11: BENEFITS OF ESTROGEN FOR LIVER HEALTH



Source: Brady, 2015

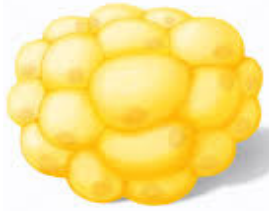
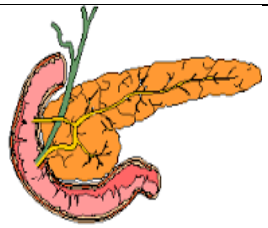
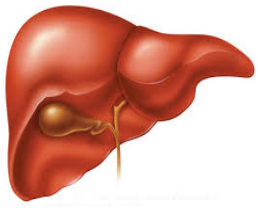
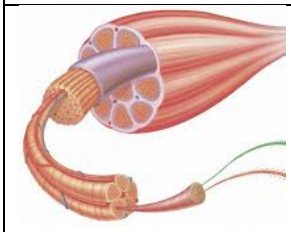
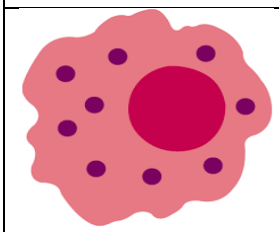
(Mankhey et al, 2005). A 10-year prospective study of post-menopausal estrogen therapy (n=1044) on renal functions indicated that females who used estrogen replacement therapy had better glomerular filtration rate and decreased risk of chronic kidney disease (Fung et al, 2011).

HORMONAL REPLACEMENT THERAPY TO COMBAT METABOLIC DERANGEMENTS

As menopause lead to decreased estrogen levels which is associated with various physiological and metabolic changes, use of hormone replacement therapy (estrogen, progesterone or combination) has been extensively studied through cohorts and randomized controlled trials to reduce the risk of chronic diseases among post-menopausal women. HRT reduces menopausal symptoms (Manson et al, 2013), prevents bone density and reduces risk of osteoporosis (Kanis et al, 2016), reduces atherosclerosis, cardiovascular diseases (Boardman et al, 2015) as depicted through different meta-analyses and systemic reviews. However there are controversies related to HRT regarding increased risk of breast (Jones et al, 2016) and overian (Li et al, 2015) cancer. According to the recommendations of British menopause society (Hamoda et al, 2016) "All women should be able to access advice on how they can optimize their menopause transition and the years beyond. There should be a holistic and individualised approach in assessing women, with particular reference to lifestyle advice and diet modification. This should be an opportunity to discuss the advantages and disadvantages of their management options including HRT and complementary therapies".

To summarize, levels of sex steroid estrogen are decreased during menopause which leads to various physiological and metabolic aberrations and further increases overall morbidity and mortality related to cardiovascular and endocrinological disorders (Table 2.2). Hormonal replacement therapy can help in reducing the risk of these diseases however it comprises of several disadvantages. Complementary therapies related to diet and physical activity should be promoted during and post menopause to maintain the health and quality of life among middle aged women.

TABLE 2.2: SUMMARY OF ESTROGEN ACTIONS IN VARIOUS ORGANS AND EFFECT OF MENOPAUSE

	Estrogen action	Estrogen deficiency/resistance
	Energy balance	Obesity
	Adipose health	Obesity, Adipose inflammation, Altered secretory profile
	β cell function survival	β cell dysfunction Type 2 diabetes
	Insulin sensitivity Lipid homeostasis	Insulin Resistance Fatty liver
	Insulin sensitivity Energy homeostasis	Insulin resistance Impaired Glucose homeostasis
	Macrophage polarization	Inflammation Adiposity Atherosclerosis

Source: Mauvais-Jarvis et al, 2013

VITAMIN B12 AND FOLIC ACID DEFICIENCY: CAUSES, PREVALENCE AND ASSOCIATION WITH NON-COMMUNICABLE DISEASES IN FEMALES

VITAMIN B12

Vitamin B12, also known as cobalamin, is the largest and the most complex out of all the types of Vitamins. The physiologically relevant B12-derivatives are coenzyme B12 (5-deoxy-5 adenosylcobalamin) and methyl-cobalamin (Krautler, 2005). The metalloenzyme structure of Cobalamin contains a corrin ring with Cobalt and four pyrrole groups (Figure 2.12). The active site of Cobalt (R) can connect to several different types of ligands like CN to form a Cyanocobalamin, to a Methyl group to form a methylcobalamin, to a 5'-deoxy adenosy group to form an adenosylcobalamin, and OH, Hydroxycobalamin (LibreTexts, 2013). Digestion and absorption of vitamin B12 is depicted in figure 2.13 (Green et al, 2017). When cobalamin is ingested, it is released from the protein complexes with the help of digestive enzymes and binds to haptocorrin which is produced by salivary glands. In stomach haptocorrin prevents B12 from acid. In duodenum it binds to intrinsic factor (IF) which is produced by gastric parietal cells. This B12-IF complex binds to cubam receptors present in enterocytes of distal ilium and mediates its uptake (Nilson et al, 2012; Aminoff et al, 1999; Tanner et al, 2003). There through lysosomal release B12 enters into circulation facilitated by multidrug resistance protein (MDR1). Transcobalamin is the carrier of B12 in the blood. (Nilson et al, 2012; Fedosov et al, 2007; Beedholm-Ebsen et al, 2010). The absorbed inert form of cobalamine is converted into two major active forms (Satyanarayana and Chakravarthy, 2006) i.e. methylcobalamin and adenosylcobalamin.

The major function of methylcobalamin is maturation of red blood corpuscles. Methyl cobalamin acts as co factor for methionine synthase enzyme which is essential for the synthesis of purines and pyrimidines. In this reaction methyl group of methyltetrahydrofolate is transferred to homocysteine to form methionine and tetrahydrofolate, therefore folate is also essential to complete this reaction (Gibson, 2005). Adenosylcobalamin is involved in healthy myelination and neuronal integrity (Kasper et al, 2008; Satyanarayana and Chakravarthy, 2006). It acts as a co-factor for the

enzyme methylmalonyl-CoA mutase. It is present in mitochondria. Isomerization of methylmalonyl-CoA to succinyl-CoA (essential for synthesis of neuronal lipids to form myelin) requires adenosylcobalamin (Kasper et al, 2008). Serum vitamin B12 is bound to proteins known as transcobalamins (IOM, 1998). Vitamin B12 is synthesized by certain bacteria in the gastrointestinal tract of animals. As it is synthesized in animal body, it is found abundant in animal sources especially tissues (Heyssel et al, 1966). Plant sources do not contain any active form of vitamin B12 (Stabler and Allen, 2004). The vitamin B12 content in plant sources is due to contamination through water, soil and fertilizers (Miyamoto et al, 2005) or in some fermented products like temphe (Denter et al, 1994) and sea alge (Food composition Japan, 2005). Recommended dietary allowance of vitamin B12 for Indians adults is 1mcg/day (NIN, 2011). However, body storage of vitamin B12 is around 1-5mg. Therefore it may take years of diminished intake/absorption of vitamin B12 to develop clinical deficiency (Harmening, 2002). Table 2.3 summarizes the pathophysiology of vitamin B12 deficiency (Hunt, 2014). At risk population for vitamin B12 deficiency are elderly, vegans or ovo-lacto vegetarians.

Clinical features of mild vitamin B12 deficiency include fatigue and anemia. Moderate deficiency may lead to macrocytic anemia and some mild neurological features. Bone marrow suppression, neurological features and risk of cardiomyopathy develop during severe deficiency (Devalia et al, 2014).

Several biomarkers are used to detect vitamin B12 deficiency. Serum (or plasma) vitamin B-12 concentration below <150 pmol/L (<200 pg/mL) indicate deficiency, and 150–221 pmol/L (200–300 pg/mL) indicate depletion (Allen, 2012). Macrocytosis is commonly used for assessing vitamin B12 status however concomitant iron deficiency or thalassaemia trait may hamper the development of macrocytosis (Harmening, 2002; Provan et al, 2010; Kaushansky et al, 2010). In the recent years holotranscobalamin is coming up as more reliable marker of impaired vitamin B12 status than is a low level of serum vitamin B12 (Valente et al, 2011). Methyl melanoic acid is the most reliable B12

FIGURE 2.12: STRUCTURE OF VITAMIN B12

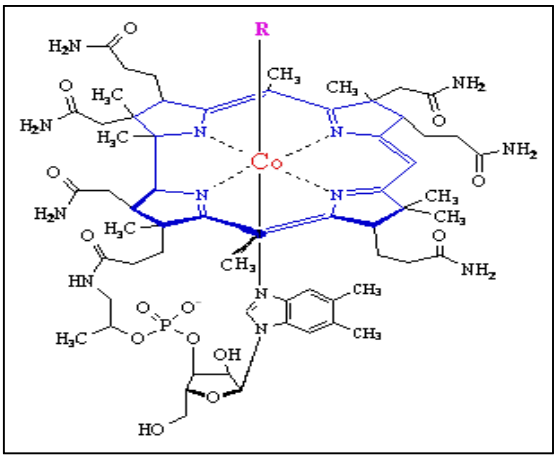
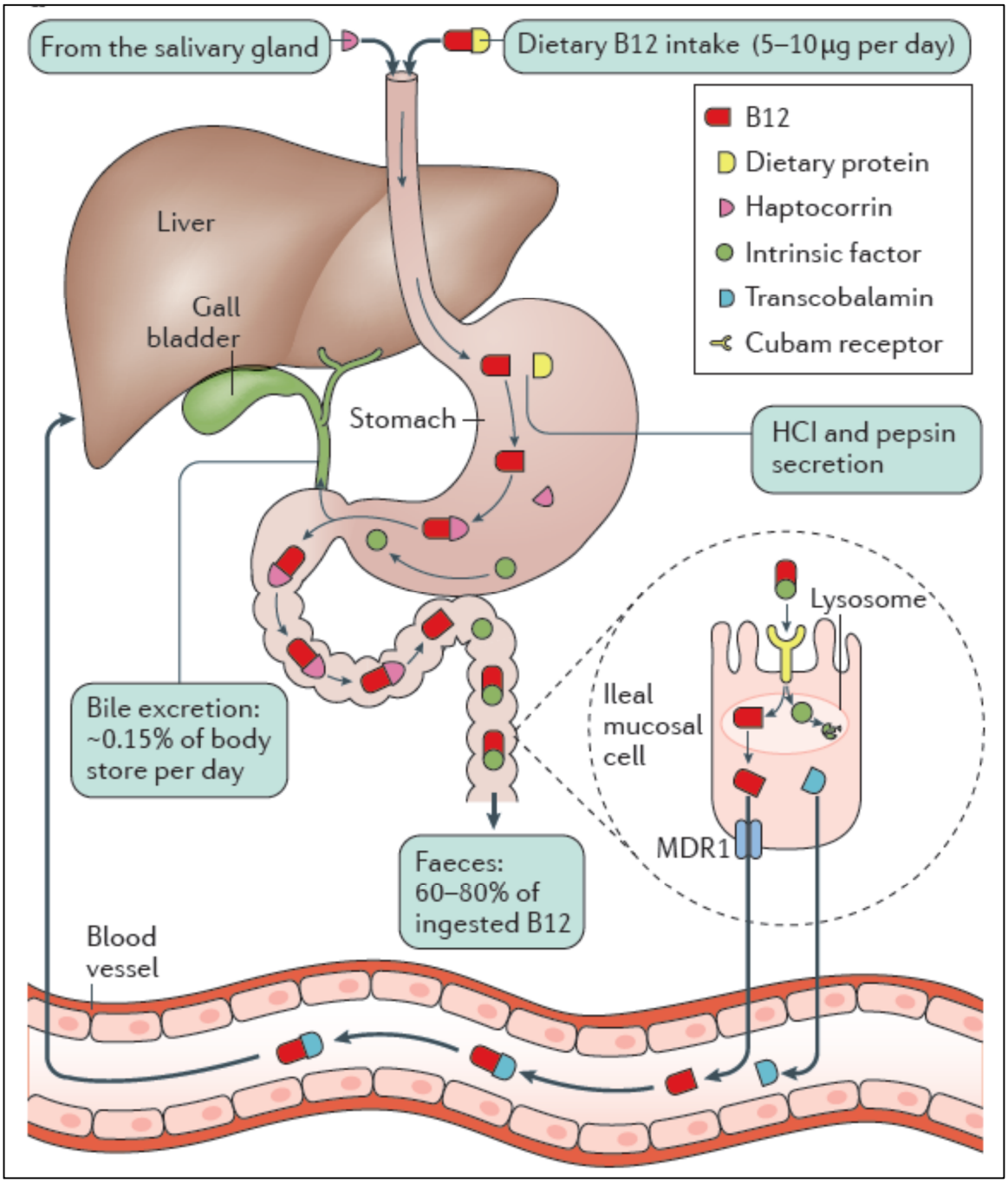


TABLE 2.3: COMMON CAUSES OF VITAMIN B12 DEFICIENCY

Factor	Related cause/disease
Impaired gastric absorption	• Pernicious anemia • Gastrectomy: partial or total • Zollinger-Ellison syndrome
Impaired intestinal absorption	• Ileal resection or disease: for example, Crohn's inflammatory bowel disease and tuberculous ileitis • Blind loop syndrome • Luminal disturbances: chronic pancreatic disease and gastrinoma • Parasites: giardiasis, bacterial overgrowth, and fish tapeworm • *Pancreatic insufficiency*
Decreased intake	• Malnutrition • Reduced intake of animal products • Strict vegan diet
Congenital/ inherited	• Intrinsic factor receptor deficiency: Imerslund-Gräsback syndrome • Congenital deficiency of intrinsic factor: "juvenile" pernicious anemia • Cobalamin mutation (C-G-1 gene) • Transcobalamin deficiency
Increased requirements	• Haemolysis • HIV
Drugs	• Alcohol • Nitrous oxide • Proton pump inhibitors • H2 receptor antagonists • Metformin • Colchicine • Slow K (potassium chloride) preparations • Cholestyramine

Source: Hunt, 2014

FIGURE 2.13: ABSORPTION AND ENTEROHEPATIC CIRCULATION OF VITAMIN B12



Source: Green et al, 2017

marker for assessing vitamin B12 deficiency. As vitamin B12 acts as a cofactor for the conversion of methylmalonic acid to succinyl-CoA, it accumulates due to unavailability. However it is an expensive method and cannot be used for elderly and patients with impaired renal function (Sobczyńska-Malefora et al, 2014). Plasma total homocysteine levels is a sensitive marker for vitamin B 12 deficiency. However it is not very much specific as the levels can also be high due to folate deficiency, renal failure, and hypothyroidism (Devalia et al, 2014).

FOLIC ACID/FOLATE

Folic acid is a member of the water soluble B-complex family of vitamins. The term `folate' is used to denote the large group of compounds with similar vitamin activity (Hoffbrand, 2001). Folic acid is composed of three primary structures, a hetero-bicyclic pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid (Figure 2.14). Folate found in food is generally a mixture of various compounds like polyglutamates, conjugate compounds, reduced folates and tetrahydrofolates. Folic acid form is primarily used in dietary supplements and food fortification (Alternative Medicine Review, 2005).

Natural folates present in food first needs to be converted to folate monoglutamates by folate reductase in the jejunal mucosa for absorption. Natural Folates can lose their activity during food processing due to their highly unstable nature. Around 40% of folate present in vegetables and 70% of present in cereals is generally destroyed by industrial processing and cooking (Benoist, 2008; Caudill, 2010). Folic acid present in supplements can be absorbed easily however it needs to be converted to other compounds like dihydrofolate and tetrahydrofolate to be biologically active. Tetrahydrofolate is converted into 5,10-methylenetetrahydrofolate by serine Hydroxymethyltransferase (Balion and Kapur, 2011). The reduced and polyglutamylated forms of folate act as coenzymes in the transfer of one-carbon groups in different reactions involving DNA biosynthesis. Tetrahydrofolate as well as its methylated forms play a crucial role as methyl(ene) donors. Folate is important for synthesis of DNA from

its precursors (thymidine and purines). It also acts as cofactor during conversion of homocysteine to methionine. Methionine is essential for the synthesis of S-adenosylmethionine (SAM), which is a methyl donor in various biological methylation reactions important for gene expression and cell differentiation (FAO/WHO, 2002).

Green leafy vegetables, sprouts, fruits, brewer's yeast, liver, and kidney are the richest sources of folate (Alternative Medicine Review, 2005). The causal factors behind folic acid deficiency are: inadequate intake from food, altered utilization, alcohol consumption, liver diseases, malabsorption, cancer, HIV infection, certain drugs and deficiencies in enzymes or cofactors required for conversion of active folic acid (Halsted, 1989; Revell et al, 1991). High risk population of folate deficiency is pregnant and lactation women due to increased needs during these physiological conditions. During pregnancy there is an increased risk of fetal neural tube defects (NTDs) which include spina bifida, anencephaly, and other similar conditions (Daly et al, 1995). Signs and symptoms of folate deficiency include macrocytic anemia, fatigue, irritability, peripheral neuropathy, tendon hyper-reflexivity, restless legs syndrome, diarrhea, weight loss, insomnia, depression, dementia, cognitive disturbances, and psychiatric disorders (Botez, 1976; Audebert et al, 1989; Young and Ghadirian, 1989; Metz et al, 1996; Quinn and Basu, 1996; Fine and Soria, 1991). Recommended dietary allowances of folate for adult population of India is 200mcg/day (NIN, 2010). The commonest indicator of folate deficiency which reflects the short term status is serum folate (Pfeiffer et al, 2010). RBC folate is a sensitive indicator of long-term folate status. It shows the folate status during the preceding 120 days, reflecting the amount of folate accumulated in RBCs during erythropoiesis (Mason, 2003; Clifford et al, 2005). The concentrations suggested for defining folate deficiencies based on these metabolic indicators are (WHO, 2008):

- < 10 nmol/L (4 ng/mL) for serum folate
- < 340 nmol/L (151 ng/mL) for RBC folate

Plasma homo-cysteine is a very sensitive indicator of folate status. During the insufficiency/deficiency of folate, plasma homocysteine levels are elevated. However it

is not a specific marker of folate status, because it will also be elevated with other B-vitamin deficiencies, lifestyle factors, renal insufficiency, and drug treatments (Refsum et al, 2004; Jacob et al, 1994). Hematological indicator like raised mean corpuscular volume, hypersegmentation of neutrophils and the first stages of anemia are important indicators of folate deficiency (Lindenbaum et al, 1990).

INTERRELATIONSHIP OF VITAMIN B12 AND FOLATE

Functions of vitamin B12 and folate are closely interlinked (Figure 2.15). For the synthesis of purines and pyrimidines methionine synthase is an essential enzyme. Methyl cobalamin acts as a co-factor in this reaction. The reaction also requires folate as the methyl group of methyltetrahydrofolate is transferred to vitamin B12 and subsequently to homocysteine to form methionine and tetrahydrofolate (Gibson, 2005). Deficiency of either vitamin B12 or folate can lead to elevated homocystine levels as recycling of homocysteine to methionine is hampered. An abnormally high plasma level of homocysteine is an independent risk factor for cardiovascular diseases (Homocystine studies collaboration, 2002). Tetrahydrofolate can then be returned to the folate pool for the generation of methylenetetrahydrofolate, which is required for DNA replication and repair. A deficiency of folate can cause interruption of this reaction and lead to megaloblastic anemia (Gibson, 2005). In the case of B12 deficiency, folate is 'trapped' in the unusable methyl-form (Green, 2017). Cobalamin is also required as a cofactor for methylmalonyl CoA mutase enzyme to convert methylmalonyl CoA to succinyl CoA. During vitamin B12 deficiency methylmalonyl CoA accumulates which is thought to be responsible for the neurological effects (Gibson, 2005). Folate fortification/supplementation can many a times mask the vitamin B12 deficiency as it allows DNA synthesis to continue which prevents megaloblastic anemia. However due to vitamin B12 deficiency homocysteine and MMA concentrations will rise and neurological damage may occur. According to Carmel (2003) about 20-30% of cases of vitamin B12 deficiency have been reported to have neurological damage without megaloblastic anemia.

FIGURE 2.14: STRUCTURE OF FOLIC ACID

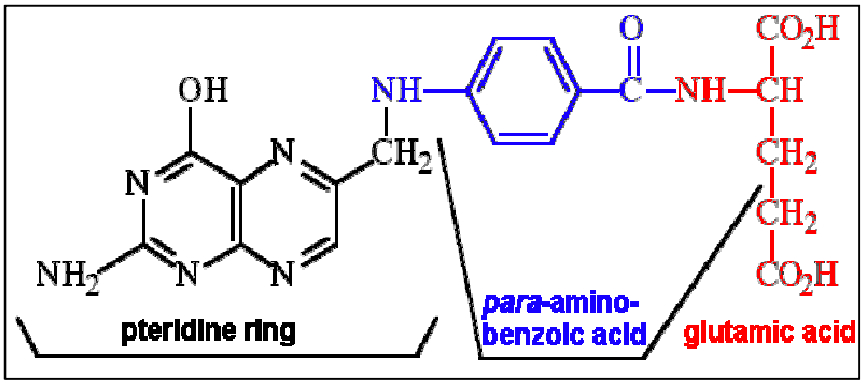
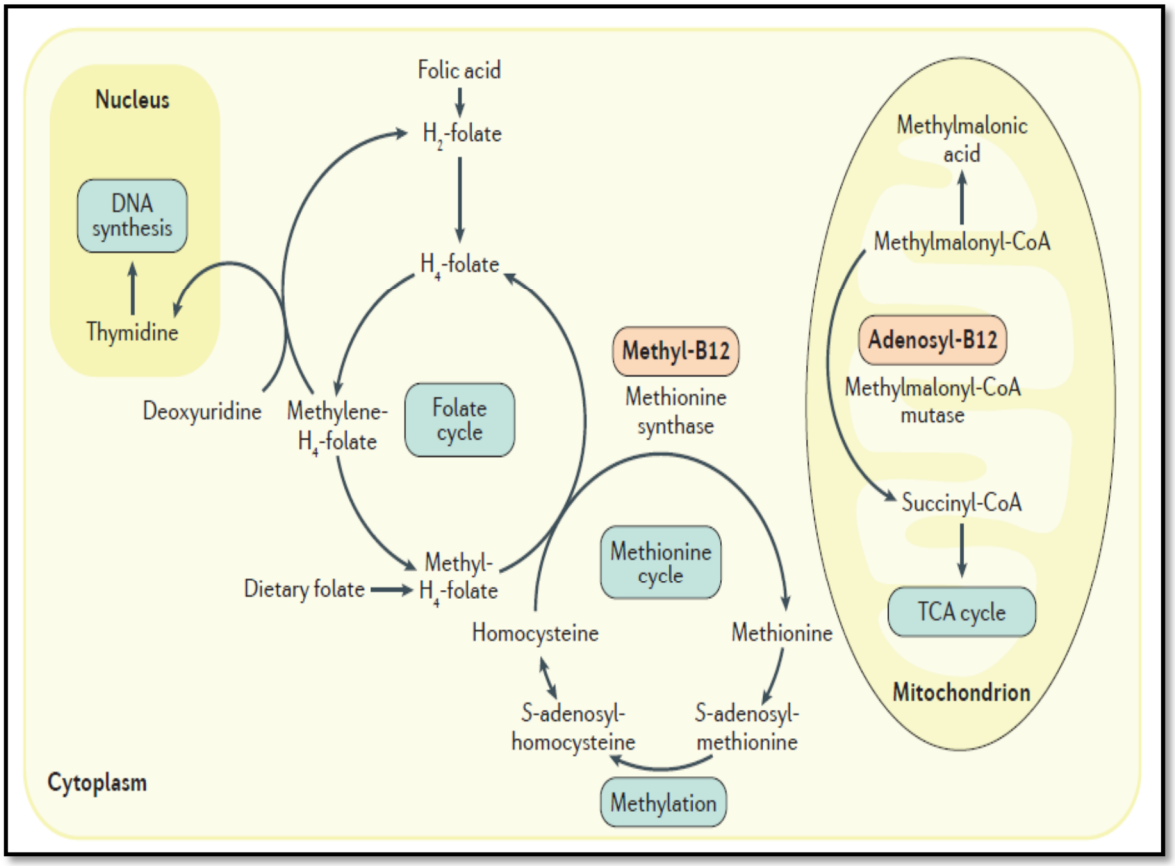


FIGURE 2.15: INTERRELATIONSHIP OF VITAMIN B12 AND FOLATE



Source: Green et al, 2017

PREVALENCE OF VITAMIN B12 AND FOLATE DEFICIENCY IN FEMALES

According to WHO various local level studies and national wide surveys across the world have shown that deficiency of vitamin B12 and folate is emerging as major public health problem which can affect millions of people (WHO, 2008). A recent meta-analysis and systemic review was performed by Obersby et al (2013) including six cohort case studies and eleven cross sectional studies (n=3230). The results concluded that omission of animal products from the daily diet can lead to vitamin B12 deficiency. According to Yajnik et al (2006) vegetarianism and urban middle class residence are two major factors of vitamin B12 deficiency. Vegetarianism in India is multigenerational, lifelong and based on religious and cultural beliefs therefore vegetarian Indians are at high risk of developing B12 deficiency.

Prevalence of vitamin B12 and folate deficiency have been studied by various Indian studies however very few focus on middle aged female population. A hospital based study on middle aged population from Delhi (n=422) revealed that women (OR: 0.62, 95%CI: 0.41 0.93) and vegetarians (OR: 4.68, 95%CI: 2.39-9.15) were found to have higher risk for developing vitamin B12 deficiency (Arora et al, 2011). A study performed on vitamin B12 deficiency among medical college employees of north-west India revealed that 53.6% of the subjects had vitamin B12 deficiency with higher prevalence seen among females (66.6% of female students and 70.6% of female employees). Males were found to have significantly higher mean vitamin B12 levels ($p<0.05$) than females (248.36 ± 98.72 vs 203.28 ± 90.16) (Chahal, 2014). Khanduri et al (2005) studied the prevalence of vitamin B12 and folate deficiency among adult population (n=96) and revealed that females (46%) had higher prevalence of vitamin B12 deficiency in comparison to males (36.9%). Prevalence of folate deficiency was found to be relatively low (17.3% and 12% in males and females respectively). A study performed by Mangukiya et al (2011) at Pipariya Gujarat indicated that the prevalence of B12 deficiency was 37.5% and 38.5% in males and females respectively. Mean serum vitamin B12 levels did not differ significantly between males (170 pg/ml) and females

(161.6pg/ml). Shobha et al (2011) studied the levels of vitamin B12 and its metabolites among elderly population and concluded that median serum B12 and red cell folate levels were 306.9 pmol/l and 1045.4 ng/ml respectively. Plasma vitamin B12 (*P*= -0.509) and folate levels (*P*= -0.550) were inversely correlated with Homocystine were inversely correlated (*P*= -0.509). Red cell folate was inversely correlated with Homocystine (*P*= -0.550). About 33.8% of the subjects tested for vitamin B12 deficiency (n=425) were found to have low serum B12 levels in a study performed by Bhatia et al (2012). Only 25% of the B12 deficient subjects had megaloblastic anemia as depicted by mean corpuscular volume. Bhardwaj et al (2013) studied the prevalence of vitamin B12 and folate deficiency in adolescent male and females of north Himalayan population and showed that all of the adolescents (male and female) were vitamin B12 deficient whereas none was having folic acid deficiency. Mean serum vitamin B12 and folate levels of overweight/obese South Asian women were found to be 227 pmol/L and 19.1 nmol/L respectively in a study performed by Gammon et al (2012). Vitamin B12 deficiency (<150 pmol/L) was observed as 24% in vegetarians in comparison to 9% in non-vegetarians. The prevalence of vitamin b12 deficiency among high socioeconomic group of Bhuj (Gujarat) was found to be 44.2% (Patel et al, 2012).

ASSOCIATION OF B12 AND FOLATE DEFICIENCY WITH NON-COMMUNICABLE DISEASES

Homocysteine is a sulfhydryl-containing amino acid, which is an intermediate product in the biosynthesis of methionine and cysteine (Faeh et al, 2006). As discussed earlier in the review, deficiency of vitamin B12 and folate can lead to elevated homocysteine levels due to inability to convert to methionine. Hyper-homocysteinemia (high levels of homocysteine) has been established as an independent marker for development of cardiovascular diseases. A meta-analysis including 30 prospective or retrospective studies involving a total of 5073 IHD events and 1113 stroke events concluded that a 25% lower usual (corrected for regression dilution bias) homocysteine level was associated with an 11% (OR 0.89; 95% CI, 0.83-0.96) lower IHD risk and 19% (OR, 0.81; 95% CI, 0.69-0.95) lower stroke risk (Homocysteine Studies Collaboration, 2002).

Homocysteine can have adverse effects on vascular endothelium and smooth muscle cells through increase in proliferation of vascular smooth muscle cells, endothelial dysfunction, oxidative damage; increase is synthesis of collagen and deterioration of arterial wall elastic material (Zhang et al, 2014). Homocysteine can initiate inflammatory response in vascular smooth muscle cells by stimulating CRP production (Pang et al, 2014).

Various studies on effect of vitamin B12 and folate supplementation on lowering CVD, CAD, stroke and other chronic disease risk have shown mixed results. A meta-analysis performed by Ji et al (2013) indicated that B vitamin supplementation can significantly reduce stroke events, through action on homocysteine levels, but limited to subjects with certain characteristics who received appropriate intervention measures. A Systemic review of prospective cohort studies to study the impact of low blood level of vitamin B12 a cardiovascular and diabetes risk factor concluded that little evidence of vitamin B12 deficiency increasing the risk of mortality and morbidity from cardiovascular diseases or diabetes in adults is available in literature (Rafnsson et al, 2011). Another meta-analysis on effect of vitamin B12 supplementation in stroke showed that B-vitamin supplements can reduce carotid intima-media thickness and (-0.10 mm, 95% CI -0.20 to -0.01 mm) and increase flow-mediated vasodilation (1.4%, 95% CI 0.7 to 2.1%). However, only short term improvement in endothelial functions was observed (Potter et al, 2008). Data of 19 studies including 47921participants in a meta-analysis (Huang et al, 2012) indicated that B vitamin supplementation has a significant protective effect on stroke (RR 0.88, 95%CI 0.82–0.95), but none on the risk of CVD (RR 0.98, 95%CI 0.94–1.03), for coronary heart disease (CHD) (0.98, 95%CI 0.92–1.05), for myocardial infarction (MI) (RR 0.97, 95%CI 0.90–1.05), for cardiovascular death (RR 0.97, 95%CI 0.91–1.02), for all-cause mortality (RR 0.99, 95%CI 0.95–1.04). In a study performed by Mahalle et al (2013) on coronary artery patients (n=216) from India, vitamin B12 levels were significantly lower in subjects with dyslipidemia ($p<0.001$), diabetes ($p<0.01$) and diabetes with hypertension ($p<0.001$). Serum vitamin B12 was inversely associated with triglyceride ($p<0.05$) and very low-density lipoprotein (VLDL) ($p<0.05$), insulin resistance

($p<0.05$) and positively with high-density lipoprotein (HDL) ($p<0.05$). Kumar et al (2009) performed a case-control study on association of CAD with vitamin B12 levels in Indians and found that CAD patients had significantly lower vitamin B12 levels than controls ($p<0.001$). A meta-analysis of 30 randomized controlled trials involving 82 334 participants on folic acid supplementation in heart diseases revealed that folic acid supplementation significantly reduced the risk of stroke (RR 0.90, 95% CI 0.84–0.96) for stroke and cardiovascular diseases (RR 0.96, 95% CI 0.92–0.99). The intervention results were more pronounced in subjects with lower folate levels as baseline (Li et al, 2016). Another similar meta-analysis (26 RCTs enrolling 58,804 participants) showed contrasting results for cardiovascular diseases (RR 0.98, 95%CI 0.95-1.02) risk reduction post folate supplementation. In the study folic acid supplementation was linked to a decreasing trend in stroke risk (RR 0.93, 0.86 to 1.00; $p = 0.05$) only (Yang et al, 2012). Zeng et al (2015) performed a meta-analysis on association of folate fortification and impact of folate supplementation in reducing stroke risk. The results indicated that folate supplementation showed significant impact on reducing risk of stroke in subgroup without folate supplementation (RR 0·88, 95% CI 0·77-1·00) in comparison to the subgroup with folate fortification (RR 0·94; 95% CI 0·58-1·54). In a retrospective study on 1743 subjects Higher folate serum levels were significantly associated with lower serum levels of LDL-C ($p < 0.001$) and lower LDL-C-C/HDL-C-ratio ($p < 0.001$), whereas, vitamin B12 was not associated with the lipoprotein profile (Semmler et al, 2010). Folate levels in hypertensive patients (n=116) were found to be significantly lower than in control subjects (6.7±5.0 ng/ml and 9.0±4.4 ng/ml respectively, $p<0.05$) in a study performed by Scazzone et al (2014).

To summarize, vitamin B12 and folate are two major B complex vitamins whose functions are interdependent. High prevalence of both the vitamins deficiency has led to consider them as public health issue. Both vitamin B12 and folate deficiency can lead to high homocysteine levels, an independent risk marker for cardiovascular diseases. Many studies also have shown their distinct association with different chronic diseases.

ASSOCIATION OF INFLAMMATION AND NON-COMMUNICABLE DISEASES AMONG WOMEN

INFLAMMATORY PROCESS AND MARKERS OF INFLAMMATION

Inflammation is a biological reaction to a disrupted tissue homeostasis (Medzhitov 2008). According to Saunders (2007) "inflammation is a localized protective response elicited by injury of destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent and the injured tissue''.

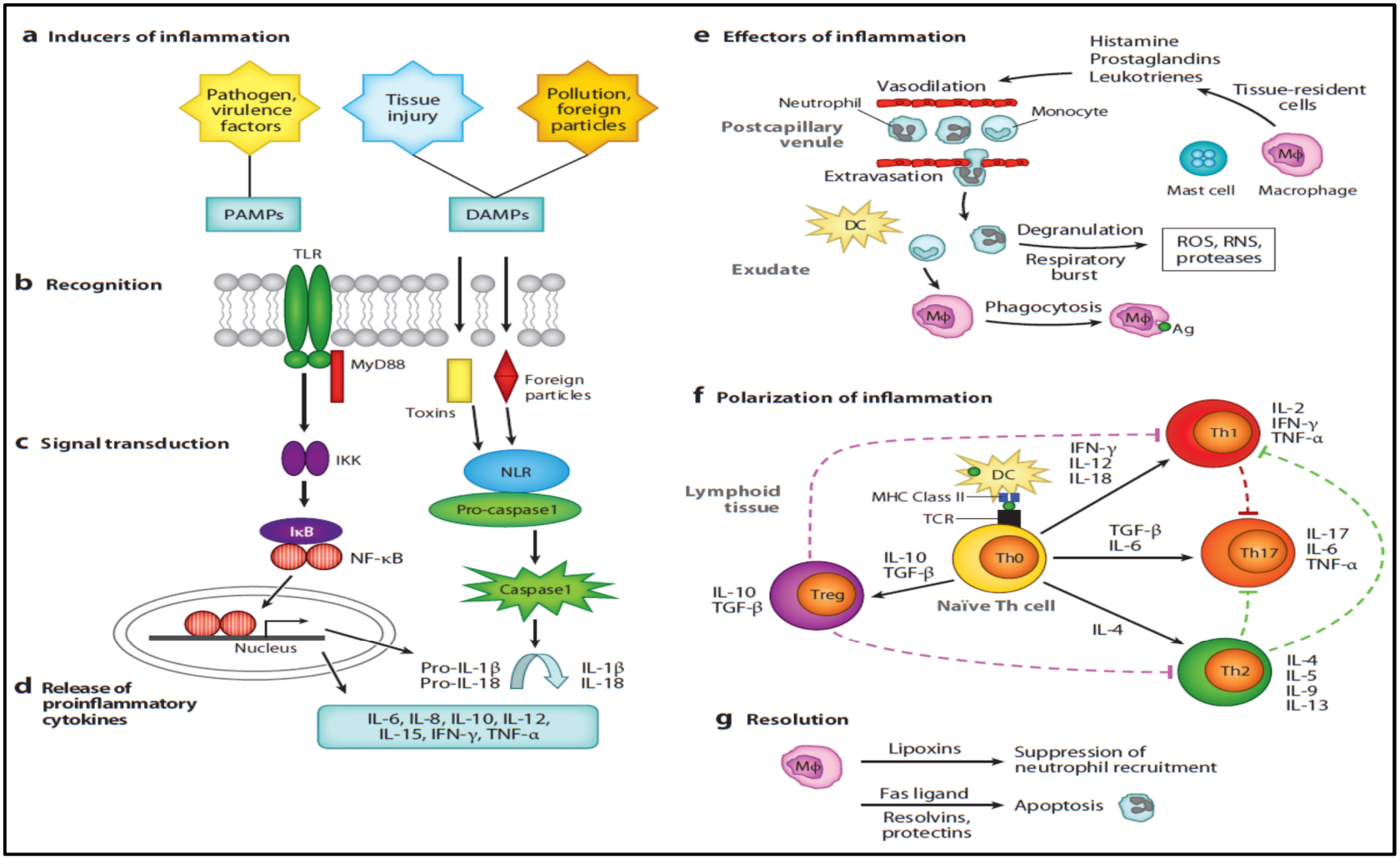
Inflammatory process involves various steps which are portrayed in figure 2.16 (Ashley et al, 2012). It starts with invasion of pathogens or foreign particles in the body or tissue injury (Bianchi 2007). The innate immune system of the body detect the signals related to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Janeway et al, 2005). Germ-line encoded receptors, like transmembrane Toll-like receptors (TLRs), intracellular nucleotide binding domain and leucine-rich-repeat containing receptors (NOD-like receptors or NLRs) can also recognize damage signals (Lange et al. 2001, Proell et al. 2008, Roach et al. 2005). Further during signal transduction TLRs activate common signaling pathways that results in the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). (Ghosh et al. 1998).

Transcription and translation of genes lead to the inducible expression of pro-inflammatory cytokines, like interleukin-1-beta (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), and others. NLRs activate caspase-1 to convert cytokines into active forms. Pro-inflammatory cytokines facilitate the mobilization macrophages and neutrophils, to the site. Neutrophils and monocytes selectively pass through endothelial cells to reach target sites (extravasation). A protein-rich fluid, known as the exudate, also accompanies causing edema (swelling).Monocytes release histamines, prostaglandins and leukotrienes which cause vasodilation. Neutrophils release toxic chemicals like highly reactive oxygen and nitrogen species (ROS and RNS, respectively) and various

proteinases from cytoplasmic granules (degranulation). This process requires glucose and oxygen, known as the respiratory burst. These toxic chemicals induce liquifaction of surrounding tissue to hinder microbial metastasis (Nathan 2002). The signs of the local inflammation include heat, swelling, redness, pain, and loss of function. Resolution, the last phase of inflammation lipoxins are released by macrophages which block further neutrophil recruitment. Apoptosis of neutrophils is promoted by fas ligand, resolvins, and protectins. Macrophages finally phagocytose the cellular debris. (Serhan & Savill 2005).

In cases when foreign bodies and indigestible particles, are too large to be phagocytosed by individual cells, polarization of inflammation occurs towards the innate immunity. (Allen & Wynn 2011). In the early stages of parasite invasion, wounded tissue is healed through the formation of granulomas (fibrous connective tissue that replaces fibrin clots) to isolate and encapsulate the invader. T-helper (Th) cells, of lymphocytes of the adaptive immune system, are largely responsible for activating and coordinating these responses. Th1 and Th17 cells are proinflammatory, Th2 cells are antiinflammatory, Tregs are regulatory cells (Abbas et al. 1996, Anthony et al. 2007). An optimization of Th1/Th2 phenotypes facilitates pathogen clearance with minimum damage to host tissues. Inflammation can be categorized based on their intensity and duration. Diseases like diabetes mellitus, atherosclerosis, neurodegenerative diseases, tumor growth, tissue damage (fibrosis) are categorized under low grade chronic inflammation (Ashley et al, 2012). Various biomarkers are used to assess the inflammatory process which include: total leucocytes, granulocytes and activated monocytes and soluble mediators (cytokines and chemokines (TNF, IL-1, IL-6, IL-8, CC chemokine ligand 2 (CCL2), CCL3, CCL5), adhesion molecules (vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin), adipokines (adiponectin) and acute-phase proteins (CRP, serum amyloid A, fibrinogen)) are frequently measured (Calder et al, 2009). Currently, there is no biomarker which can differentiate between acute and chronic inflammation or between various phases of inflammatory responses. Therefore there is no consensus

FIGURE 2.16: A PRIMER OF INFLAMMATORY PROCESS



Source: Ashley et al, 2012

regarding the best marker representing low-grade inflammation (Calder et al, 2011). However, the most widely used 'universal' marker of inflammation is C-reactive protein. It is an 'acute-phase reactant' produced in the liver in response to IL-6 (Rietzschel and Buyzere, 2012). It can be affected by other factors like smoking, physical stress etc. which reduces its specificity (Chandrashekara, 2014).

INFLAMMATION AND NON-COMMUNICABLE DISEASES AMONG WOMEN

Inflammation is coming up as a strong risk factor in development of non-communicable diseases among women. According to Ridker et al (2005), the hazard ratio of high HsCRP levels for development of future CVDs was 2.98 (95% CI, 1.90-4.67) among 15 632 initially healthy US women aged 45 years or older. A gender specific study (n= 2749) performed by Khera et al (2005) revealed that women had higher CRP levels than men (median, 3.3 vs. 1.8 mg/l; $p < 0.001$). A prospective nested case control study on 28,263 apparently healthy postmenopausal women for three year resulted that out of various markers like high-sensitivity C-reactive protein (hs-CRP), serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule type 1 (sICAM-1), homocysteine, lipid and lipoprotein profile, HsCRP was found to be the strongest univariate predictor of the risk of cardiovascular events (RR 4.4, 95%CI 2.2-8.9) (Ridker et al, 2000). Bermudez et al (2002) performed a cross sectional study on 340 healthy females to study the association between inflammation and CVD risk factors among women. The study indicated that IL-6 levels were significantly associated with age, BMI, smoking, systolic blood pressure, alcohol use, presence of diabetes, and frequency of exercise. Whereas CRP was associated with age, BMI, systolic blood pressure, high density lipoprotein, smoking, and hormone replacement therapy in multivariate analysis.

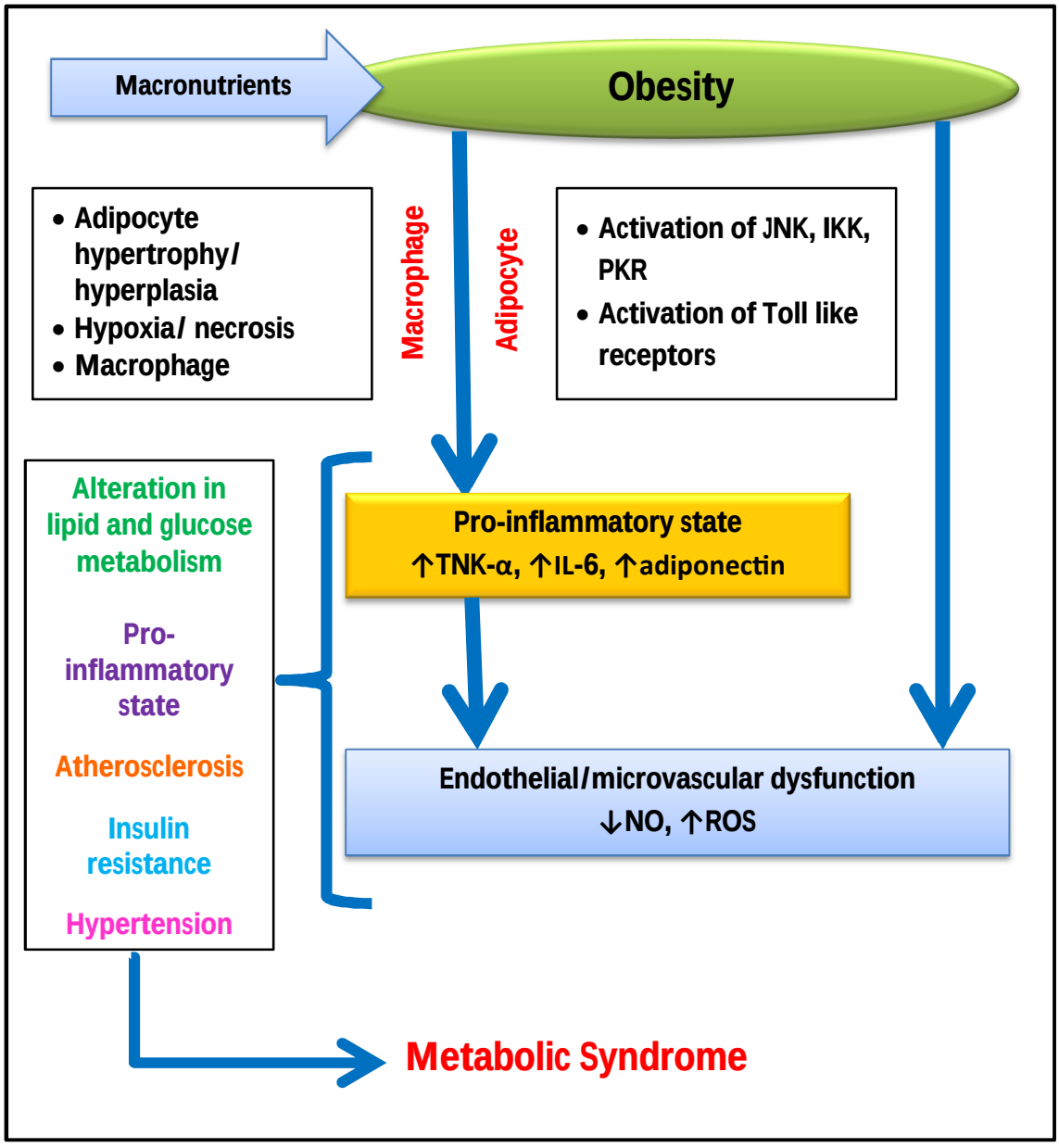
According to Emanuela et al (2012) obesity leads to initiation of proinflammatory process and result in release of inflammatory cytokines (TNF-α, IL-6, adiponectin, etc.). Obesity-induced inflammatory process may lead to various physiological characteristics of metabolic syndrome (Figure 2.17). Increase in inflammation may lead to increased cardiovascular events through reduction in nitric oxide (Teixeira et al, 2014). HsCRP *can*

increase the expression of vascular endothelial plasminogen activator inhibitor-1 (PAI-1) and other adhesion molecules and alter LDL uptake by macrophages (Libby, 2013).

Females were found to have significantly higher odds of developing high hsCRP levels than males (OR 1.63, 95%CI 1.02-2.58), in a study performed by Jeemon et al (2011) on 581 subjects from the sentinel surveillance study in Indian industrial population (SSIP). Singhania et al (2013) reported 59.5% of women subjects from Mumbai having high hsCRP levels. CRP levels among women showed a negative association with HDL-C levels ($p<0.05$) in this cross sectional study including 74 women (30-75y). HsCRP levels were found to be independently associated with obesity among women (n=110) independent of their age in a study performed by Dev and Marcus (2012). Similar trends were observed in another study performed by Ganguli et al (2011) among women (n=100) from Kolkata. A cross-sectional study including 9517 Indian subjects indicated that hsCRP levels were higher in females than males. HsCRP levels were strongly related to the obesity and insulin resistance among the subjects (Mahajan et al, 2012). High HsCRP levels posed a significant higher risk of developing metabolic syndrome among the subjects (OR 1.65, 95%CI 1.41-1.92). Vidhyasagar et al (2013) also indicated increased levels of hsCRP with increase in number of components of metabolic syndrome ($p< 0.008$) among subjects in a hospital based study. High hsCRP levels were also found to be associated with type 2 diabetes (OR 1.66, 95%CI 1.21-2.28) independent of obesity status of the subjects in another population based study (n=2520) (Mahajan et al, 2009). A review performed by Kamath et al (2015) indicated that the normal values of hsCRP seem to be higher in Indians than the western population. There is a need of population based longitudinal studies with large sample size to derive risk cut offs of hsCRP for Indian population.

To summarize, inflammation is a major physiological factor behind the development of NCDs. HsCRP is the most established biomarker of inflammation to predict CVDs. High prevalence of elevated HsCRP levels in women and its association with metabolic syndrome poses pronounced risk of NCDs among middle aged women.

FIGURE 2.17: INTERRELATIONSHIP OF INFLAMMATION, OBESITY AND METABOLIC SYNDROME



Source: Emanuela et al, 2012

ASSOCIATION OF INSULIN RESISTANCE AND NON-COMMUNICABLE DISEASES AMONG WOMEN

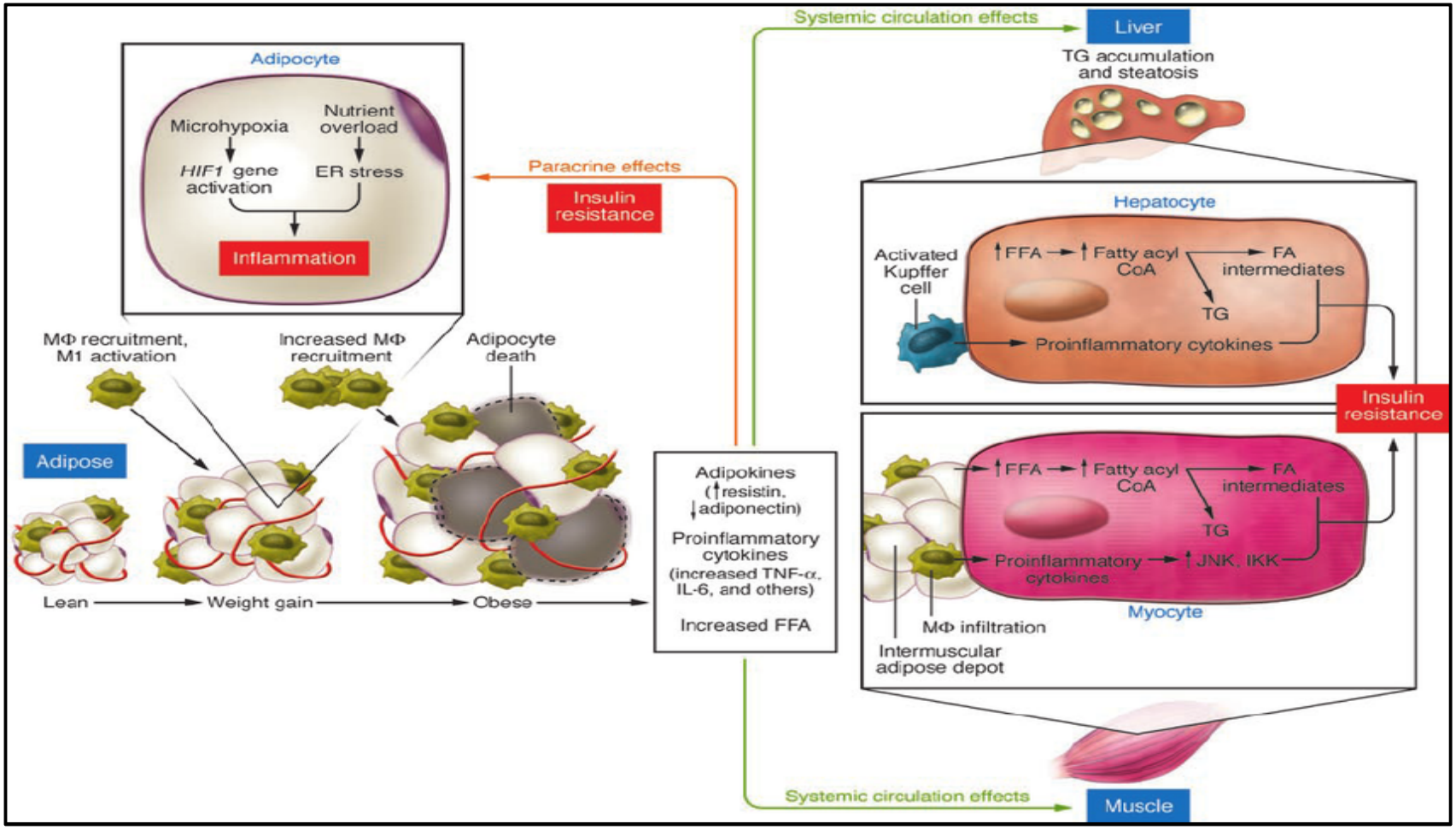
PATHOPHYSIOLOGY AND BIOMARKERS OF INSULIN RESISTANCE

Insulin is an anabolic hormone which facilitates intra-cellular transport of glucose into insulin-dependent tissues such as muscle and adipose tissue (Burks and White, 2001). It helps muscles to utilize carbohydrates, as a primary energy source for muscle contraction. It also suppresses lipolysis and gluconeogenesis through its anabolic activity (Karam, 1997). Insulin resistance is defined as attenuated biological response to elevated insulin levels (Cefalu, 2001). In other terms insulin resistance is impaired sensitivity to glucose disposal facilitated by insulin (Reaven, 2004). Insulin is secreted by β cells of the pancreatic islets of Langerhans as its inactive form i.e. pro-insulin. Proinsulin is synthesized in the ribosomes of the rough endoplasmic reticulum (RER) and transferred to golgi apparatus where it form complex with zinc (Dodson and Steiner, 1998). During transfer of this complex outside golgi apparatus, enzymes convert proinsulin to insulin (Malaisse, 1997). In response to a stimulus like glucose, initial a rapid insulin secretion takes place which is transformed to less intense sustained releases after a while (Bratanova-Tochkova et al, 2002). There are various factors which can affect the insulin secretion like glucose, arginine, fatty acids, mixed meals and gastrointestinal peptide (GIP) and GLP-I hormones. Various counter-regulatory hormones include glucagon, glucocorticoids and catecholamines also regulate the insulin secretion (Karam, 1997). There are various insulin and glucose receptors facilitating entry of glucose into the cells. It is hypothesized that insulin resistance manifests at cellular level due to defects in insulin signaling. Down-regulation, deficiencies or genetic polymorphisms of tyrosine phosphorylation of the insulin receptor, insulin receptor substrate (IRS) proteins or Phosphatidylinositol 3-phosphate kinase, or abnormalities of glucose transporter type 4 (GLUT 4) function may be the possible mechanisms behind insulin resistance (Wheatcroft et al, 2003). Insulin resistance can affect functioning of various organs like muscles, liver, adipose tissues,

endothelium tissues, pancreas, gonads, kidney, brain, bone and pituitary gland (Wilcox, 2005). Within the adipocytes, nutrient excess, weight gain, and obesity can lay down the path for insulin resistance (Figure 2.18) through microhypoxia and endoplasm reticulum stress (Schenk et al, 2008). As adipocytes are not dependent on glucose, insulin resistance may lead to increased β oxidation and free fatty acid flux towards liver which promotes very low density lipoprotein (VLDL) production (Grundy, 2004). However peripheral uptake of triglycerides from VLDL is diminished as lipoprotein lipase activity requires insulin which leads to hypertriglyceridaemia (Krauss and Siri, 2004). Insulin resistance also upregulates the production of pro-inflammatory cytokines like IL-6, TNFα, plasminogen activator inhibitor 1 (PAI-1), angiotensinogen and leptin (Devaraj et al, 2004). In liver, glucose output increases via increased gluconeogenesis during insulin resistance. Compensatory hyper-insulinemia depresses sex hormone binding globulin (SHBG) production and promotes insulin's mitogenic effects. Production of VLDL and TG is also increased as discussed above. Synthesis of C-reactive protein, fibrinogen and PAI-1 is stimulated in response to adipocyte-derived pro-inflammatory cytokines such as TNFα and IL-6 (Devaraj et al, 2004). In insulin resistance, due to diminished capacity of intracellular glucose translocation, muscle glycogen synthesis is also impaired (Hunter and Garvey, 1998).

Measurement of insulin resistance can provide an early insight regarding the risk of various metabolic consequences like type II diabetes, CVDs etc. Gold standard for assessing insulin resistance is Hyperinsulinemic euglycemic clamp (HEC). However, this method is time consuming and quite expensive. Therefore various indices are used to predict insulin sensitivity among non-diabetic population using the values of fasting insulin, glucose and oral glucose tolerance test (OGTT). Indices which use fasting insulin and glucose are HOMA-IR, QUICKI, Matsuda index and McAuley index. Other indices also require OGTT values to predict insulin resistance which include Belfiore index, Avignon index, Stumvoll index and Gutt index (Gutch et al, 2015). HOMA-IR index is widely used in epidemiological studies to assess insulin resistance in comparison to other indices.

FIGURE 2.18: INSULIN RESISTANCE, TISSUE INFLAMMATION AND OBESITY



Source: Schenk et al, 2008

HOMA-IR was found to be a convenient method for evaluating insulin resistance, especially in subjects with visceral fat accumulation in a study performed by Ikeda et al (2001). It was first developed by Matthews et al (1985). They developed a computed-solved model of interaction between the steady-state basal plasma glucose and insulin concentrations in a feedback loop. Deficient β-cell function indicates diminished response of β-cell to glucose-stimulated insulin secretion whereas insulin resistance is reflected by the diminished suppressive effect of insulin on hepatic glucose production. According to Matthews et al (1985) insulin resistance values obtained by HOMA-IR model well correlated with values of the euglycaemic clamp (Rs = 0.88, p less than 0.0001), the fasting insulin concentration (Rs = 0.81, p less than 0.0001), and the hyperglycaemic clamp, (Rs = 0.69, p less than 0.01). A prospective study of 3.5 years on 1449 subjects indicated that HOMA IR index is an effective model in assessing insulin resistance and β cell functions (Haffner et al, 1996). HOMA-IR was also found to a useful tool in predicting glucose intolerance in Thai women of reproductive age having poly cystic ovary disease (Wongwananuruk et al, 2012).

INSULIN RESISTANCE AND NON-COMMUNICABLE DISEASES AMONG WOMEN

HOMA-IR was found to be an independent predictor of cardiovascular disease risk among diabetics in Verona Diabetes Complications Study (n=1326) (Bonora, 2002). In this longitudinal study a 1-unit increase in (log)HOMA-IR value was associated with significant higher odds for prevalent CVD at baseline of (OR 1.31, 95%CI 1.10–1.56) and for incident CVD during follow-up (OR 1.56, 95%CI 1.14–2.12). Tosi et al (2015) performed a study on 116 Caucasian women with PCOD and resulted insulin sensitivity was inversely correlated ($p<0.001$) with parameters of obesity like fat mass, truncal obesity. A population based longitudinal study (20 years) comparing the incidence of diabetes in three ethnic groups indicated that in comparison to Europeans, age-adjusted sub-hazard ratio for developing diabetes was significantly higher in Indian Asian women (HR 1.91, 95%CI 1.18–3.10). Mean levels of HOMA-IR index were significantly higher ($p<0.01$) in Asian Indians (0.7) than Europeans (0.6) in non-diabetics. Whereas, in

subjects who developed diabetes, non-significant high mean levels of HOMA-IR were observed in Indian Asians (0.9) than Europeans (0.8). The results revealed that insulin resistance and truncal obesity together doubled the incidence of diabetes among Asian Indian women (Tillin et al, 2013). Coronary Risk of Insulin Sensitivity in Indian Subjects (CRISIS) Study performed by Yajnik et al (2008) on 149 rural, 142 urban slum and 150 urban middle-class male subjects HOMA-IR, levels increased progressively from rural to urban slum and urban middle-class men. HOMA-IR and adiposity were significantly correlated ($r = 0.57$, $p < 0.001$). Despite a relatively low BMI, adiposity was very prevalent in men and was strongly associated with insulin resistance ($r^2 = 32.5\%$, $p < 0.001$). CRP was positively correlated with age, body mass index (BMI), waist, fasting glucose and insulin, HOMA-IR was found to be significantly correlated with hsCRP, a marker for inflammation in adult women aged between 18-60y in a study performed by Pannacciulli et al (2001). In a study performed by Vikram et al (2006) HOMA-IR levels were found to be independently associated with obesity, sub scapular skinfold thickness however no association was found between HOMA-IR and hsCRP levels. HOMA-IR was found to be positively associated with blood pressure ($p<0.001$), lipid aberrations ($p<0.01$ for TC, TG, LDL-C) family history ($p<0.05$) (beta = 0.100, $p < 0.001$), generalized and abdominal obesity ($p<0.001$) among 1550 subjects from the Chennai Urban Rural Epidemiology Study [CURES-66] irrespective of their age and BMI. Subjects with high HDL-C levels ($p<0.001$) and engaged in heavy physical activity ($p<0.001$) had significantly lower HOMA-IR values than their counterparts (Sandeep et al, 2011).

To summarize, macronutrient overload and obesity is one of the primary causes of insulin resistance. Lipid abnormalities and inflammation are the associated metabolic derangements with insulin resistance and a cluster of them can lead to development of non-communicable diseases. HOMA-IR is a convenient and reliable method to assess insulin resistance. There is a lack of epidemiological studies on association of insulin resistance and other risk factors of NCDs among women.

APPROACHES TO PREVENT EARLY METABOLIC AND INFLAMMATORY ABERRATIONS

From the review so far, it is evident that menopause can lead to various biophysical and metabolic derangements. The presence of obesity at the time of menopause can aggravate the menopausal symptoms and risk factors of non-communicable diseases in the later life. Inflammation and insulin resistance can be the key factors related to various metabolic risk factors of CVDs. Therefore there is a need to focus on the early preventive strategies for obesity, biophysical, metabolic and inflammatory aberrations. Promotion of health lifestyle habits should be the primary focus of various intervention programs. Major lifestyle components for early prevention of risk factors for non-communicable diseases are as follows:

PHYSICAL ACTIVITY

The prevalence of physical inactivity is about 31.1% around the world and 17·0% in south-east Asia (Hallal et al, 2012). According to Lee et al (2012) physical inactivity causes 3.2% of the burden of disease from coronary heart disease, 3.9% of type 2 diabetes, 5.6% of breast cancer, and 5.7% of colon cancer in south-east Asian population. The life expectancy of the world's population can be increased by 0·68 years through eradication of physical inactivity. In a large scale prospective study of 25 years (Kubota et al, 2017) including 7252 women it was observed that the respective lifetime risks of CVD in women with no moderate to heavy activity was 42.4% (39.5-44.9) in comparison to 30.5% (27.5-33.1) in those who performed physical activity at recommended levels. According to WHO (2010) recommendation, an adult should perform at least 150 minutes of moderate-intensity aerobic physical activity, or at least 75 minutes of vigorous-intensity aerobic physical activity per week. A systemic review of long term longitudinal studies with large sample size (500 or more) and more than 5 years of follow up resulted that physical activity have a positive long term impact on coronary heart disease, type 2 diabetes mellitus, Alzheimer's disease and dementia (Reiner et al, 2013).

ALCOHOL, SMOKING AND TOBACCO

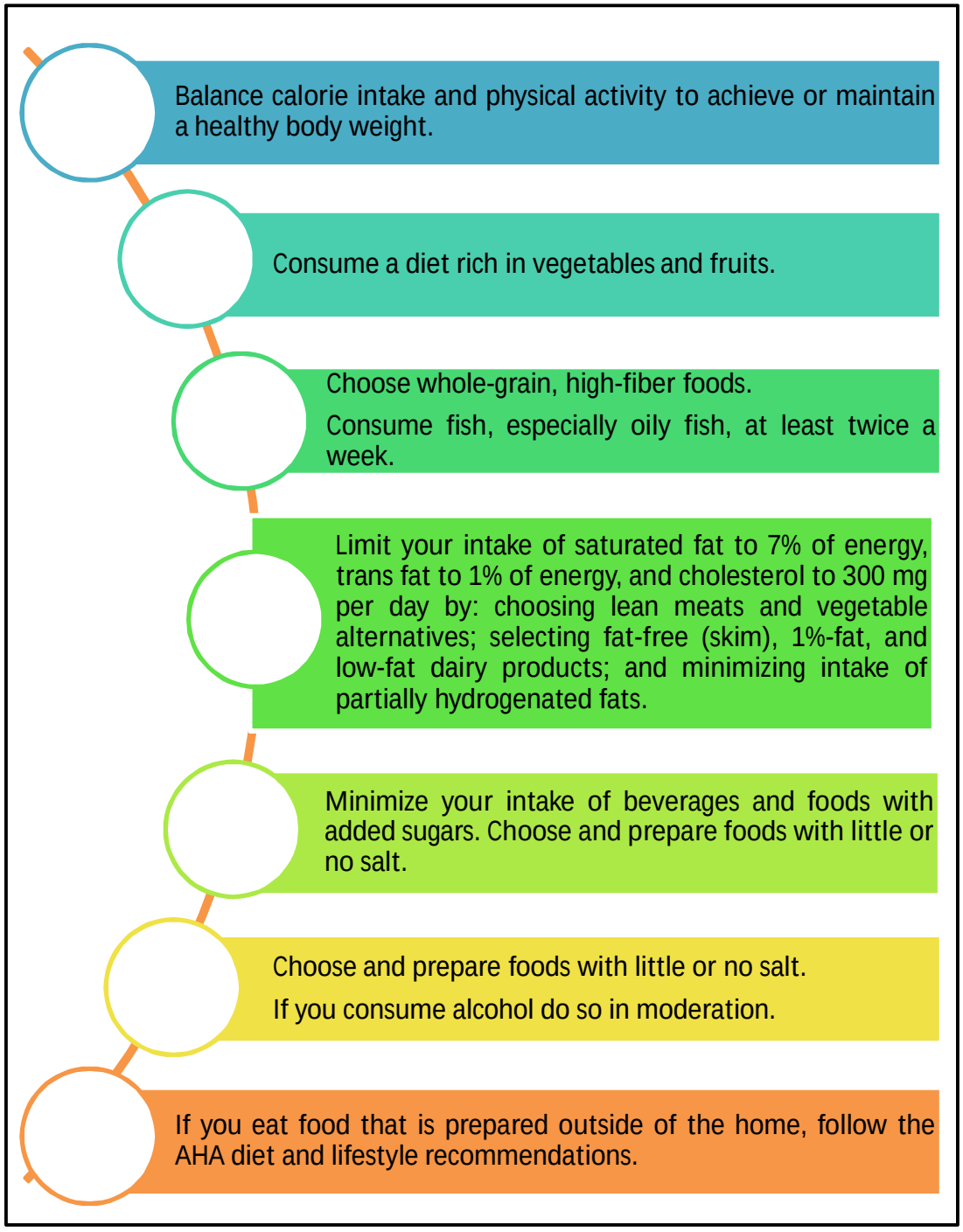
Alcohol consumption leads to a significant percent for the global burden on NCDs. About 33.4% of the alcohol-attributable deaths are due to cardiovascular disease and diabetes whereas 12.5% are due to cancer in both male and females (WHO, 2014). Smoking is the single greatest preventable cause worldwide (WHO, 2009). Out of all deaths due to non-communicable diseases among women all over the world, 9% are attributable to tobacco intake (WHO, 2012). Therefore life style modifications are required to quit/avoid smoking and tobacco chewing and limiting alcohol intake to moderation (30ml for women and 60ml for men) (Lichtenstein et al, 2006).

DIETARY MODIFICATION

Appropriate quantity as well as quality of the diet is very important for prevention of non-communicable diseases. According to Hawkes and Popkin (2015) a transformation towards a healthy and sustainable diet is necessary to meet many of the sustainable development goal (SDGs) set under millennium development goals. American Heart Association (Lichtenstein et al, 2006) focusses on consumption of fruits and vegetables, whole grain, high fiber diet, fish oil, limit saturated and trans fat intake, limit intake of sweetened beverages and salt (Figure 2.19). Replacement of whole plant foods (fruit, vegetables, nuts, seeds, unrefined whole grains) by refined carbohydrates, fast foods/snack foods/processed foods, and fried foods in the vegetarian diet due to nutrition transition is the biggest culprit along with use of cooking oils having athrogenic activity for the epidemic of CVDs in India(Singh et al, 2014). A comparative study of risk factors of NCDs among six different countries revealed that India had highest prevalence of low fruit and vegetable intake in comparison to China, Ghana, Russian federation, Mexico and South Africa (Wu et al, 2015).

According to the results of Nurses' Health Study (n=121700) females who did not smoke, were having normal weight, maintained the healthful diet i.e. low in trans fat and glycemic load, high in cereal fiber, marine n–3 fatty acids, and folate, and with a high ratio of polyunsaturated to saturated fat; exercised moderately or vigorously for

FIGURE 2.19: AHA 2006 DIET AND LIFESTYLE RECOMMENDATIONS FOR CARDIOVASCULAR DISEASE RISK REDUCTION



Source: Lichtenstein et al, 2006

half an hour a day, and consumed alcohol moderately had an incidence of coronary events >80 percent lower than other females of the study (Stampfer et al, 2000). Therefore there is a need to promote health lifestyle and inclusion of variety of food in the diet having functional properties which can help in prevention of metabolic derangements on the long run among women.

FUNCTIONAL FOODS

According to the International Life Sciences Institute (1999) "foods that, by virtue of the presence of physiologically-active components, provide a health benefit beyond basic nutrition" are defined as functional food. As per the definition of American Dietetic Association (1999) foods that are "whole, fortified, enriched, or enhanced," and consumed as "part of a varied diet on a regular basis, at effective levels" for their maximum health benefit. Bioactive compounds present in functional food helps in improving obesity through regulation of food intake, adipocyte lifecycle and function, and the lipid metabolism via genetic and epigenetic mechanisms (Lai et al, 2015). Majority of the functional food belong to plant origin like fruits and vegetables, whole grains, nuts, legumes, tea etc. However different foods from some animal sources also contain bioactive compounds like n-3 fatty acid, probiotics, conjugated linoleic acid (CLA) etc. (Hasler, 2002). Table 2.4 categorizes various functional food based on their bioactive components and describes their potential health benefits. Along with these bioactive compounds there are various vitamins (vitamin B complex, A, C, D and E) and minerals (calcium, potassium, magnesium and selenium) which exert functional properties (International Food Information Council Foundation, 2009). ADA has focused on consumption of whole functional food on regular basis. However the pharmaceutical market has developed the concept of 'nutraceuticals'. Nutraceuticals are "concentrated form of a presumed bioactive agent from a food, presented in a nonfood matrix, and used to enhance health in dosages that exceed those that could be obtained from normal food" (Zeisel, 1999).

TABLE 2.4: VARIOUS BIOACTIVE COMPOUNDS, THEIR FUNCTIONAL SOURCES AND POTENTIAL HEALTH BENEFITS

(a)

Bioactive compound	Functional food	Potential health benefit
CAROTENOIDS		
Beta-carotene	carrots, pumpkin, sweet potatoes, spinach, tomatoes	Neutralizes free radicals Which may damage cells; bolsters cellular antioxidant defenses; acts as vitamin A precursor
Lutein, Zeaxanthin	spinach, corn, eggs, citrus fruits, asparagus, carrots, broccoli	Supports maintenance of eye health
Lycopene	tomatoes and processed tomato products, watermelon	Supports maintenance of prostate health
DIETARY FIBER		
Insoluble fiber	wheat bran, corn bran, fruit skins	Supports maintenance of digestive health; may reduce the risk of some types of cancer
Beta glucan	oat bran, oatmeal, oat flour, barley, rye	May reduce risk of Coronary heart disease
Soluble fiber	psyllium seed husk, peas, beans, apples, citrus fruits	May reduce risk of CHD and some types of cancer
Whole grains	cereal grains, whole wheat bread, oatmeal, brown rice	May reduce risk of CHD and some types of cancers; supports maintenance of healthy blood glucose levels
FATTY ACIDS		
MUFAs	tree nuts, olive oil, canola oil	May reduce risk of CHD
Omega-3 fatty acids-ALA	walnuts, flaxseeds, flaxseed oil	Supports maintenance of heart and eye health; supports maintenance of mental function
Omega-3 fatty acids-DHA/EPA	salmon, tuna, marine and other fish oils	May reduce risk of CHD; supports maintenance of eye health and mental Function
CLA	beef and lamb; some cheese	Supports maintenance of desirable body composition and immune health

(b)

Bioactive compound	Functional food	Potential health benefit
FLAVANOIDS		
Anthocyanins- Cyanidin, Pelargonidin, Delphinidin, Malvidin	berries, cherries, red grapes	Bolster cellular antioxidant defenses; supports maintenance of healthy brain function
Flavanols- Catechins, Epicatechins, Epigallocatechin	tea, cocoa, chocolate, apples, grapes	Supports maintenance of heart health
Procyanidins and Proanthocyanidins	cranberries, cocoa, apples, strawberries, grapes, red wine, peanuts, cinnamon, tea, chocolate	Supports maintenance of urinary tract health and heart health
Flavanones- Hesperetin, Naringenin	citrus fruits	Neutralizes free radicals which may damage cells; bolster cellular antioxidant defenses
Flavonols- Quercetin, Kaempferol, Isorhamnetin, Myricetin	onions, apples, tea, broccoli	Neutralizes free radicals which may damage cells; bolster cellular antioxidant defenses
ISOTHIOCYANATES		
Sulforaphane	Cauliflower, Broccoli, Cabbage	May enhance detoxification of undesirable compounds; bolsters cellular antioxidant defenses
PHENOLIC ACIDS		
Caffeic Acid, Ferulic Acid	Apples, Pears, Citrus Fruits, Some Vegetables, Whole Grains, Coffee	Bolsters cellular antioxidant defenses; supports maintenance of eye and heart health
PLANT STANOLS/STEROLS		
Free Stanols/Sterols	Corn, Soy, Wheat, Fortified Foods And Beverages	May reduce risk of CHD
Stanol/Sterol Esters	Dietary Supplements, Fortified Foods	May reduce risk of CHD

(c)

Bioactive compound	Functional food	Potential health benefit
POLYOLS		
Sugar Alcohols-Xylitol, Sorbitol, Mannitol, Lactitol	Some Chewing Gums And Other Food Applications	May reduce risk of dental caries
PREBIOTICS		
Inulin, Fructooligosaccharides (FOS), Polydextrose	Whole Grains, Onions, Some Fruits, Garlic, Honey, Banana, Fortified Foods	Supports maintenance of digestive health; supports calcium absorption
PROBIOTICS		
Yeast, *Lactobacilli, Bifidobacteria* And Other Specific Strains Of Beneficial Bacteria	Certain Yogurts And Other Cultured Dairy And Non-dairy Applications	Supports maintenance of digestive and immune health; benefits are strain specific
PHYTOESTROGENS		
Isoflavones-Daidzein, Genistein	Soybeans And Soy-Based Foods	Supports maintenance of bone and immune health, and healthy brain function; for women, supports menopausal health
Lignans	Flax Seeds, Some Vegetables, Seeds And Nuts, Lentils, Broccoli, Cauliflower, Carrot	Support maintenance of heart and immune health
SOY PROTEIN		
Soy Protein	Soybeans And Soy-Based Foods Like Milk, Yogurt, Cheese, Tofu	May reduce risk of CHD
SULFIDES/THIOLS		
Diallyl Sulfide, Allyl Methyl Trisulfide	Garlic, Onions, Leeks,	May enhance detoxification of undesirable compounds; supports maintenance of heart, immune and digestive health
Dithiolthiones	Cruciferous Vegetables	May enhance detoxification of undesirable compounds; supports maintenance of healthy immune function

Source: International Food Information Council Foundation, 2009

In a natural complex food system there are hundreds of bioactive compound. There compounds may interact with each other as well as with the gastro intestinal environment to exert the desired effect which may not be achieved even with the large doses of nutraceuticals (Fardet, 2015). For example data from various human intervention studies have failed to establish the beneficial impact of multivitamin and mineral supplements to prevent cancer and chronic disease in healthy people (Huang et al, 2006). Therefore there is a need to follow the holistic approach of incorporation of different functional food in the diet is such amount which can be consumed on a regular basis.

FLAXSEED: A PROMISING FUNCTIONAL FOOD

TAXONOMY AND VARIETIES

Flaxseed also known as linseed is emerging as a major functional food to prevent non-communicable diseases. Its botanical name is *Linum usitatissimu and belongs to the family* Linaceae. The length of the mature plant is around 60cm with a fibrous stem. The flower is bright blue in colour having 3cm diameter (Pradhan et al. 2010). Flaxseed measures around 4-6mm with a flat, oval shape and sharp tip (Daud et al, 2003). The flavor of seeds is nutty with crisp texture (Carter, 1996). Based on its pigments it can be divided into two varieties: brown and golden. Flax is generally produced for human consumption, industrial oil production and fiber-linen fabrication and its variety vary widely according to purpose of production (BeMiller et al, 1993). Highest yield of world's total production of flaxseed comes from Canada, followed by China, United States and India (Rubilar et al, 2010). The major traditional varieties of flaxseeds are Surbhi, Nagarkot, Jeevan, Janaki, Himalini, Him Alsi-I, Him Alsi-2 (Kumar, 2012), Shekhar, Suyog, Meera, Parvati, Neelum, Neela etc. However, hundreds of varieties of flaxseed are being developed and released under project "All India coordinated Research Project on Linseed" (ICAR, 2011) at different agriculture research institutes of India. These varieties are aimed to produce better yield, disease resistant and having short maturity period.

NUTRIENT COMPOSITION

Flaxseed is a rich source of fats, proteins and fiber. Indian and Canadian flaxseed varieties provide around 444±3.8 kcal and 450kcal/ 100g (NIN, 2017; Morris, 2007). The average carbohydrate, protein and fat content of Indian flaxseeds (100g) are 10.99±0.86g, 18.55±0.15g and 35.67±0.70g respectively (Table 2.5a). The Canadian flaxseeds contain around 29g carbohydrate (including dietary fiber), 20g protein and 41g fats. Total fiber content is around 35.67±0.70g/100g with a fraction of 4.33±0.84g soluble and 21.83±0.60g insoluble fiber (Table 2.5c). Major fibers present in flaxseeds are cellulose, mucilage gums and lignins. Other carbohydrates present in flaxseeds are starches, fructose, galactose and raffinose. Amino acid profiles of the Indian flaxseeds indicate that it contains all essential amino acids with fair amount of leucine (Table 2.5d). According to Rubilar et al (2010) the chemical composition of flaxseeds can vary according to the variety as well as the environment in which plant has grown.

Flaxseeds are rich in α linolenic acid (ALA). As described in table 2.5b Indian varieties of flaxseeds contain lower amount of ALA (12956±467mg or 13g /100g) in comparison to Canadian flaxseed (23g/100g). Around 36.4% of the total lipid fraction of Indian flaxseed is made up of ALA. Other major fatty acids present Indian flaxseeds (100g) are linoleic acid (3191±144mg), oleic acid (5049±303mg), palmitic acid (1503±68.1mg) and steric acid (1503±68.1mg). Overall it contains around 3g of saturated fats, 5g of MUFA and 16g of PUFA. Indian flaxseed also contain different B-complex, fat soluble vitamin and minerals, though is small quantities (Table 2.6a and c).

FUNCTIONAL COMPONENTS

α linolenic acid

Flaxseeds are the richest source of α linolenic acid (ALA). Table 2.7 gives the % ALA content of in different flaxseed varieties in India, which ranges from 33.97- 53.10% of the total fats. ALA, an essential fatty acid which cannot be synthesized in our body, was

TABLE 2.5: COMPOSITION OF MACRONUTRIENT AND THEIR CONSTITUENTS IN INDIAN FLAXSEED (BROWN) PER 100g

(a) MACRONUTRIENTS	
Energy (Kcal)	443.83±3.8
Carbohydrate (g)	10.99±0.86
Protein (g)	18.55±0.15
Total Fats(g)	35.67±0.70

(b) TYPES OF CARBOHYDRATE	
Total Fiber (g)	26.17±0.39
Soluble Fiber (g)	4.33±0.84
Insoluble Fiber (g)	21.83±0.60
Total Starch (g)	7.55±0.13
Fructose (g)	0.20±0.02
Glucose (g)	0.40±0.05
Total Free Sugars (g)	0.60±0.04

(c) FATTY ACID PROFILE	
Palmitic (C16:0) (mg)	1503±68.1
Stearic (C18:0) (mg)	1323±65.6
Arachidic (C20:0) (mg)	59.74±4.28
Behenic (C22:0) (mg)	51.21±3.89
Lignoceric (C24:0) (mg)	31.93±2.83
Palmitoleic (C16:1) (mg)	25.61±1.29
Oleic (C18:1n9) (mg)	5049±303
Eicosaenoic (C20:1n9) (mg)	36.60±1.43
Linoleic (C18:2n6) (mg)	3191±144
α-Linolenic (C18:3n3) (mg)	12956±467
Total Saturated Fatty Acids (TSFA) (mg)	2968±48.4
Total Mono Unsaturated Fatty Acids (TMUFA) (mg)	5112±303
Total Poly Unsaturated Fatty Acids (TPUFA) (mg)	16147±378

(d) AMINO ACID PROFILE	
Histidine (g)	2.62±0.10
Isoleucine (g)	4.47±0.22
Leucine (g)	6.81±0.26
Lysine (g)	4.63±0.22
Methionine (g)	1.75±0.05
Cystine (g)	1.61±0.10
Phenylalanine (g)	5.58±0.26
Threonine (g)	3.88±0.10
Tryptophan (g)	1.57±0.12
Valine (g)	5.31±0.13
Alanine (g)	4.89±0.25
Arginine (g)	11.15±0.62
Aspartic Acid (g)	10.87±0.25
Glutamic Acid (g)	17.70±1.07
Glycine (g)	5.71±0.20
Proline (g)	4.39±0.17
Serine (g)	5.25±0.12
Tyrosine (g)	3.48±0.12

Source: Indian Food Composition Table (NIN), 2017

TABLE 2.6: COMPOSITION OF MICRONUTRIENT AND OTHER COMPOUNDS IN INDIAN FLAXSEED (BROWN) PER 100g

(a) VITAMINS	
Thiamine (mg)	0.28±0.018
Riboflavin (mg)	0.05±0.001
Niacin (mg)	1.09±0.05
Pantothenic acid (mg)	0.37±0.03
Total B6 (mg)	0.35±0.020
Biotin (mcg)	21.25±0.49
Total folates (mcg)	86.50±2.75
Ergocalciferol (D2) (mcg)	0.55±0.13
α Tocopherol (mg)	7.79±0.81
Gama Tocopherol (mg)	1.51±0.13
α Tocotrienol (mg)	1.12±0.01
α-Tocopherol Equivalent (mg)	8.28±0.81
Phylloquinones (K1) (mcg)	19.17±0.70
Lutein (mcg)	5.67±0.81
Zeaxanthin (mcg)	7.22±0.70
β-Carotene (mcg)	1.05±0.03
Total Carotenoids (mcg)	92±22.8

(b) MINERALS AND TRACE MINERALS	
Arsenic (mcg)	8.25±3.18
Cadmium (mg)	0.009±0.002
Calcium (mg)	257±14.0
Chromium (mg)	0.001±0.000
Cobalt (mg)	0.033±0.014
Copper (mg)	1.34±0.09
Iron (mg)	5.44±0.69
Lead (mg)	0.004±0.002
Magnesium (mg)	349±19.8
Manganese (mg)	2.14±0.23
Molebdeum (mg)	0.022±0.009
Nickel (mg)	0.171±0.052
Phosphorus (mg)	445±20.1
Potassium (mg)	655±45.6
Selenium (mcg)	46.87±9.22
Sodium (mg)	32.93±6.45
Zinc (mg)	4.86±0.25

(c) ORGANIC ACIDS AND POLYPHENOLS	
Total oxalate (mg)	5.85±1.00
Soluble Oxalates (mg)	1.91±0.34
Insoluble oxalates (mg)	3.95±1.09
Citric Acid (mg)	9.73±0.53
Fumaric Acid (mg)	7.06±1.03
Mallic Acid (mg)	11.46±2.65
Succinic Acid (mg)	1.87±0.19
Caffeic acid (mg)	2.07±0.31
Chlorogenic Acid (mg)	1.44±0.19
Ferulic acid (mg)	1.44±0.12
Resvertrol (mg)	1.82±0.61
Total Polyphenols (mg)	31.60±13.38

(d) OLIGOSACCHARIDES PHYTOSTEROLS PHYTATES AND SAPONINS	
Raffinose (g)	3.52±0.359
Campesterol (mg)	31.48±0.23
Stigmasterol (mg)	7.25±0.03
β-Sitosterol (mg)	112±0.2
Phytate (mg)	1859±15.8
Total Saponin (g)	2.21±0.29

Source: Indian Food Composition Table (NIN), 2017

first identified by Burr and Burr in 1930. ALA can be converted to eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3) in our body. n-3 Fatty acids is a major component of phospholipids, as well as sphingolipids and plasmalogens of cell membranes. Therefore unsaturated long-chain n_3 fatty acids help in maintaining eg, fluidity, thickness, and deformability of the plasma membrane and affect transmembrane proteins activity (Rajamoorthi et al, 2005). DHA is important in accommodating transitional changes associated with transmembrane protein activation due to its flexibility (Gawrisch et al, 2003; Salem et al, 2001). The relative proportion of different long chain fatty acids fatty acids also determines their availability for cyclooxygenases and lipoxygenases synthesis, which balances the eicosanoids and other anti-inflammatory autocoids, like resolvins (Serhan, 2005). N-3 fatty acids can also influence gene regulation through action as ligands for nuclear receptors (Li et al, 2005).

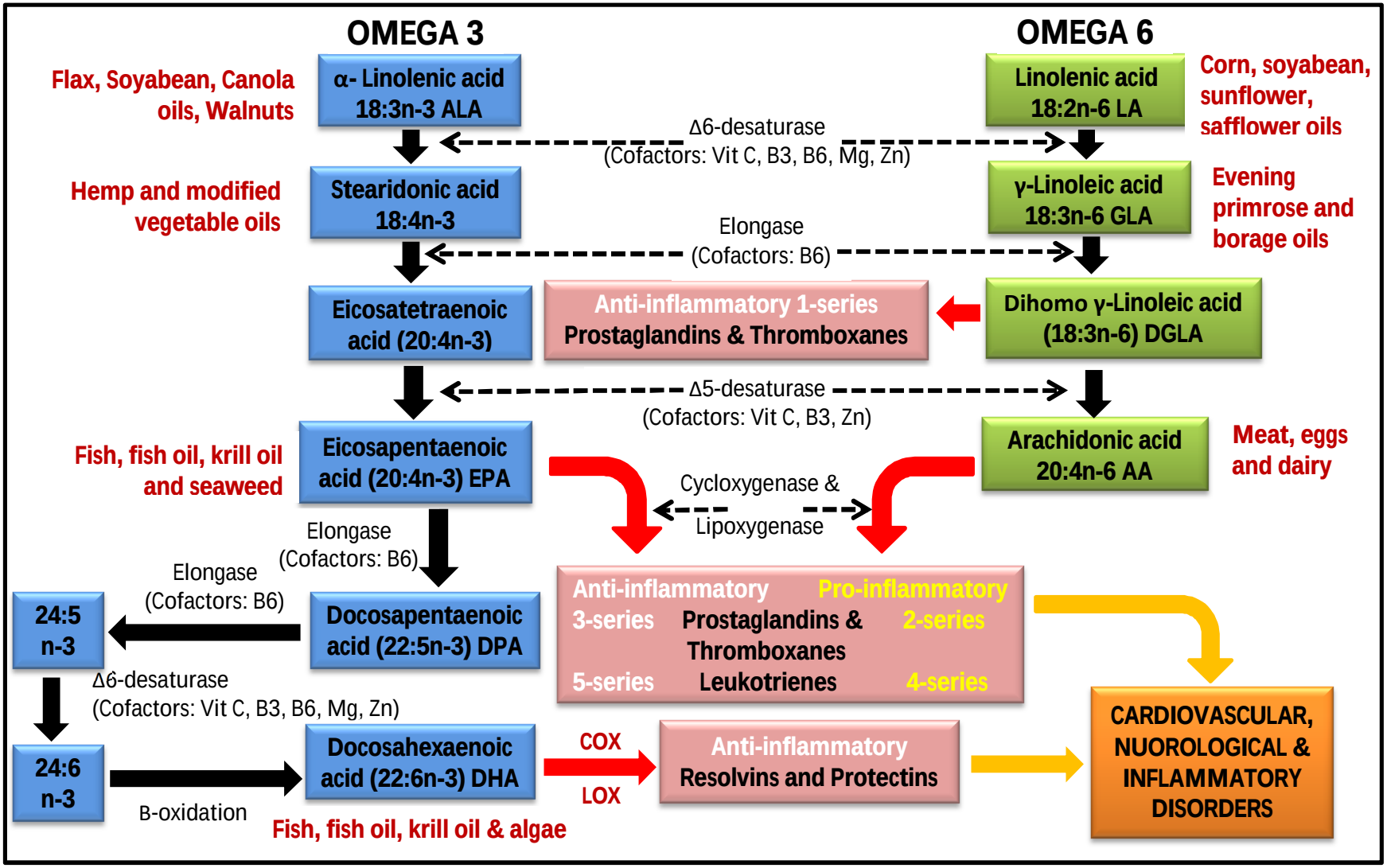
The conversion of ALA to EPA and DHA occurs through a series of elongation and desaturation reactions. The process takes place in endoplasmic reticulum of the liver. Different elongase enzymes increase carbon chain length through sequential addition of 2 carbon units whereas desaturase enzymes remove hydrogen and create double bonds (Figure 2.20). The final step for conversion into DHA involves peroxisomal β oxidation. (Arterburn et al, 2006). The enzymes required for long chain n-3 and n-6 fatty acids are same. Though desaturase enzymes have a higher affinity for (Plourde and Cunnane 2007), high levels of dietary LA can compete for the enzymes and inhibit EPA/DHA formation (Liou et al, 2007). EPA synthesizes anti-inflammatory eicosanoids through action of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Sprecher 2000). Resolvins and protectins are two major anti-inflammatory substances produced by metabolism of DHA (Adkins and Kelley, 2010). The conversion of ALA into EPA/DHA in the body is quite limited. About 15–35% of dietary ALA is catabolized for energy (Vermunt et al, 2000; Burdge et al, 2003) in the body. Conversion rate of ALA into EPA is approximately 5%. However, conversion of ALA to DHA is almost negligible (<0.5-1%) (Plourde and Cunnane, 2007; Goyens et al, 2005). The conversion of DPA to DHA is the rate-limiting step in this process (Pawlosky et al, 2003).

TABLE 2.7: ALA CONTENT OF FORTY EIGHT DIFFERENT FLAXSEED VARIETIES OF INDIA

Name of Variety	% Oil content of flaxseed	% ALA content of total fat	Name of Variety	% Oil content of flaxseed	% ALA content of total fat
Kiran	36.60	33.14	RLC-128	37.08	40.48
Deepika	42.27	46.63	RLC-129	36.93	46.59
Kartika	41.11	43.39	RLC-132	38.31	49.09
Indira Alsi-32	42.12	38.74	RLC-133	40.38	49.60
Shekhar	39.76	46.13	RLC-134	37.34	53.01
Neela	33.97	36.85	RLC-135	36.80	38.14
Rashmi	37.82	38.36	RLC-137	40.11	49.97
Sharda	41.12	34.41	R-552	36.52	52.41
Meera	39.65	38.00	CI-229	37.96	42.79
PKDL-43	39.65	42.04	NL-97	36.15	37.76
PKDL-58	38.48	43.66	Polf-22	37.76	37.94
PKDL-62	38.04	41.69	T-397	37.01	40.85
JRF-5	38.22	46.53	LC-54	35.81	37.77
JLS-9	40.50	39.31	LCK-88068	37.59	41.04
KL-1	39.48	44.20	FRW-12	37.30	44.36
KL-168	40.28	39.75	Gcwargi 1-2	37.66	39.50
GS-27	36.74	43.57	R-2678	36.94	46.07
GS-61	39.34	46.45	R-4129	39.91	45.36
GS-64	39.12	50.49	R-4140	38.30	51.14
GS-129	39.18	46.64	R-4141	36.72	44.33
RLC-92	40.23	54.82	R-4152	36.85	47.98
RLC-94	36.74	49.71	R-4154	38.98	49.65
RLC-122	38.55	51.08	R-4158	38.76	38.47
RLC-123	39.82	43.21	R-4168	38.04	49.79

Source: Pali and Mehta, 2014

FIGURE 2.20: METABOLISM OF OMEGA-3 AND OMEGA-6 FATTY ACIDS



Source: Gillingham, 2013

The oxidation rate of ALA is also quite high in comparison to other unsaturated fatty acids (Nettleton, 1991). There are various factors which can affect the conversion of ALA to EPA/DHA like amount of EPA/DHA, n-6 fatty acid and trans fats in the diet, micronutrients, gender and genetics (Gillingham, 2013). Dietary DHA and EPA down-regulate this step of conversion of ALA to EPA/DHA even up to 70% (Pawlosky et al, 2003; Vermunt et al, 2000; Burdge et al, 2003). According to Kummerow (2004) trans fats can also inhibit the synthesis of ω-3 long-chain PUFA. According to Emken et al (1994) a diet high in LA can reduce the conversion of ALA to ω-3 long-chain PUFA up to 40%. Vitamins B3, B6, and C, magnesium and zinc can also affect the synthesis of ω-3 long-chain PUFA in the body as they act as cofactors to the Δ5- and Δ6-desaturase enzymes (Lorgeril and Defaye, 2005; Harris et al, 2009). The conversion of ALA to EPA in males is around 0.3-8% and ALA to DHA undetectable-4% in various studies (Burdge et al, 2003; Burdge et al, 2002; Hussain et al, 2005; Emken et al, 1994). This conversion appears better in the females (21% for EPA and 9% for DHA) (Burdge and Wootton, 2002).

The Institute of Medicine, Washington (2002) has established the recommended dietary allowance for ALA as 1.1d/day. American Heart Association has recommended 1.5-3g of ALA intake daily to exert cardio protective effect. According to WHO/FAO recommendations (2008), the lower accepted macronutrient dietary range (L-AMDR) for ALA intake is >0.5g/day. The AMDR for ALA is set as 0.5-2% of total energy intake by Indian Institute of Medical Research, India (2010). Because of the low availability and consumption of salty fish in Indian diet, major source of n-3 fatty acid is ALA (Ghafoorunissa, 1996). According to National Nutrition Monitoring Bureau (2002) the average ALA intake of Indian rural men is around 0.24% of total energy per day. Most of the oils consumed in India do not provide the AMDR levels for ALA if consumed according to balanced diet provided by ICMR and there is a high chance of skewed ratio of n-3/n-6 ratio in the diet if oil not consumed in blended form (Mani and Kurpad, 2016). Inadequate ALA intake with high levels of LA in the diet is associated with increased risk of various non-communicable diseases. Various animal studies indicate that high LA and

low ALA intake can lead to pro-inflammatory condition and an increased deposition of adipose tissue (Alvheim et al, 2012; Ailhaud et al, 2006; Pouteau et al, 2010). A meta-analysis of five prospective studies and three clinical trials indicated that high ALA intake was associated with reduced risk of fatal heart disease (combined relative risk 0.79, 95%CI 0.60 –1.04) and it may provide protection against heart disease (Brouwer et al, 2004). Another recent meta-analysis and systemic review was performed by Pan et al (2012) which included twenty-seven prospective studies (251,049 individuals and 15,327 CVD events). The results of 13 comparisons in which dietary ALA was used as exposure indicated significant lower risk with high ALA consumption (pooled RR: 0.90; 95% CI 0.81-0.99). Similar trends were seen in comparisons using ALA biomarkers as exposure, however was not statistically significant (pooled RR: 0.80; 95% CI 0.63-1.03).

Lignan and other polyphenols

Flaxseeds are richest source of lignans also, which are bioactive compounds belonging to phenolic acid group. Their chemical structure involves dimers of hydroxycinnamic acids (*p*-coumaric, ferulic, and sinapic acids (Peterson et al, 2010). They act as antioxidant and phytoestrogen in human body. The chemical structure of phytoestrogens is similar to natural and synthetic estrogens. Depending on their concentration they can act like weak estrogens by binding to the estrogen receptor (Morris, 2007). Secoisolariciresinol diglucoside (SDG) is the major lignan of flaxseeds. Other flaxseeds lignans include matairesinol, pinoresinol, lariciresinol, isolariciresinol and secoisolariciresinol (SECO) (Smeds et al, 2007; Thompson et al, 2006). Flaxseeds have 47 times higher lignan content than sesame seeds and more than 600 times higher lignan than garlic (Thompson et al, 2006). The concentrations of SDG in flaxseed may vary with different varieties. Table 2.8 shows the SDG content of different flaxseed varieties of India. The data shows high variability in SDG content ranging from 1.12-7.9mg/g of flaxseeds (BAIF-NAIP, 2013). Lignans are hydrolyzed by some of the anaerobic microbes in the gut to lignan aglycones and further into enterolignans.

TABLE 2.8: SDG (LIGNAN) CONTENT OF FIFTY DIFFERENT FLAXSEEDS VARIETIES OF INDIA

Name of Variety	Lignan Content (mg/g)	Name of Variety	Lignan Content (mg/g)
Surabhai	2.99	K-2	3.44
Neelum	3.03	Himalsi-1	6.26
Jawahar-17	5.14	RLC-95	5.58
Meera	6.58	LMS-95-4	3.68
R-552	7.9	BAU-9906	4.83
Garav	1.63	LC-2279-4	4.19
RLC-76	2.5	T-397	5.82
Neela	1.93	Shikha	5.61
Pusa-2	2.23	C-429	4.83
KL-224	7.49	JLS-9	3.14
Jeevan	3.19	KL-43	4.04
LC-54	1.23	Grima	4.79
Nagrkot	3.19	SLS-27	2.6
KL-221	1.12	RLU-6	2.5
Shekhar	2.32	LMS-4-27	2.09
Rashami	4.45	Jawahar-7	3.08
LS-3	1.63	Laxmi-27	2.09
Parvati	3.06	Shubhra	6.12
RL-914	2.67	S-36	6.1
RLC-81	2.63	Sweta	4.93
LC-2063	4.42	Himalini	1.98
Kiran	3.33	Heera	3.16
Mukta	2.69	Suyog	2.21
Jawahar-23	3.37	Himalsi-2	3.92
Sheela	3.85	LC-185	2.02

Source: BAIF-NAIP, 2013

Secoisolariciresinol diglucoside is metabolized in the gut to secoisolariciresinol, then to the enterolignan enterodiol and finally to enterolactone (Clavel et al, 2006a; Clavel et al, 2006b; Clavel et al, 2005). The metabolic efficiency depends upon genetic or other factors (Low et al, 2005a; Low et al, 2005b). Enterodiol and enterolactone can be directly excreted in the feces; can be taken up by epithelial cells lining the human colon, conjugated with glucuronic acid or sulfate and excreted in the feces or enter the circulation (Jansen et al, 2005); or can be absorbed from the gut and transported to the liver, conjugated and released into the bloodstream (Raffaelli et al, 2002). Ultimately, they enter enterohepatic circulation, secreted into bile and reabsorbed from the intestine and are excreted in the urine in conjugated form (Axelson et al, 1982). Other polyphenols present in flaxseeds are caffeic acid, chlorogenic acid and fumaric acid and resvertrol (NIN, 2017).

The flaxseed lignan secoisolariciresinol (SECO) and its diglucoside Secoisolariciresinol Diglucoside (SDG) can exert various health benefits due to their antioxidant properties (Hosseinian et al, 2007; Rajesha et al, 2010). SECO and SDG are hypothesized to possess chemo-preventive properties (Muir and Westcott, 2003; Pattanaik and Prasad, 1998). Dietary flaxseed supplementation with SDG can reduce the growth of established human breast tumors in athymic mice with low circulating estrogen concentrations (Chen et al, 2009). SDG was found to be a potent Angiotensin-Converting Enzyme (ACE) inhibitor in lowering blood pressure in a study performed by Prasad (2013) in rats. SDG can prevent the development of atherosclerosis and diabetes (Fukumitsu et al, 2008; Prasad, 2009) and can reduce blood lipids and cholesterol levels (Fukumitsu et al, 2010).

Fiber

Around 28% of the flaxseed weight is made up of fiber. Flaxseed contains both soluble fiber (Mucilage gums) and insoluble fibers (Cellulose and lignins). Lignin is a highly branched fiber which provides strength and rigidity to the plant cell walls (Morris, 2007). Soluble fiber can reduce the risk of cardiovascular diseases through its lipid lowering effect (Singh et al, 2011). Insoluble dietary fibre can help in maintaining bowel

movement and exert beneficial impact on insulin resistance. High fiber diet can also increase healthy intestinal bacteria (Cummings & Mann, 2012). The mechanism of action of different bioactive components of flaxseed has been projected in Figure 2.21.

Other bioactive compounds

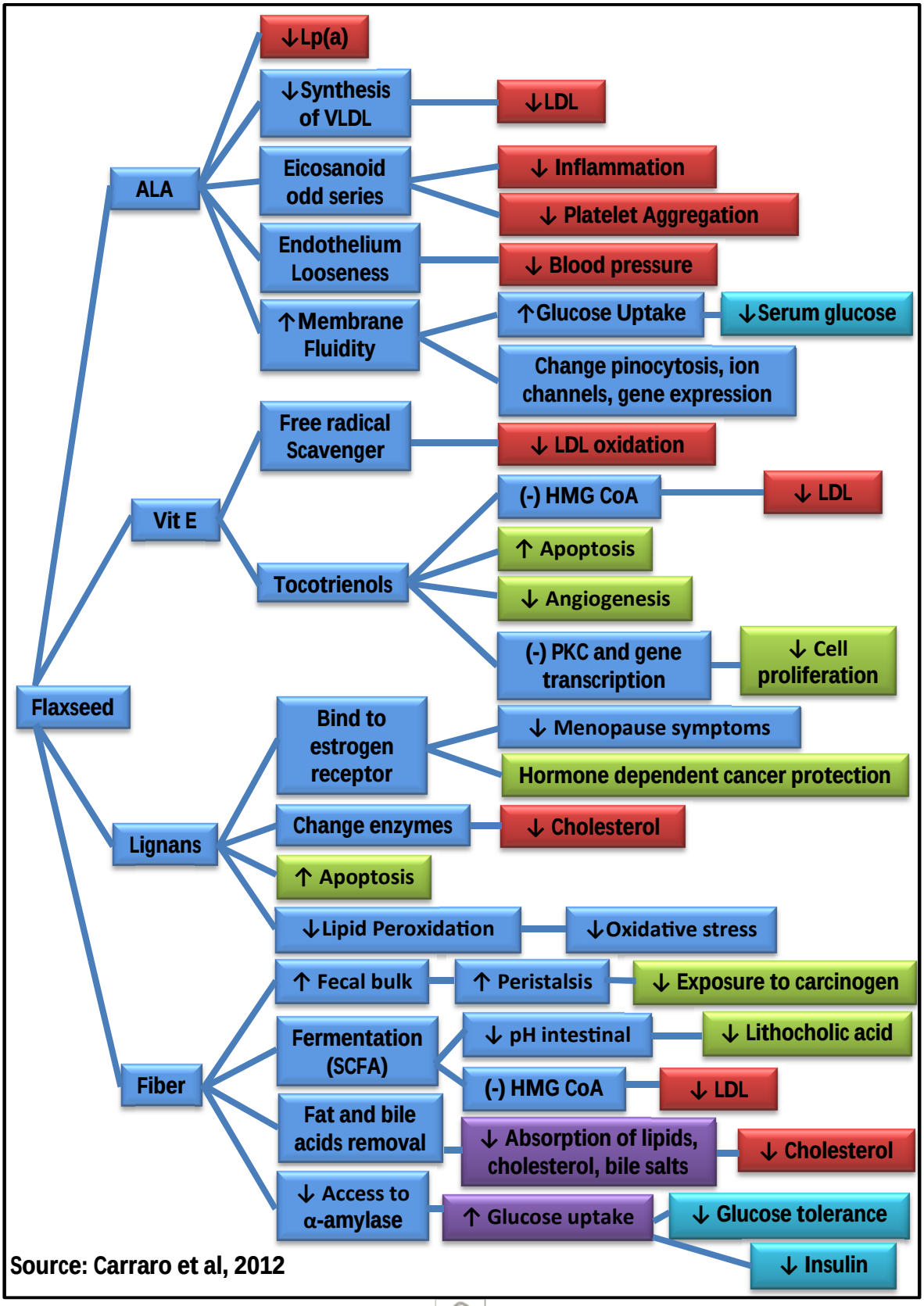
There are various other bioactive compounds in flaxseeds that can be potentially harmful if consumed in large quantities. Linatine (vitamin B antagonist), cyanogenic glycosides, cadmium, cyclolinopeptides, oxalates, phytates, saponins are such compounds which can exert anti nutrient/ toxic effect. However a moderate consumption of flaxseeds does not pose any risk of toxicity due to these compounds (Shim et al, 2014). Flaxseed have been provided the status of generally recognized as safe (GRAS) by US-FDA (2009).

To summarize, flaxseed has three potential bioactive components i.e. ALA, lignans, vitamin E and fiber. Flaxseed is the most promising source of ALA in the vegetarians' diet. Sufficient amount of ALA in the diet can reduce the pro inflammatory activity in the body. Lignans can act as potent antioxidant and weak estrogen to impart health benefits. These anti-inflammatory, antioxidant and estrogenic activity make flaxseed ideal food choice to reduce the risk of non-communicable diseases among women.

HEALTH BENEFITS OF FLAXSEED AND ITS BIOACTIVE COMPONENTS: SCIENTIFIC EVIDENCE

Different forms of flaxseeds like whole, roasted, grounded, flax oil, SDG or flaxseed incorporated food products are used in various studies to study its health benefits. Different processing can affect the nutritive profile of the flaxseeds. ALA reacts with oxygen 5 times faster than linoleic acid therefore quality of ALA in flaxseed oil depends upon its shelf life which is quite limited (3 months). Flaxseed powder has a longer shelf life than flaxseed oil (nine months) and should be refrigerated once the packing is opened (Holstun and Zetocha, 1994).

FIGURE 2.21: ACTION MECHANISMS OF FLAXSEED BIOACTIVE COMPOUNDS ON RISK REDUCTION OF NONCOMMUNICABLE CHRONIC DISEASES



Source: Carraro et al, 2012

According to Brevi et al (2011) flaxseed oil enriched with SDG should be stored at dark and low temperature to preserve SDG content. Levels of ALA in flaxseed fortified macroni were found to be unchanged with a slight reduction in SDG (80-95% recovery) after 32 weeks of storage (Hall et al, 2005). In a study performed by Schorno et al (2003) roasting at 140, 160 and 180C in an air impingement oven (4, 8, 16 and 24 min.) significantly reduced the peroxide value ($p<0.05$) and free fatty acid content ($p<0.05$), showing better shelf life capacity. Linolenic acid content was reduced when subjected to high temperatures and long processing times. Hydrogen cyanide (HCN) content of flaxseeds was reduced by 82% 27%, 52% and 100% through microwave roasting, autoclaving, solvent extraction (once) and water boiling respectively in a study performed by Yang et al (2004). According to Moknatjou et al (2015) seed oil content was higher in roasted brown flaxseeds (53.31±0.30%) in comparison to unroasted brown one (45.20±0.20%) and for roasted and flax, respectively. The calculated oxidizability (COX) value was found as 13.19 ± 0.01 % for the unroasted brown flaxseed and 12.79±0.01 % for the roasted brown seed at 350^{o}C. Milled flaxseed can be stored up to 4 months at ambient temperatures (23±2°C) without noticeable changes in quality (Malcolmson et al, 2000). The bioavailability of flax oil and milled flaxseed was found to be better than whole flaxseed in a study performed by Austria et al (2008) after 1 month supplementation of 30g milled/ground flaxseed/6g ALA from oil (in muffin form). Whole seed and oil preparations induced adverse gastrointestinal effects among the subjects. Therefore different forms of processing have different advantages and disadvantages. The form of flaxseed supplementation can be chosen based on the feasibility for consumption, dietary pattern of the population and individual preferences. The effect of different forms of flaxseeds in reducing major risk factors of various non-communicable diseases through clinical trials worldwide is summarized in Table 9.

HYPERLIPIDEMIA

Hyperlipidemia is one of the major risk factor for development of cardio vascular diseases. The effect of flaxseeds and its various forms on hyperlipidemia have been

extensively studied. A meta-analysis performed by Pan et al (2009) indicated that whole flaxseeds (20.21 and 20.16 mmol/L, respectively) and lignans (20.28 and 20.16 mmol/L, respectively) can significantly reduce total cholesterol and LDL-C levels. However flaxseed oil was not found to decrease lipid levels in the analysis. The overall reduction in TC and LDL-C observed was 0.10 mmol/L (95% CI: 20.20, 0.00 mmol/L) and 0.08 mmol/L (95% CI: 20.16, 0.00mmol/L), respectively. HDL-C and TG levels were not found to be significantly changed by any form of flaxseed supplementation. Flaxseed supplementation in females (especially post-menopausal women) and subjects with high initial cholesterol concentrations showed better cholesterol lowering effect. No significant changes in the concentrations of HDL cholesterol and triglycerides were observed. Study with good experimental design, as indicated by high Jaded score, showed better results. Most of the randomized control trials summarized in table 2.9 with primary of cholesterol reduction included hypercholesterolemic/dyslipidemic subjects, with few including apperantly healthy subjects and post-menopausal women (Cassani et al, 2015; Machado et al, 2015; Torkan et al, 2015; Dhruv, 2014; Gandhi, 2014; Saxena and Katare, 2014, Chauhan and Kansara, 2012; Parmeshwari and Nazni, 2012; Gillingham et al, 2011; Fukumitsu et al, 2010; Wu et al, 2010; Coulman et al, 2009; Kaul et al, 2008; Zhang et al, 2008; Hallund et al, 2006a; Harper et al, 2006, Dodin et al, 2005; Stuglin and Prasad, 2005). Different forms like SDG, flax oil, ground, roasted, bread, muffins using flaxseeds were used for different studies with doses of 15-40g of flaxseeds/ 20-600g SDG/ 2-3g of ALA. The randomized control trials showed mixed results regarding the lipid lowering effect on flaxseeds. Results varied based on study population, form of flaxseeds and duration of intervention.

INFLAMMATION

Whole flaxseed supplementation can prevent the development/diminish the effect of atherosclerosis through action upon inflammatory markers in rats (Nounou et al, 2012). However there is inconsistency in the results of human trails regarding impact of flaxseed in reducing inflammation. Ren et al (2016) performed a meta-analysis to

evaluate the effectiveness of flaxseed and its derivatives on inflammatory marker CRP including 20 studies. The results revealed that overall, flaxseed interventions had no effects on reduction of CRP ($p = 0.428$) however whole flaxseed reduced CRP levels to some extent (borderline significant). Body mass index (BMI) was found to be a significant source of heterogeneity (P-interaction <0.05), as CRP levels significantly reduced (0.83 mg/L, $p<0.01$) among subjects with a BMI of ≥30. According to the author the CRP lowering impact of whole flaxseed can be due to mixed effect of ALA, lignans and dietary fiber present in flaxseeds as purified forms failed to exert any desired impact. Table 2.9 includes various studies assessing effect of flaxseeds and its components on inflammation (Cassani et al, 2015; Machado et al, 2015; Dhruv, 2014; Gandhi, 2014, Barre et al, 2012; Dewell et al, 2011; Faintuch et al, 2011; Gillingham et l, 2011; Rhee and Brunt, 2011; Cornish et al, 2009; Kaul et al, 2008; Hallund et al, 2006b). These studies included different study populations and the results of various forms of flaxseed supplementation showed diverse results.

DIABETES AND INSULIN RESISTANCE

According to Elshal et al (2012) dietary flaxseed supplementation may reduce the incidence of diabetic vascular complications through improvement of insulin sensitivity, vascular permeability and lipid profile. Most of the studies included Table 2.9 with primary outcome as insulin resistance showed positive impact of flaxseed supplementation on insulin resistance (Yari et al, 2016; Rhee and Brunt, 2011; Muramatsu et al, 2010; Pan et al, 2007; Lamay et al, 2002). The major forms of flaxseed used in these studies were whole/ground flaxseed or SDG. Another set of randomized control trials focused on glycemic control using flaxseeds supplementation in different populations (Machado et al, 2015; Barre et al, 2012; Kapoor et al, 2011; Wu et al, 2010; Zhang et al, 2008). These studies also used whole/ground flaxseed or lignans and showed positive impact in form of bold glucose levels of HbA1c. No meta-analysis on impact of flaxseed on insulin resistance/diabetes has been performed by any researcher till date.

HYPERTENSION

Flaxseeds have shown a beneficial impact in reducing blood pressure through various clinical trials in humans. Ursoniu et al (2016) performed a meta-analysis including 15 trials (comprising 19 treatment arms) and 1302 participants. The results showed that flaxseed or its derivative's supplementation significantly reduced SBP ($p<0.05$) and DBP ($p<0.01$) by 2.85mmHg and 2.39mmHg respectively. Studies with longer duration (>12 weeks) showed a better impact in lowering blood pressure than studies with shorter duration (<12 weeks). The reduction was 3.10mmHg for SBP and 2.62mmHg for DBP in long duration studies in comparison to 1.60 mmHg for SBP and 1.74 mmHg for DBP in short studies. Flaxseed powder was found to be effective in significantly reducing systolic blood pressure (1.81 mmHg, $p < 0.001$) whereas flax oil and lignans were ineffective in exerting such impact. Diastolic blood pressure was significantly reduced by use of both flaxseed powder and flax oil.

Another similar meta-analysis (Khalesi et al, 2015) including 11 studies (14 trials) concluded that flaxseed supplementation significantly reduced systolic blood pressure by 1.77 mmHg ($p<0.05$) and diastolic blood pressure by 1.58 mmHg ($p<0.01$). The results were more pronounced in participants with initial systolic blood pressure level <130mmHg. Further sub group analysis indicated that whole flaxseed supplementation showed a significant reduction in DBP with non-significant reduction in SBP. Studies with longer duration >12 weeks significantly reduced DBP (2.17mmHg; $P < 0.05$). Most of the studies in table 2.9 aiming blood pressure reduction used whole/ground flaxseed or its bakery products and showed a positive impact of blood pressure (Yari et al, 2016; Machado et al, 2015; Caligiuri et al, 2014; Dhruv, 2014; Gandhi, 2014; Saxena and Katare, 2014; Chauhan and Kansara, 2014; Wu et al, 2010; Paschos et al, 2007).

BODY COMPOSITION AND WEIGHT LOSS

A meta-analysis performed by Sartang et al (2017) including 45 randomized control trials indicated that a flaxseed supplementation significantly reduced body weight by 0.99kg

(p<0.05), BMI by 0.30 (p<0.05) and waist circumference by 0.80cm (p<0.05). Use of whole flaxseed (≥30 g/day), longer duration of interventions (≥12 weeks) and higher BMI (≥ 27) showed positive effects on body composition in sub group analysis. Mixed results of flaxseed supplementation on body weight and central obesity have been observed in different randomized control trials included in Table 2.9 (Yari et al, 2016; Torkan et al, 2015; Saxena and Katare, 2014; Barre et al, 2012; Gillingham et al, 2012; Kapoor et al, 2011; Wu et al, 2010; Cornish et al, 2009). Ibrugger et al (2012) showed that flax fiber drink or capsule can significantly increase the satiety and subsequently decrease energy intake. However contrasting results were showed by Cohen et al (2013), where whole flaxseed meal did not have any impact on hunger, satisfaction, fullness, and desire to eat as well as basal and postprandial glucose and leptin levels.

To summarize, whole/ground flaxseeds and not the purified form like flax oil (ALA) and lignans have found to be effective in various meta-analysis and randomized control trial to impact lipid levels, blood pressure, body weight, body composition and insulin resistance. Flaxseeds have not found to be effective in reducing inflammation in various studies. Most of the studies have used around 30g of flaxseeds as dose which may be difficult to consume regularly on long term basis. However no major side effects of consuming 30-40g of flaxseed have been reported in these studies.

SUMMARY AND RATIONALE

The review suggests that the prevalence of non-communicable diseases among women is escalating in the recent years. Menopause is emerging as a major risk factor for development of these diseases among women. Estrogen deficiency post menopause can lead to various physiological and metabolic changes like dyslipidemia, insulin resistance, glucose intolerance, high blood pressure and increase in fat mass, central obesity etc. Along with menopause, vitamin B12 and folate deficiency among women can also increase the risk of non-communicable diseases. Inflammation has found to be a distinctive feature in females with metabolic syndrome. Insulin resistance which is a major pathophysiological factor in development of diabetes, metabolic syndrome and

CVDs is understudied among the female population. As per the review, there are very few female centric studies on non-communicable diseases, especially in Indian population till date. Therefore there is a need for comprehensive studies focusing on female specific issues of non-communicable diseases in Indian population.

Moreover early lifestyle and dietary modifications as well as inclusion of functional foods in regular diet are important in this context. Flaxseed is a plant derived source of n-3 fatty acids, lignans and dietary fiber. India is a major producer of flaxseeds. Consumption of whole or ground form of flaxseeds have been associated with reduction in cholesterol and blood pressure levels, maintenance of glucose homeostasis, improvement in body composition, weight loss and anti-inflammatory process. However these associations are associated with higher doses of flaxseed (30-40g/day) consumption. Therefore there is a need to study the impact of rationalized low doses of flaxseeds on the metabolic aberrations which can be feasibly incorporated into the regular diet for sustained consumption.

TABLE 2.9(a): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Yari et al, 2016	randomized controlled clinical trial	Subjects with metabolic syndrome	44	Physical activity + brown milled flaxseed	30g/day	11 Weeks	• Significant reduction in prevalence of MS, central obesity, insulin resistance, Body weight, waist circumference, and body mass index • No significant changes in blood pressure
Cassani et al, 2015	Single blinded placebo controlled trial	Adult men	27	Flaxseed powder	60g/day	42 days	• A significant decrease in inflammatory markers (CRP and TNF-α) (mean decrease of 25% and 46% respectively) and triglycerides
Machado et al, 2015	Randomized, parallel, single-blind trial	Overweight adolescents	75	Brown and golden flaxseed	28g/day	12 weeks	• Significant reduction in diastolic BP in both brown and golden flaxseed group • No significant impact on plasma lipid, glucose and inflammatory profile.
Torkan et al, 2015	Randomized controlled clinical trial	Hyperlipidemic subjects	70	Raw flaxseed powder	30g/day	40 days	• Significant reduction in total cholesterol, low density lipoprotein and triglycerides • Non-significant reduction in weight and body mass index
Dhruv, 2014	Randomized controlled trial (Departmental study)						
Gandhi, 2014	Randomized controlled trial (Departmental study)						

TABLE 2.9(b): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Caligiuri et al, 2014	Randomized, double-blinded, controlled clinical trial	Subjects with arterial disease (75% hypertensive)	110	Milled flaxseed	30g/day	6 months	• significant decrease in 8 plasma oxylipins, and significant decrease in systolic blood pressure in such subjects • out of these eight, six were products of soluble epoxide hydrolase
Saxena and Katare, 2014	Randomized control trial	Dyslipidemic subjects	50	Roasted flaxseed powder	30g/day	3 months	• Significant reduction in body weight and BMI)($p< 0.01$), systolic and diastolic BP ($p< 0.05$), TC, TG, LDL-C, VLDL-C ($p<0.01$) • Significant increase in HDL-C levels
Cohen et al, 2013	Single-blind crossover and randomized study	Women in post-operative state of gastric bypass surgery	18	Whole flaxseed meal, defatted flaxseed meal	5.5g fiber and 3g ALA 7g fiber and 1.6g ALA	3 test meal with 1 week washout	• No difference between test meals for the variables of hunger, satisfaction, fullness, and desire to eat. • No significant difference in the basal and postprandial glucose and leptin levels • Defatted flaxseed showed increased postprandial leptin levels ($p = 0.044$).
Barre et al, 2012	Double-blind, randomized crossover placebo-controlled study	Type 2 diabetics	16	SDG	600mg/ day	3 months	• Significant decrease in fasting plasma glucose, HbA1c, inflammation, CRP, IL-6 • Significant increase in bleeding time • Significant reduction in central obesity gain
Chauhan and Kansara, 2012	Randomized control trial (Departmental study)	Geriatrics	45	Roasted flaxseed (Laddo)	20g	8 week	• Significant rise in BMI, Significant reduction in TC , LDL, DBP and increase in HDL

TABLE 2.9(c): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Gillingham et al, 2012	Randomized crossover design	Hypercholesterolemic subjects	34	Controlled meal with 37% energy from fats	70% of fat from flax and canola oil	28 days	• No significant difference in resting and postprandial energy expenditure and substrate oxidation, body composition • The android-to-gynoid ratio increased (P=.055) after flaxseed canola oil diet
Ibrugger et al, 2012	Single-blinded randomized crossover acute study	Healthy adults	20	Drink with flax fiber, flax fiber tablet	2.5g fiber	One time effect	• Significant increase in satiety using flax drink and tablet. • Significant decrease in subsequent energy intake
Parmeshwari and Nazni, 2012	Randomized control trial	Hyperlipidemic subjects	40	Roasted flaxseed	15g/day	4 weeks	• Significant (P<0.001) reduction of serum total cholesterol, triglyceride, LDL-C and increase serum HDL-C
Dewell et al, 2011	Randomized parallel arm placebo control study	Subjects with metabolic syndrome	100	ALA	2.2 and 6.6 g/d	8 weeks	• No significant differences in plasma inflammatory marker i.ie monocyte chemotactic protein-1 (MCP-1), IL-6, and soluble intercellular adhesion molecule-1
Faintuch et al, 2011	Double blind randomized control trial	Morbid obese candidates for bariatric surgery	12	Flaxseed powder (60g)	10g ALA/day	13 Weeks	• Non-significant decrease in neutrophils, • Fibrinogen, complement C4, pro-thrombin time and carotid diameter remained stable in flaxseed group whereas further elevated in placebo group
Gillingham et al, 2011	Randomized crossover design	Hypercholester-olemic subjects	36	Controlled meal with 36% energy from fats	70% of fat from flax and canola oil	28 days	• Significant reduction in LDL-cholesterol, Total cholesterol HDL-cholesterol (p<0·001) and LDL:HDL ratio (p<0.01) • Significantly decreased E-selectin concentration (p<0.05 0·02).

TABLE 2.9(d): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Kapoor et al, 2011	Randomized, parallel control trial	Non-insulin dependent menopausal diabetic female	90	Flaxseed powder	15g and 20g	2 months	• Decrease in FBS, PP2BS, weight and BMI in both flaxseed groups • Hot flushes, depression, night sweats, lack of sleep and burning sensation relieved through flaxseed supplementation
Rhee and Brunt, 2011	Randomized crossover design	Obese glucose intolerant subjects	9	Ground flaxseed	40g/day	12 weeks and 4 weeks washout	• Significant decrease in TBARS ($p = 0.022$) and HOMA-IR ($p = 0.038$). • No effect on inflammatory biomarkers-CRP, TNF-α, and IL-6
Fukumitsu et al, 2010	Randomized placebo controlled trial	Men with TC 180-240 mg/dL	30	SDG capsules	20 and 100mg	12 weeks	• Significant reduction in LDL/HDL ratio ($P < .05$) in 100g group • Decreased hepatic disease risk in 100g group
Muramatsu et al, 2010	Cross sectional	Japanese government workers (35-66y)	3383	--	--	--	• The odds of insulin resistance decreased across the quartiles of ALA intake ($P < 0.01$) and the association was observed only in subjects with a BMI <25
Wu et al, 2010	Randomized, controlled trial	Subjects with metabolic syndrome	243	Life style counselling + flaxseed (bread)	30 g/d	12 weeks	• Prevalence of MS decreased significantly • Significant reduction in weight, WC, serum glucose, total cholesterol, LDL cholesterol, apolipoprotein (Apo) B, ApoE, and blood pressure from baseline
Cornish et al, 2009	Randomized double-blind placebo controlled study	Older adults ≥50 years	100	SDG	543 mg/ day	6 months	• Significant increase in metabolic syndrome composite *Z*-score ($p < 0.05$) in placebo males • No differences in bone measures, body composition, lipoproteins, or cytokines.

TABLE 2.9(e): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Coulman et al, 2009	Randomized crossover study	Post-menopausal women	16	Unground flaxseed	25g/day	4 week with 4 week washout	• Significant increase in total serum n-3 fatty acids and urinary lignans ($p<0.05$) • No effect on Plasma lipids and several antioxidant markers remained unaffected
Kaul et al, 2008	Randomized double-blind placebo controlled trial	healthy male and female	86	flaxseed oil	2g/day	12 Week	• No significant change in TC, HDL-C, LDL-C, TG and oxidative LDL • No significant change in collagen or thrombin stimulated platelet aggregation and level of inflammatory markers
Zhang et al, 2008	Randomised, double-blind, placebo-controlled study	Hypercholesterolemic subjects	55	SDG	300 and 600mg/ day	8 weeks	• Significant decrease in TC, LDL-C and glucose concentrations • Colesterol-lowering values were correlated with plasma secoisolariciresinol (SECO), enterodiol (ED) ($P < 0{\cdot}05$ to $< 0{\cdot}001$).
Pan et al, 2007	Randomized, double-blind, placebo-controlled, cross-over trial	Type 2 diabetics with mild hypercholes-terolemia	73	Flaxseed lignan capsule	360 mg/day	12 week with 8 week washout	• Significant improvement in HbA_{1c} (-0.10±0.65 % vs. 0.09±0.52 %, $P = 0.001$) • no significant changes in fasting glucose, insulin resistance and lipid profiles
Paschos et al, 2007	Prospective, parallel-arm design	Middle-aged dyslipidaemic men	59	Flaxseed oil	8g/day	12 week	• Significant reduction in systolic and diastolic blood pressure
Hallund et al, 2006a	Randomized, double-blind, placebo-controlled, crossover study	Healthy Post-menopausal women	22	Low fat muffins fortified with SDG	500mg/day	6 week and 6 week washout	• No effect on TC, LDL-C, HDL-C, TG, lipoprotein oxidation lag time, trolox-equivalent antioxidant capacity (TEAC) and ferric reducing ability of plasma (FRAP)

TABLE 2.9(f): EFFECT OF FLAXSEEDS AND ITS VARIOUS INGREDIENTS ON METABOLIC ABERRATIONS

Author and year	Study design	Study population	N	Form of Flaxseed	Doses	Duration	Effects
Hallund et al, 2006b	Randomized, double-blind, placebo-controlled, crossover study	Healthy Post-menopausal women	22	Low fat muffins fortified with SDG	500mg/ day	6 week and 6 week washout	• Slight decrease in flow-mediated, endothelium-dependent vasodilatation • No effect on plasma nitrite and nitrate, endothelin-1 (ET-1), and asymmetric dimethylarginine (ADMA)
Harper et al, 2006	Randomized double-blind placebo controlled trial	subjects (49 women, 7 men) without CHD	56	Flaxseed oil (ALA)	3g/day	26 week	• No significant change in LDL, HDL, or IDL particle size
Dodin et al, 2005	Randomized, double-blind, placebo-controlled clinical trial	Healthy post-menopausal women	199	Flaxseed (half in bread form and half in ground form)	40g/day	12 months	• Significant reduction in TC and LDL-C ($p<0.05$) • 1-yr incorporation of flaxseed into the diet produced a favourable, but not clinically significant
Stuglin and Prasad, 2005	Randomized trial	Healthy men 22-47y	15	Flaxseed muffins	32.7 g of flaxseed	4 week	• BP, heart rate, hemoglobin, RBC, WBC, and neutrophils remained unaltered. • Serum total cholesterol, HDLC, LDL-C, and VLDL-C remained unchanged, • Liver and kidney functions remained unchanged
Lemay et al, 2002	Randomized crossover study	hypercholesterolemic Menopausal women	25	Crushed flaxseed	40g/day	2 months with 2 months washout	• Flaxseeds produced similar decreases in menopausal symptoms, glucose and insulin levels as hormonal replacement therapy but not for lipid levels