Chapter 11
The Menopause and Oxidative Stress

Lucky H. Sekhon and Ashok Agarwal

Abstract Reproductive aging resulting in menopause is characterized by the permanent cessation of ovarian follicular activity. The signs and symptoms resulting from estrogen withdrawal can significantly disrupt a woman’s activities of daily living and sense of well being, while predisposing them to osteoporosis and heart disease. Current medical therapies are targeted at symptomatic relief or alleviating the hormonal deficiency itself to prevent its harmful sequelae. The progressive loss of estrogen and its protective effects, combined with deficient endogenous antioxidant, results in oxidative stress—which is implicated in the pathogenesis of vasomotor disturbances, loss of bone mass and heart disease in menopause. The link between oxidative stress and estrogen deficiency has been demonstrated by numerous studies. Based on this, hormonal replacement therapy, antioxidant supplementation, and lifestyle modification have been investigated for their efficacy and safety in the treatment and prevention of menopause-related symptoms and chronic disease processes.

Keywords Reproductive aging • Menopause • Antioxidant vitamins • Deficient endogenous antioxidant • Loss of estrogen • Herbal extracts • Vitamin C • Vitamin E • Vitamin A • Phytoestrogens • Curcuma longa • Lycopene • Grape polyphenols • Melatonin

L. H. Sekhon
Mount Sinai School of Medicine, OB/GYN, New York, NY, USA
e-mail: lucky.sekhon@mountsinai.org

A. Agarwal (✉)
Lerner College of Medicine, Cleveland Clinic, Center for Reproductive Medicine, Cleveland, OH, USA
e-mail: agarwaa@ccf.org

11.1 Introduction

Reproductive aging involves the permanent cessation of the primary female reproductive functions—the ripening and release of ova and the release of hormones that modulate the endometrial proliferation and shedding. This loss of ovarian follicular activity can be a natural process or a result of an iatrogenic insult such as surgery, chemotherapy, or radiotherapy. In the US, menopause is typically reached at an average of 51 years and affects approximately 40 million women. Premature menopause occurs when a women experiences menopause before 40 years of age, and can result from gynecologic disorders such as polycystic ovaries and endometriosis. In certain women, the changes that can occur during the menopause transition years can significantly disrupt their daily activities and their sense of well being. These may include irregular menses, vasomotor instability (hot flashes and night sweats), genitourinary tissue atrophy, increased stress, breast tenderness, vaginal dryness, forgetfulness, mood changes and sometimes osteoporosis and heart disease. These effects are a direct result of estrogen decline and may affect each woman to a different extent. Currently, established medical treatment targets the altered hormonal milieu of women experiencing menopause. Therapy may also include lifestyle modifications, such as exercise and dietary measures. Free radicals and oxidative stress have been implicated in the pathogenesis of various menopause-related symptoms and complications. As such, vitamins and foods rich in antioxidant compounds might be an effective strategy to alleviate oxidative stress and the associated symptoms and complications affecting women experiencing menopause.

11.2 The Pathophysiology of Hormonal Changes in Menopause

The transition from reproductive to non-reproductive is the result of a major reduction hormone production by the ovaries. This transition is normally not sudden or abrupt, tends to occur over a period of years, and is a natural consequence of aging. The early phase of postmenopause consists of the first 5 years. The late phase of postmenopause is the time from 5 years after the onset of menopause until death [1].

The terminal phase of reproductive aging is preceded by many hormonal changes. These hormonal changes result in age-related fertility decline and a gradual decrease in the number of ovarian follicles and have physical manifestations which often negatively impact the quality of life of perimenopausal and postmenopausal women. The earliest hormonal alteration noted in the perimenopause is the rise in follicle stimulating hormone (FSH) levels, followed several years later by a rise in luteinizing hormone (LH) levels [2, 3]. Inhibin, a dimeric glycoprotein known to suppress FSH, shows a marked decline at and before
menopause. Therefore, the decrease in inhibin B is a hormonal change that is an early indicator of reproductive aging [4]. Inhibin B exhibits greater potency than estradiol in exerting negative feedback on pituitary FSH secretion [4]. Thus, increased FSH levels may be related to a decrease in total inhibin in both follicular and luteal phases of the cycle. Along with the changes in the levels of FSH, inhibin and LH, a marked decrease in estrogen concentration occurs in the menopause [5]. This disrupted ovarian function leads to changes in the pattern of menstrual bleeding during the perimenopausal phase.

Estrogen is the major reproductive hormone in the female body and promotes the development of female secondary sex characteristics. In women, naturally occurring estrogen is produced from androgens via enzymatic reactions which yield three major forms: estradiol, estriol, and estrone. In the perimenopausal years, 17β-estradiol, is the most potent and predominant estrogen, whereas the weaker form, estrone, is the predominant estrogen in the postmenopausal phase. The synthesis of estrogen is stimulated by FSH and LH and takes place primarily in developing follicles in the ovaries and the corpus luteum. Estrogen is also produced in small amounts by the liver, adrenal glands, fat cells, and breasts. In postmenopausal women, estrone is formed as a result of the peripheral conversion of androstenedione in both adipose tissue and the liver. Estrogen metabolites have been proven to exert both antioxidant [6, 7] and pro-oxidant effects [7]. Methoxyestrogen is seen to have the most potent antioxidant properties of the various forms of estrogen [7]. Some believe that estrogen’s antioxidant properties are derived from the phenolic ring in its structure [5]. Markides et al. [7] proposed that estrogen has antioxidant activity through the inhibition of 8-hydroxylation of guanine bases of DNA. Estrogen metabolites significantly increased the concentrations of 8-hydroxyguanine bases by 54–66 % [7]. The concentration and chemical structure of estrogen metabolites determines whether it will have an antioxidant or pro-oxidant effect. At high concentrations, estrogen metabolites tend to produce antioxidant effects—whereas at lower concentrations, estrogen metabolites are more likely to produce pro-oxidant effects. Estrogen metabolites that possess a catechol structure act in a pro-oxidant manner [7]. In one study, estrogen supplementation led to a decrease of the oxidation of LDL cholesterol in postmenopausal women [8]. According to Pansini et al. [9], supplementing postmenopausal women with estrogen can improve their lipid profile, by increasing HDL levels and decreasing LDL and lipoprotein A levels. However, further studies are needed to assess the direct implications of this finding on the cardiovascular complications often seen in postmenopausal women [10].

11.3 The Role of Oxidative Stress in the Menopause

Oxidative stress, which is defined as an imbalance between oxidants and antioxidants, plays a well-established role in normal aging and has been implicated in the pathogenesis of a number of disease processes, including age-related degenerative
processes such as atherosclerotic cardiovascular disease [11], non-alcoholic liver cirrhosis, and various pathologies afflicting the female reproductive system. Various studies have shown that vasomotor disturbances [12], osteoporosis [13] and cardiovascular diseases [14] significantly correlate with the progressive loss of estrogen and its protective effects, combined with deficient antioxidant defense leading to a pronounced redox imbalance.

Vural et al. [15] compared follicular phase levels of serum TNF-α, IL-4, IL-10, and IL-12 in premenopausal women, ages 19–38, to the levels seen in postmenopausal women, ages 37–54. Higher serum concentrations of TNF-α, IL-4, IL-10, and IL-12 were seen in postmenopausal women compared to premenopausal women [15]. Levels of TNF-α and inflammatory cytokines have been established to be elevated in the presence of oxidative stress. Therefore, it can be speculated that oxidative stress is present in increased amounts in postmenopausal women. This study also demonstrated a compensatory relationship between TNF-α and IL-4. Elevated levels of IL-4, with its anti-inflammatory effects, may act to counter the pro-inflammatory state induced by increased TNF-α levels [15].

Signorelli et al. [16] also reported findings that show a high degree of oxidative stress is experienced by postmenopausal women. Blood serum levels assessing for malonaldehyde (MDA), 4-hydroxynenal (4-HNE), oxidized LDL, and glutathione peroxidase (GSH-Px) were compared in two groups of women: fertile women, between the ages of 30–35 and postmenopausal women, between the ages of 45–55. The postmenopausal group demonstrated significantly higher levels of the pro-oxidant biomarkers MDA, 4-HNE, and oxidized LDL, whereas levels of the antioxidant GSH-Px were significantly decreased when compared to premenopausal control subjects.

Estrogen is involved in a number of physiological processes in the tissues of the cardiovascular system. It is known to be protective against cardiovascular disease by way of endothelial and non-endothelial mediated effects, favorable effects on lipoprotein, glucose, and insulin homeostasis, changes in extracellular matrix composition, atherosclerotic plaque destabilization and the facilitation of collateral vessel formation [9]. Postmenopausal estrogen deficiency is associated with higher blood levels of free fatty acids, which contribute to the pathogenesis of the metabolic syndrome and insulin resistance. Menopause complicated by poorly controlled diabetes is linked to an elevated risk of atherosclerosis and cardiovascular disease. The risk of cardiovascular disease is present even in non-diabetic postmenopausal women in the presence of recognized risk factors such as elevated lipid and glucose concentrations in plasma [17]. Atherogenesis is considered to be an inflammatory, fibroproliferative process [18]. The incidence of atherosclerosis is increased in menopause, as the antioxidant influence of estrogen is lost, leading to increased oxidation of LDL cholesterol. Moreau et al. [19, 20] demonstrated elevated levels of plasma oxidized LDL in postmenopausal women compared to premenopausal women. The administration of antioxidant vitamin C was shown to reverse this effect, with the decrease in oxidized LDL concentrations leading to an improvement in parameters of vascular health such as blood flow and vascular conductance [20].
Elevated cholesterol coupled with vascular endothelial injury contributes to the development of atherosclerotic plaques. Angiotensin type I (AT-1) receptor activation is thought to be a predominant source of free radical production in vasculature. In a study conducted by Wassmann et al. [21], treatment of spontaneously hypertensive rats with the AT-I receptor antagonist irbesartan normalized the vascular production of free radicals and reverse endothelial dysfunction. These findings suggest that menopause-induced oxidative stress may be mediated by overexpressed AT-I receptor, resulting in an enhanced vasoconstriction and endothelial dysfunction. Increased breakdown of nitric oxide (NO) may be another mechanism by which oxidative stress contributes to the pathogenesis of cardiovascular disease in postmenopausal women [22]. NO, which is derived from the endothelium, is an important physiological regulator of blood flow and regulates blood pressure by inducing vascular relaxation [23–25]. It also demonstrates anti-aggregative, anti-inflammatory, fibrinolytic, thrombolytic, cardio-protective, and cyto-protective properties [23, 25, 26]. NO acts to suppress smooth muscle proliferation, and exerts an anti-atherogenic influence on the vasculature. NO levels in men and postmenopausal women are found to exist at lower levels than those measured in premenopausal women [27, 28].

Leal et al. [29] implicated oxidative stress in the pathogenesis of menopausal symptoms including hot flashes. Hot flashes are characterized by a generalized, transient increase in metabolic rate which may manifest clinically as sweating, irritability, and panic, as well as cardiovascular alterations which cause an increase in blood flow and heart rate. Repetitive increases in metabolic activity are thought to contribute to the development of oxidative stress, possibly by exhausting the antioxidant capacity to regulate reactive oxygen species production. Postmenopausal women experiencing vasomotor symptoms were shown to have lower plasma antioxidant activity than postmenopausal women of the same age without hot flashes [29].

Postmenopausal osteoporosis is a progressive loss of bone density which results in pathological fracture within 10–20 years of the onset of menopause [13]. However, the reason why the incidence of osteoporosis is higher in postmenopausal women and the mechanism by which osteoporosis occurs is not yet completely understood. Iqbal et al. [30] analyzed various markers and cells present in bone marrow samples from mice to characterize the mechanism of osteoporosis development in postmenopausal women. Results demonstrated that mice deficient in the β subunit of FSH are protected from excessive bone turnover despite experiencing a state of severe estrogen deficiency. Furthermore, these FSH-β deficient mice were found to have significantly lower levels of TNF-ζ. Thus, TNF-ζ production may be regarded as being dependent on FSH. Decreased TNF-ζ appears to render mice resistant to hypogonadal bone loss, suggesting TNF-ζ may be critical to the action of FSH on bone. Estrogen normally prevents bone loss by way of multiple effects on bone marrow and bone cells which cause decreased osteoclast formation, increased osteoclast apoptosis, and decreased capacity of mature osteoclasts to resorb bone [13]. In estrogen deficiency, TNF-ζ is most
likely produced from macrophages and granulocytes, and induces osteoclast and osteoblast formation leading to increased bone turnover [30].

A study conducted by Vural et al. [13] demonstrated that the plasma cytokines—TNF-α, IL-4, IL-10, and IL-12, and markers of bone turnover—urinary hydroxyproline and calcium were elevated in postmenopausal women compared to premenopausal controls. A weak but significant correlation was found between IL-4 and TNF-α, suggesting that anti-inflammatory cytokines such as IL-4, IL-10, and IL-12 serve to counteract pro-inflammatory TNF-α, helping to balance oxidative stress and osteoclast activity. TNF-α contributes to increased osteoclast formation by direct stimulation of osteoclast precursor proliferation and enhancement of pro-osteoclastogenic activity of stromal cells [13]. The role of pro-inflammatory cytokine TNF-α in bone resorption implicates oxidative stress as a key factor in the age-related decline of bone mass density.

The high FSH level in menopause stimulates osteoclast differentiation and TNF-α production from bone marrow macrophages and granulocytes. This leads to the activation of three mechanistic pathways: an increase in oxidative stress, increased M-CSF levels, and M-CSF receptor expression which increase osteoclast precursors and macrophages inducing the proliferation of activated T lymphocytes, leading to an increase in receptor activator of nuclear factor kappa B ligand (RANK-L) expression, resulting in a further increase in TNF-α production. This cycle of increased TNF-α production results in a greater number of osteoclast precursors, giving rise to the bone resorption characteristic of osteoporosis. This process may be inhibited by various substrates. Selective estrogen receptor modulators (SERMs) can prevent an increase in FSH and interact selectively with either α or β estrogenic receptors to activate protective estrogen-signaling pathways in skeletal tissue. The antioxidant vitamin C can block TNF-α production from macrophages and granulocytes, while suppressing high levels of FSH to halt and reverse increased bone turnover. A recombinant RANK-L antagonist or osteoprotegerin can block the RANK-L expression [30] and bisphosphonates, such as alendronate and risedronate, inhibit resorption and are mainstays in the treatment of osteoporosis [9]. Another prophylactic measure or treatment is the synthetic steroid tibolone, which has been reported to decrease urinary markers of bone resorption [15].

Based on the evidence which shows a strong relationship between oxidative stress and estrogen deficiency, hormone replacement and antioxidant supplementation have been investigated for their efficacy and safety in the treatment and prevention of menopause-related symptoms and complications.

11.4 Medical Management of Menopause

The medical treatment of menopause has been extensively studied. It is difficult to clearly distinguish which compounds may be superior in alleviating OS and menopause-associated symptoms and diseases. Several pharmacotherapeutic
agents and compounds have been evaluated for their efficacy in alleviating oxi-
dative stress and menopause-related symptoms and associated disease, with the
aim to provide clinicians with evidence-based treatment options.

11.4.1 Hormone Replacement Therapy

Estrogen supplementation has been thoroughly investigated as a treatment for the
myriad of symptoms and long-term degenerative effects of menopause. The use of
Hormone Replacement Therapy (HRT) to improve the redox status in postmen-
opausal women has been debated by the clinical and research community,
as estrogen can exhibit both antioxidant and pro-oxidant properties. Many studies
have attempted to determine the relationship between HRT and oxidative stress,
and although the studies’ conclusions may differ, more studies favor the use of
HRT.

Unfer et al. [5] compared the serum levels of superoxide dismutase (SOD),
catalase (CAT), GPx, and thiobarbituric acid reactive substances (TBARS) in
premenopausal women with levels in postmenopausal women, both with and
without HRT. HRT consisted of differing regimens containing conjugated estro-
gens, estradiol or estrogen plus progestin. Postmenopausal women without HRT
demonstrated significantly lower SOD activity, not related to aging, and similar
levels of CAT, GPx, and TBARS activity compared with premenopausal women
and postmenopausal women on HRT. Therefore, HRT estrogen supplementation
may boost SOD activity, thereby antagonizing oxidative stress. Leal et al. [29]
compared 6 postmenopausal women without hot flashes to 12 menopausal women
with hot flashes. All subjects were administered transdermal estradiol (17-β E2;
50 µg per day, twice a week) and medroxyprogesterone acetate (MPA) (5 mg per
day for the first 12 days of each month). Postmenopausal women with hot flashes
has lower baseline total antioxidant status (TAS) and higher baseline levels of
lipoperoxides compared with women without hot flashes. After 4 months treat-
ment with HRT, postmenopausal women with and without hot flashes experienced
a significant increase in TAS and decrease in lipoperoxides. However, the corre-
lation of vasomotor symptoms with increased oxidative stress was seen to persist,
as the subjects with hot flashes continued to display lower TAS and higher
lipoperoxide levels even after HRT administration. Therefore, in addition to
decreasing oxidative stress in postmenopausal women, HRT is effective in
reducing the frequency and severity of hot flushes [29].

Estrogen is hypothesized to increase NO levels, by stimulating NO synthase
[31, 32] or through other indirect mechanisms. The antioxidant properties of
estrogen are also thought to modulate the levels of NO [27, 33–35]. However, the
precise mechanism by which estrogen affects NO levels remains unclear. A study
by Cincinelli et al. [36] provided evidence that estrogen modulates NO concen-
tration as higher NO levels were demonstrated during the follicular phase
compared to the secretory phase of the menstrual cycle [36].
The effect of estrogen/estrogen-progestin therapy (ET/EPT) on plasma NO was studied in 80 postmenopausal women, including 26 with surgically induced menopause and 54 with physiological menopause compared with 40 healthy premenopausal women [37]. The group with surgically induced menopause was treated with 4 months of ET and those with physiological menopause were given 4 months of EPT. Transdermal E2 (50 μg twice weekly) and oral MPA (5 mg daily for 12 days) were used in the treatment. The pre- and post-treatment levels of serum E2, NO, lipid peroxide, and FSH were measured and compared to the controls. The pretreatment NO levels were lower in the postmenopausal women compared with controls, with these levels increasing significantly after hormonal therapy. As a result of treatment, the levels of total cholesterol, LDL cholesterol, triglycerides, and apolipoprotein B levels decreased to the levels seen in the control group. Interestingly, there was no correlation between increased levels of NO and the improvement in lipid profile (especially LDL) in postmenopausal women taking ET/EPT. This finding is in disagreement with the hypothesis that an improved lipid profile may promote the generation of NO. No significant difference in NO levels was observed between the ET and EPT treated groups, suggesting that progesterone does not have a significant action in the regulation of NO levels [37]. Furthermore, some studies have suggested that the addition of progesterone may actually antagonize the beneficial NO-mediated effects of estrogen on blood flow [38, 39].

Kurtay et al. [40] studied the effects of transdermal infusion of estradiol hemi-hydrate (2 mg) and norethisterone acetate (NETA) (0.25 mg) in 80 postmenopausal women. Plasma NO levels were monitored at 1, 3, 6, and finally at 12 months. A significant increase in NO levels was observed in postmenopausal women receiving HRT transdermally over a 12 month period. However, no significant change in serum NO was seen in postmenopausal women that were given oral HRT. Therefore, the route of administration of HRT may have a direct bearing on the mechanism by which supplemental hormones are metabolized by the body and influence the degree to which oxidative stress is counteracted [40].

Many researchers have also assessed the specific effects of progestin as part of HRT. In a study by Rosselli et al. [39], 26 postmenopausal women were randomized into a group that received HRT in the form of a transdermal patch of 17β-estradiol and an oral progestin supplement of 1 mg of NETA and another group which served as a control. The levels of NO were not significantly altered from baseline levels when measured at 6, 12, and 24 month intervals. Therefore, progestin supplementation did not appear to have a favorable effect on the NO levels and the redox status of postmenopausal women [39].

The use of progestin supplementation in HRT to prevent and improve cardiovascular disease in postmenopausal women was further investigated by Imthurn et al. [41]. A subject group of 26 postmenopausal women received orally administered estradiol valerate tablets for 21 continuous days. On days 12 through 21 of the treatment cycle, this treatment was supplemented with one of two chemically distinct progestins: cyproterone acetate (CPA) or MPA. Following day 21, treatment was followed by a 7-day treatment-free interval. Blood samples of
the postmenopausal women receiving HRT were collected while the subjects were being treated with estradiol valerate alone and estradiol valerate plus CPA or MPA. After 12 months of treatment with estradiol valerate alone, NO levels were significantly increased. However, when estradiol valerate was supplemented with CPA or MPA, no significant difference in NO levels was seen. Therefore, progestin supplementation may have reversed the cardioprotective effects provided by estrogen in postmenopausal women [41]. The conflicting results of the above studies regarding the interaction of progesterone with the beneficial effects of estrogen on the NO-mediated blood may be attributed to the fact that various studies tested different types of progestin.

Vasodilation is also mediated by the effect of estrogen on the synthesis of prostacyclin and endothelin, blocking calcium channels and interfering with the potassium conductance [18]. Estrogen may oppose atherosclerosis by downregulating inflammatory markers, such as cell adhesion molecules and chemokines. In addition, estrogen inhibits smooth muscle cell proliferation and downregulates angiotensin receptor gene expression. Estrogens may also stabilize atherosclerotic plaques, by reducing the expression of matrix metalloproteinases, and may decrease the thrombogenic potential of ruptured plaques by downregulating the synthesis of plasminogen activator inhibitor-1 [18].

A study by Archer et al. [42] randomized 1,147 postmenopausal subjects to groups who received either 1 mg of estradiol alone or in combination with 0.5, 1, 2, or 3 mg of drospirenone. Drospirenone is a progestin derived from spironolactone with anti-minerocorticoid and anti-androgen actions. The combination treatment group had decreased incidence of endometrial hyperplasia as compared to the group treated with estradiol alone. Furthermore, endometrial thickness remained stable over time in the combination treatment group. The combination regimen had a favorable effect on lipid profile as it reduced the total cholesterol, triglyceride and LDL levels. Due to the anti-aldosterone action of drospirenone, these patients were able to maintain or even lose weight. Urogenital and vasomotor symptoms improved in all treatment groups. Combination treatment was able to achieve an increase in the bone mineral density, thus lowering the risk of osteoporosis. Interestingly, a post-hoc analysis of a subgroup of hypertensive women in this study demonstrated a significant reduction in blood pressure in women receiving drospirenone and estradiol, in combination [43]. This finding may be attributable to the anti-minerocorticoid action of drospirenone. Drospirenone and estradiol combination treatment was reported to improve the quality of life in postmenopausal women. Overall, combination therapy was considered more effective in treating menopause-related symptoms and complications compared to estrogen monotherapy [43]. Since the use of progestin provides varied results, further studies are required to arrive at a general consensus.

There have been several studies which have failed to find a relationship between HRT and oxidative stress. Maffei et al. [44] randomized 15 postmenopausal women to receive either 2 mg oral micronized 17β-estradiol daily or transdermal estradiol therapy (1.5 mg 17β estradiol gel) The oxidative stress biomarker 8-epi PGF₂α were evaluated over 12 months and was not found to be
significantly altered in response to treatment. However, the sample size in this study was considerably small and the reliability and significance of 8-epi PGF$_{2\alpha}$, as a biomarker of oxidative stress, is not confirmed. Another form of HRT, tibolone, is a synthetic steroid with combined progesterogenic, weak estrogenic and androgenic properties. In a study by Vural et al., postmenopausal women were treated with oral tibolone daily for 6 months. Treatment failed to demonstrate any modifying effect on the levels of cytokines TNF-$\alpha$, IL-4, IL-10, and IL-12 in postmenopausal women [15]. Vassalle et al. [45] confirmed the idea that tibolone has no effect on the biochemical parameters of oxidative stress, as 2.5 mg per day for 3 months did not significantly alter the levels of IL-6, C-reactive protein or antioxidant status in both pre- and postmenopausal women. However, treatment was reported to significantly lower diastolic and systolic BP, TNF-$\alpha$ and glucose, and HDL. Despite the fact that HDL was reduced, tibolone may lower the overall cardiovascular risk in postmenopausal women because of a beneficial effect on blood pressure, inflammation, and glycemic control [45].

There are a considerable number of risks and side-effects associated with HRT use, including higher incidence of estrogen-dependent breast, ovarian, and endometrial cancers, and increased risk of thromboembolism, cardiovascular, and cerebrovascular events [22]. There is thought to be a certain time frame which is a window of opportunity in postmenopausal life, during which HRT is beneficial, and outside of which harm may be caused. The timing of HRT is relevant, as longer periods of estrogen deficiency lead to reduced number and activity of estrogen receptors which contributes to more extensive atherosclerotic damage or endothelial dysfunction, resulting in decreased vascular responsiveness and lowered efficacy of HRT. If HRT is given early enough, it may protect postmenopausal women by maintaining their vascular health, improving vascular reactivity to estrogen’s effects and delaying the clinical manifestations of atherosclerosis [44].

According to the International Menopause Society (IMS) [46], women who start late HRT may have a transient, slightly increased risk of cardiovascular events [46]. Thus, age after menopause may be considered an important factor in determining the individualized risk–benefit ratio of HRT use. Mares et al. [47] studied the relationship between the risk of heart disease and HRT. This prospective cohort study compared 2,693 women currently taking HRT or stopped HRT 5 years or less with an unexposed group of 2,256 women who had never taken HRT or stopped taking HRT for more than 5 years. After 2 years, no significant increased risk of heart disease was observed in the exposed group as compared with the unexposed group. The authors concluded that the time to menopause is a crucial factor [47].

The current recommendation is that postmenopausal women should use the lowest possible dose of HRT, with treatment being based on clear indications. The long-term data regarding fracture risk and cardiovascular implications is considered insufficient. However, HRT may prevent cardiovascular disease if started in young women at the onset of menopause, with long-term administration. The benefits of HRT are thought to generally outweigh the risks for women under the age of 60 years [46].
11.4.2 Selective Estrogen Receptor Modulators

SERMs are a class of compounds that act on the estrogen receptor, with the possibility to selectively stimulate or inhibit the effects of estrogen in various tissues. Raloxifene was the first SERM to be used to prevent and treat osteoporosis [9]. The compound functions in the breast and uterus as an estrogen antagonist [9, 48]. Raloxifene shares properties similar to those of estrogen, particularly in its capacity to reduce oxidative stress. The antioxidant activity of raloxifene is attributed to the presence of phenolic rings in its structure [9, 49]. The mechanism of action targets NADPH oxidase, an enzyme responsible for generating free radicals [9]. In normal physiologic conditions, NADPH oxidase requires activation of a particular subunit by GTPase rac 1. Raloxifene was shown to downregulate rac1 protein expression in the aortic membrane, further reducing the activity of GTPase rac1. The effects of raloxifene to ultimately decrease NADPH oxidase activity result in a less oxidative stress due to hindered ROS production [50].

Raloxifene was shown to reduce blood pressure and improve endothelial dysfunction in male spontaneously hypertensive rats. Furthermore, treatment was seen to cause a significant increase in SOD levels and the release of NO and upregulation of endothelial NOS in spontaneously hypertensive rats [48]. Raloxifene has also been shown to prevent the accumulation of cholesterol in ovariectomized cholesterol-fed rabbits and inhibit macrophage lipid oxidation [51]. A recent study by Ozbasar et al. [52] studied the effect of daily raloxifene administration in a group of 24 postmenopausal women who were undergoing long-term hemodialysis for the treatment of chronic renal failure. A regimen of 60 mg per day for 3 months lead to significantly lower levels of serum MDA and NO levels, with favorable effects on the lipid profile. The results of these studies illustrate the protective effect of raloxifene on the vascular endothelium.

Oviedo et al. [49] reported that levels of myeloperoxidase and F2α-isoprostane, markers of oxidative stress, did not change in a cohort of 30 postmenopausal women treated with raloxifene, at a dose of 60 mg per day for a 6 month period. However, the results of this study should be taken with caution as myeloperoxidase and F2α-isoprostane have not yet been proven to be reliable indicators of oxidative stress [49].

Based on the evidence, raloxifene is thought to serve a vasoprotective role by decreasing blood pressure levels and improving endothelial function as well as providing preventing hypogonadal bone loss. These effects are mediated via estrogen-receptor pathways and may result in protection against oxidative stress.

11.5 Exercise

Exercise training is thought to modulate oxidative stress by suppressing the production of free radicals and upregulating antioxidant production, resulting in an augmented antioxidant capacity [53]. Campbell et al. [54] assessed the impact of
regular aerobic exercise on the levels of F2-isoprostane, a specific marker of lipid peroxidation and general oxidative stress. After 12 months of intervention, previously sedentary postmenopausal women who exercised exhibited marked gains in aerobic fitness and decreased oxidative stress compared with non-exercising control subjects. Menopause is generally accompanied by an increase in body weight, particularly in the upper body [55]. In an observational clinical study of 90 women, total body fat mass of postmenopausal women was significantly increased by 22 %, compared to premenopausal control subjects. Furthermore, both antioxidant status and hydroperoxide levels were significantly correlated with trunk fat mass [55]. In a study by Mittal et al. [56], postmenopausal women were found to have greater body weight and a higher degree of oxidative stress compared with menstruating and perimenopausal control subjects. There was a highly significant association between weight greater than 60 kg and increased levels of SOD and MDA and decreased CAT. Karolkiewicz et al. [57] reported that an 8-week intervention of moderate intensity physical workout enhanced insulin sensitivity and improved the redox balance in healthy, postmenopausal women.

Exercise training is conservative, cost-effective strategy that may have a beneficial role in the treatment of menopausal symptoms such as hot flashes, sweating, anxiety, and depression [58, 59]. Exercise training may be useful in alleviating the symptoms of menopause, without the potential risks associated with long-term HRT use. Attipoe et al. [60] evaluated the combined effect of HRT and exercise training on oxidative stress. The study included 48 previously sedentary postmenopausal women placed into two groups: 21 women using HRT and 27 women not using HRT. Pre-exercise training and post-exercise training levels of plasma TBARS, a sensitive biomarker of lipid peroxidation and oxidative stress, were measured to assess exercise intensity. The results demonstrated a significant decrease in the plasma TBARS levels in both groups; however, no significant difference existed between the two groups. The authors concluded that a 24-week aerobic exercise training regimen significantly decreased oxidative stress in postmenopausal women regardless of HRT use [60]. However, in this study the HRT administration was not standardized, dietary intake of antioxidants was not strictly assessed in the present study, and the independent effects of HRT and exercise on oxidative stress were not assessed.

11.6 Dietary Factors and Antioxidant Supplementation

As oxidative stress has been implicated in the pathophysiology of various menopause-associated disorders, supplementing postmenopausal women with substances with antioxidant properties may serve as a useful adjunct to enhance the beneficial effect of pharmacological treatments often prescribed to postmenopausal patients. Furthermore, postmenopausal women predisposed to developing estrogen-dependent cancers based on either personal or family history, and women who
suffer harsh side effects of HRT may instead benefit from dietary changes. Supplementing the diet of postmenopausal women might serve to prevent antioxidant deficiency, preserving the health of women who are exposed to high levels of oxidative stress due to either genetic factors, lifestyle elements such as poor diet, smoking, excessive alcohol intake, and psychological stress.

**11.6.1 Vitamin C and Vitamin E**

Vitamins C (ascorbic acid) and E (α-tocopherol) are well-known antioxidants that can be obtained through one’s diet. They are thought to counteract oxidative stress through their ability to scavenge free radicals, and this effect can be harnessed to prevent and reverse the symptoms and disorders associated with age-related estrogen decline. Vitamins C and E are thought to protect against and alleviate the damaging effects of oxidative stress on the cardiovascular system of postmenopausal women. In a study conducted by Naziroglu et al. [17], 40 postmenopausal women were studied in comparison to 20 postmenopausal women with type 2 diabetes. Diabetic postmenopausal women had increased plasma and RBC lipid peroxide levels and decreased activity of key antioxidants, such as GSH-Px. Six weeks supplementation of vitamins C and E plus HRT resulted in significant decreases in levels of MDA, LDL-cholesterol, total cholesterol, and triglyceride levels in both diabetic and non-diabetic postmenopausal women. Furthermore, treatment improved fasting glucose levels. Therefore, vitamin C and E might help in lowering the risk of cardiovascular disease (with or without diabetes) in postmenopausal women by inhibiting the biosynthesis of cholesterol and oxidation of LDL-cholesterol as well as by improving glycemic balance and lipid profiles [17].

Kushi et al. [61] studied 34,486 postmenopausal women to assess the effect of vitamin E on the risk of acquiring cardiovascular diseases. After 7 years, 242 of these women died of coronary heart disease. Kushi et al. [61] reported an inverse relationship between vitamin E consumption and cardiovascular mortality and morbidity. Therefore, vitamin E obtained through dietary intake may have a significant antioxidant effect which may be helpful in decreasing cardiovascular risk [61].

Moreau et al. [62] assessed the effect of ascorbic acid on large elastic arteries in postmenopausal women. The compliance of large arteries in the cardiothoracic region decreases with age and has an important role in the increased prevalence of cardiovascular disease in postmenopausal women. The study demonstrated the ability of ascorbic acid to selectively improve large elastic artery compliance, increasing vascular conductance and blood flow in postmenopausal women, suggesting that oxidative stress might contribute to the reduced large elastic artery compliance in sedentary, estrogen-deficient postmenopausal women [62].

Furthermore, Moreau et al. [20] analyzed the relationship of oxidative stress with lower limb vasoconstriction in estrogen-deficient postmenopausal women. It should be noted that this study was limited by a small sample size as it compared a
group of only 20 postmenopausal women with 9 premenopausal women. The subjects were administered an oral pharmacological dose of ascorbic acid followed by a drip infusion of ascorbic acid and saline. Lower limb vascular conductance increased by 15% in postmenopausal women while no effect was seen on the lower limb vascular conductance of premenopausal women [20]. Vitamin C is thought to improve vascular function through its activation of the endothelial l-arginine-NO pathway. In a study by McSorley et al. [63], a 1.5 g dose of vitamin C was sufficient to induce relaxation of vascular smooth muscle via release of NO, resulting in improved vascular function [63].

Conversely, some studies failed to yield results which validate the adjuvant use of antioxidants with HRT to prevent postmenopausal women from acquiring coronary atherosclerosis. A large randomized, controlled, double-blind clinical trial evaluating the effects of HRT and antioxidant vitamin supplementation on coronary atherosclerosis in 423 postmenopausal women having baseline coronary stenosis at angiogram, reported both fatal and non-fatal myocardial infarctions during the first 2 years of treatment in patients with cardiovascular disease [64].

Low intake of ascorbic acid has been linked to increased rates of bone loss via enhanced osteoblast and osteoclast function, which result in accelerated bone turnover. This property of vitamin C has prompted investigation of its potential role in the prevention and treatment of osteoporosis in postmenopausal women. According to Iqbal et al. [30], ascorbic acid may prevent FSH-induced hypogonadal bone loss by modulating the destructive action of TNF-α, limiting its stimulatory effects on osteoclast formation. The efficacy and safety of vitamin C and E in the prevention and treatment of postmenopausal cardiovascular disease and osteoporosis should be further investigated in large-scale, double-blinded randomized, controlled trials.

11.6.2 Phytoestrogens

Phytoestrogens are weakly estrogenic compounds contained in soybeans. They are derived from the diet in the form of soymilk, soy protein, and beverages. Dietary phytoestrogen are also known as isoflavones, a broad group of polyphenolic compounds that are distributed widely among foods of plant origin. Isoflavones may be considered as natural SERMs [60] due to their structural similarity with 17β-estradiol which allows binding to both types of estrogen receptors: ERα and ERβ [65].

Isoflavones have been thought to act protectively against cardiovascular disease, osteoporosis, and cancers of the breast and prostate through their prevention of LDL oxidation and inhibition of DNA damage. Phytoestrogens may decrease the risk of cardiovascular disease by lowering the levels of oxidized LDL and decreasing the frequency of hot flushes in postmenopausal women [12]. Furthermore, phytoestrogens have been shown to exhibit defensive
immunoprotective properties, such as their role in B cell stimulation and in the inhibition of oxidative damage of DNA in postmenopausal women [66].

Engelman et al. [67] evaluated the effect of isoflavone treatment in a 55 postmenopausal women. The subjects were administered varying proportions of soy proteins and isoflavones. After 6 weeks of supplementation, neither phytate nor isoflavone demonstrated any effect on redox status. Hence, additional studies employing higher doses of soyflavones in a greater sample size should be conducted to arrive at a conclusion [67].

Another study reported that the consumption of soy milk and supplemental isoflavones in 52 postmenopausal women led to decreased plasma levels of 8-hydroxydeoxyguanosine (8-OHdg) and 8-isoprostane [66]. Hallund et al. [65] verified the benefits of phytoestrogens in postmenopausal women by examining the effects of soy cereal bar consumption for an 8 week period. Specific markers of cardiovascular health, including plasma nitrate concentrations, the nitrate: endothelin-1 ratio, and the amount of nitroglycerine-mediated endothelium-independent vasodilatation, were found to be significantly increased in postmenopausal women who consumed soy cereal bars in comparison to the control group that received a placebo. Flow-mediated endothelium-dependent vasodilation was not affected [65]. Isoflavone supplementation was reported to be beneficial, in conjunction with regular exercise, in regulating weight gain, lipid profiles, and oxidative stress in the ovariectomized rat model [68]. After 12 weeks of intervention, isoflavone treatment, both alone and with exercise, led to a significant decrease in total cholesterol, triglycerides and LDL-cholesterol compared to ovariectomized control subjects [68].

A recent study by Beavers et al. [69] conducted a single-blind, randomized, controlled trial that found no significant alteration in markers of inflammation or oxidative stress in 16 postmenopausal women who consumed soymilk 3 times a day for 4 weeks, compared with 15 postmenopausal control subjects that consumed reduced fat dairy. The duration and dosage of isoflavone treatment in this study was comparable to that studied in the literature. However, the indices used to measure oxidative stress were not, which may explain the contradictory findings. The results may have been confounded by lifestyle factors that influence the expression of plasma markers of oxidative stress. It is possible that soy supplementation is efficacious only in those having significantly elevated biomarkers of oxidative stress. However, the patients in this study were not selected according to baseline oxidative stress status [69].

The role of isoflavones in reducing the risk of cardiovascular disease through oxidative stress-induced pathways must be further assessed. Studies have suggested that phytoestrogens may have a protective effect against osteoporosis through their intrinsic growth-promoting activity which stimulates osteoblasts. This action of phytoestrogens could be a new therapeutic approach toward prevention and treatment of osteoporosis. More research is required to arrive at a consensus on the use of isoflavones in the therapeutics of menopause.
11.6.3 *Curcuma longa*

*C. longa* is an herbal extract with phenolic antioxidant properties. The compound has powerful free radical-neutralizing properties and was shown to decrease the levels of oxidized HDL and LDL in women (40–90 years) without inducing hepatic or renal problems [14].

Apolipoprotein A (Apo A) is involved with the metabolism of HDL-cholesterol and is a component of the body’s anti-atherogenic defense. Conversely, Apo B has pro-atherogenic effects as it induces the formation of LDL cholesterol. In a study analyzing apolipoproteins in relation to postmenopausal subjects, the ratio of apo A and B was significantly altered after treatment with curcuma longa and it was concluded that *C. longa* may normalize the apo B/apo A ratio [70]. *C. longa* extract has also been reported to decrease abnormally high levels of plasma fibrinogen to normal values [71].

11.6.4 *Lycopene*

LycoRed, a form of lycopene, is thought to decrease the risk of cardiovascular diseases in postmenopausal women. In healthy women ranging from 31 to 75 years, circulating lycopene levels were seen to exhibit an inverse relationship with arterial stiffness, as measured by brachial-ankle pulse wave velocity [72]. This effect may be mediated by lycopene’s capacity to reduce the oxidative modification of LDL. Misra et al. [73] reported that supplementation led to a decrease in serum HDL, LDL, MDA and an increase in GSH compared to the pretreatment serum levels. The decrease in levels of MDA and LDL (a risk factor for atherosclerosis) and the increase in protective antioxidant glutathione suggest an overall decline in oxidative stress as a result of LycoRed administration [73].

11.6.5 *Grape Polyphenols*

Grape polyphenols have also been considered to be used as an alternative treatment to reduce oxidative stress. In both premenopausal and postmenopausal women, grape polyphenols was reported to reduce indices of oxidative stress such as plasma F2-isoprostane and plasma TNF-α, as well as resulting in reduced triglycerides, LDL and apo-B levels [74].
11.6.6 Acanthopanax senticosus

*A. senticosus* is a common Asian herb also referred to as “Siberian Ginseng” or “Eleutherococcus senticosus”. It has been shown to have antioxidant effects in rats [75]. Lee et al. [76] studied the effects of *A. senticosus* supplementation on serum lipid profiles, biomarkers of oxidative stress, and lymphocyte DNA damage in postmenopausal women. A significant decrease in the concentration of LDL, LDL/HDL ratio, serum MDA concentration, serum protein carbonyl levels, and lymphocyte DNA damage was observed. Additionally, no side effects were reported [76].

11.6.7 Vitamin A

Behr et al. [77] conducted a recent, inaugural study of low-dose retinol palmitate, a vitamin A supplement, in the treatment of menopause symptoms and associated oxidative stress. The subjects of this study, Wistar rats, were bilaterally ovariec-
tomized and subsequently exhibited characteristics of menopause, including increases in body weight, uterine atrophy, altered lipid profile, increased blood peroxidase activity and decreased plasma antioxidant status. Low-dose supple-
mentation with vitamin A was shown to reverse some of these effects, by restoring the levels of enzymatic and non-enzymatic antioxidant defense and decreasing the degree of oxidative damage incurred by proteins [77]. The results of this study are compelling and should promote further research to elucidate whether vitamin A is safe and effective in the treatment of menopause and associated oxidative stress. Safety is a concern as high doses of vitamin A may have embryotoxic and teratogenic effects [78].

11.6.8 Klamin

Klamin is an algae extract that is rich in potent algal antioxidant, Aphanizomenon flos-aquae (AFA) phycocyanin, and natural neuromodulators, such as phenyleth-
ylamine and selective monoamine oxidase inhibitors. Klamin has been proposed as an alternative treatment for psychological, somatic, and vasomotor symptoms related to menopause. Scoglio et al. [79] investigated the effect of Klamath algae on the general and psychological health of 21 postmenopausal women that did not take HRT. Treatment led to significantly reduced MDA levels, indicating decreased plasma lipid peroxidation. An increase in antioxidants such as carote-
noids, tocopherols, and retinols was observed. Furthermore, treatment was reported to improve the overall and psychological well-being of subjects, as indicated objectively by a decreased average Green Scale score. A favorable
side-effect profile was suggested by the fact that Klamin did not exhibit any steroid-like effects on hormonal parameters. Therefore, Klamin may be having a role as a complementary treatment or as a plausible, natural alternative for patients who wish to avoid hormonal therapy [79].

11.6.9 Melatonin

Melatonin is secreted by the pineal gland and exhibits anti-oxidant properties. Melatonin is thought to arrest lipid peroxidation and protein oxidation in a dose-dependent manner. Up until now, the effect of melatonin on oxidative stress and symptoms in the postmenopausal state has only been studied in the ovariectomized rat. Baeza et al. [80] reported that, as part of a combination with growth hormone, estrogens, and phytoestrogens, melatonin supplementation led to a significant reduction in oxidative stress, represented by a decrease in MDA levels and the degree of glutathione depletion [80]. Melatonin was shown to influence oxidative stress in the blood and brain of ovariectomized rats [81]. In comparison with the non-treated, ovariectomized control group, melatonin supplementation for 30 days decreased lipoperoxide levels, while increasing erythrocyte glutathione, vitamin A, C, and E levels, and the concentration of the 2B subunit of the hippocampal N-methyl-D-aspartate receptor (NMDA) [81]. Therefore, by boosting antioxidant defense and upregulating the NMDA receptor, melatonin may prevent the excess oxidative stress seen in the postmenopausal state. The results of these preliminary animal studies warrant further investigation into the efficacy of melatonin supplementation in the treatment of postmenopausal women.

11.7 Conclusion

Estrogen is an established antioxidant; therefore, in menopause, estrogen deficiency leads to the development of oxidative stress. Various studies have demonstrated increased oxidative stress marker levels and decreased antioxidant levels in postmenopausal women. Oxidative stress has been linked to the development of osteoporosis and increased cardiovascular risk in these women. HRT decreases oxidative stress in women with menopause by increasing the TAS and preventing the breakdown of NO. HRT can be effective in reducing the frequency and severity of hot flushes and may be protective against osteoporosis and cardiovascular complications during menopause. HRT may also delay the clinical manifestations of atherosclerosis.

In addition to HRT, various dietary changes, exercise training, and SERMs are potential therapeutic alternatives which have been assessed in postmenopausal women for their potential role in alleviating the oxidative stress underlying the symptoms and complications of menopause. The use of antioxidant vitamins and
herbal extracts may prove to be beneficial in postmenopausal women by normalizing the redox status of the cell. Further investigations are required to study their efficacy and safety before they can be implemented for clinical use in postmenopausal women. Wide varieties of treatment options are now available to prevent and reverse the effects of oxidative stress associated with reproductive aging in postmenopausal women, and treatment should be tailored according to personal circumstances with periodic reviews.

References


