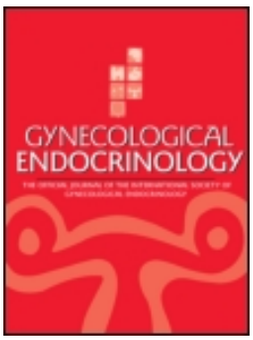

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MENOPAUSE

The effect of menopause on the skin and other connective tissues

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Cutaneous ageing manifests itself as a progressive reduction in function and reserve capacity of skin tissue. Collagen atrophy is a major factor in skin ageing. There is a strong correlation between skin collagen loss and oestrogen deficiency due to the menopause. Skin ageing is associated with a progressive increase in extensibility and a reduction in elasticity. With increasing age, the skin also becomes more fragile and susceptible to trauma, leading to more lacerations and bruising. Furthermore, wound healing is impaired in older women. Oestrogen use after the menopause increases collagen content, dermal thickness and elasticity, and it decreases the likelihood of senile dry skin. Large-scale clinical trials are necessary to help make informed recommendations regarding postmenopausal oestrogen use and its role in the prevention of skin ageing. Oestrogen has profound effects on connective tissue turnover, no matter the site. It has been shown that menopause has similar effects on the connective tissue of the carotid artery media, intervertebral discs and bones.

Keywords: Collagen, connective tissue, oestrogen, hormone replacement therapy, menopause, skin

Anatomy of the skin

The skin is the largest organ in the body. It is stratified into two layers: epidermis and dermis, apart from hair follicles, sebaceous and sweat glands. The epidermis forms the thin outer layer and consists of keratinocytes and melanocytes. The dermis is the deeper layer and forms the main bulk of the skin. Its function is to provide a tough matrix to support the blood vessels, nerves and appendages. The fibres present in the dermal connective tissue are predominantly collagen and elastin [1].

About 80% of the dry weight of adult skin consists of collagen. The predominant form of collagen found in adult human skin is Type I, followed by Type III [2]. Collagen fibres are arranged parallel to the skin surface. This gives the skin a high tensile strength and prevents it from being torn by overstretching. Collagen is synthesized by fibroblasts from procollagen molecules by the action of neutral endoproteases. The fibrils of collagen undergo a series of post-translational modifications in order to enhance their stability and tensile strength.

In contrast, elastin constitutes about 5% of the dermis, providing the skin with elasticity and resilience [3]. Elastin fibres are also produced by fibroblasts, and are arranged as a thinly distributed sub-epidermal network. The dermal connective tissue also contains sensory receptors, together with the supportive glycosaminogylcans (GAGs) [4].

Effects of menopause on structural components and physical characteristics of the skin

The skin becomes thinner, with changes in structure and function with age [5,6]. Skin quality deteriorates with age due to the synergistic effects of time and photo-ageing, together with hormonal deficiency, environmental factors and a decline in several metabolic activities [4]. The dermis undergoes morphological, physical and chemical changes [1]. Overall, many of the skin's functions are known to decline with age. The effects of postmenopausal oestrogen deficiency are thought to include: atrophy; decreased collagen and water content; decreased sebaceous secretions; loss of elasticity and manifestations of hyperandrogenism. The cumulative effects of oestrogen deficiency on the skin are thought to contribute to poor wound healing in older patients. Oestrogen deficiency may account for the accelerated skin ageing; however, it is difficult to distinguish between the changes that occur specifically with age, and those occurring with oestrogen deprivation.

Collagen

Collagen atrophy is a major factor in skin ageing. In the elderly, the skin contains thickened, clumped basophilic collagenous material, indicating partial degradation of collagen. There is also a significant decline in the dermal quantity of collagen with ageing. There is a decrease in the amount of enzymes involved in post-translational processing of collagen, together with a decline in the amounts of hydroxyproline and glycosylated hydroxylysine in Type I collagen. Collagen ageing results from progressive cross-linking between the collagen molecules. A larger proportion of the cross-links between collagen molecules become non-reducible, while there is a decline in the number of immature and reducible cross-links. There is a reduction in the number of fibroblasts that synthesize collagen and vessels that supply the skin. This contributes to an increase in laxity, making wrinkles more evident [1].

There is a strong correlation between skin collagen loss and oestrogen deficiency secondary to the menopause [7,8]. As much as 30% of skin collagen is lost in the initial five years after the menopause. Total collagen content declines with an average of 2.1% per postmenopausal year over a period of 15 years [9].

One study showed a closer correlation between collagen loss and chronologic age than between skin collagen loss and time since the menopause [10]. This finding may be explained by the fact that the study participants were between 40 and 55 years of age and had recently undergone surgical menopause, and consequently had not been oestrogen deficient for long enough [11].

The decline in skin collagen content can be prevented with oestrogen [12]. A beneficial effect of subcutaneous, topical or oral oestrogens has been demonstrated on skin collagen content [13–16]. There is a variation in the extent of the oestrogen-induced increase

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in collagen content, depending on the route of administration, dose and duration of hormone treatment. As there are differences in the methods employed to assess collagen levels, results between studies are often not comparable [17]. The increase in collagen with oestrogen is proportionate to baseline collagen levels. One study observed no change in collagen levels of postmenopausal women following one year of hormone replacement therapy (HRT) [18]. This can be explained by the fact that, given the short time since menopause, the amount and synthesis of collagen may have not yet fallen [19]. Collagen levels and metabolism do not change immediately after the menopause [20].

Elastin

Elastin fibres are closely linked and interwoven with the collagen fibrils so that they can recoil after transient stretching, preventing overstretching. Young women with a premature menopause were shown to have accelerated degenerative changes in dermal elastic fibres [4]. Histological studies demonstrate that topical oestrogen can increase the number and thickness of skin elastic fibres [21]. Clinical trials demonstrate no observable improvement in elastin fibre content from the baseline with systemic oestrogen therapy [9,13,18]. However, these studies are small, short or treat very early postmenopausal women.

Water

Healthy skin needs a substantial water content, which is determined by cutaneous evaporation and epidermal hydration [17]. There is a decrease in transepidermal water flux with age [10]. Glycosaminoglycans have a high water-binding capability, and are essential for normal skin hydration. There are minor quantities of various glycosaminoglycans (including versican and heparan sulphate) in the dermis, which are closely associated with skin collagen. Total dermal glycosaminoglycan content decreases significantly with ageing [1]. Collagen and glycosaminoglycans may interact to produce the age-related changes in the properties of collagen. It is possible that hydration of the dermis, influenced by glycosaminoglycans, may be more important than the extent of collagen cross-linking.

Dry skin is one of the commonest dermatological conditions in older women [14]. An epidemiological survey of 3875 postmenopausal women aged 40 years and over found that 36.2% had dry skin. Oestrogen use was associated with a statistically significant decrease in the likelihood of senile dry skin (odds ratio, 0.76; 95% confidence interval, 0.60–0.97) [15]. These positive effects may be related to oestrogen-stimulated increases in mucopolysaccharides and hyaluronic acid levels in skin which correlate to an increased dermal water content [10], which may also lead to an increase in skin thickness.

Sebaceous glands and hair

Sebum secretion decreases with age. Overall, there is a 38% increase in sebum production in postmenopausal women taking HRT, when compared with controls, with an increase in skin surface lipids [16].

Possible changes in postmenopausal women include an increase in facial hair and a decrease in body and/or pubic hair. The onset of the menopause can lead to a diffuse or an androgenic alopecia [22–24]. Another type of hair loss includes frontal fibrosing alopecia, which is a variant of lichen planopilaris. The latter has been associated with the postmenopause; however, hair loss may persist despite hormone treatment [25].

During the ageing process, there is also a decrease in the production of glycosaminoglycans in the connective tissue. There is also a higher intracellular concentration of protocollagen lysylhydroxyproline transferase, which is the enzyme responsible for collagen breakdown [26]. The loss of connective tissue in cutaneous ageing results in increased distensibility and loss of tonicity, leading to a progressive deepening of facial creases and wrinkling [17]. In untreated perimenopausal women, there is a rapid increase in skin extensibility, as detected in computerized measurements of skin deformability [27]. The mechanical properties of the skin improve with oestrogen [28,29]. However, few clinical studies have specifically examined HRT and facial wrinkling, due to technical challenges in quantitating a visual end point such as facial wrinkles [17].

Skin thickness

There is an increase in skin thickness up to the age of 35 to 49 years, followed by an age-related thinning [17]. For the initial 15 to 18 postmenopausal years, the decrease in skin thickness accelerates with as much as 1.13% annual decline [5,7]. The thinning effect is due to decreases in collagen, water and glycosaminoglycans [17]. Most clinical trials have shown that postmenopausal women who take HRT have greater skin thickness when compared to non-users [30–33].

Elasticity

Skin ageing, especially in the face, is associated with a progressive increase in extensibility and a reduction in skin elasticity [17]. In postmenopausal women, skin elasticity declines 0.55% per year after the menopause. The HRT for 12 months increases elasticity by 5.2% [34]. The HRT delays the deterioration in the extensibility of the skin, slowing the progress of cutaneous slackening that follows the menopause [16,28].

Wrinkles

The loss of connective tissue in cutaneous ageing results in increased distensibility and loss of skin tone, leading to a progressive deepening of facial creases and wrinkling [17]. In untreated perimenopausal women, there is a rapid increase in skin extensibility, as detected in computerized measurements of skin deformability and the mechanical properties of the skin improve with oestrogen [28,29]. Facial wrinkling improves with oestrogens but worsens with smoking [35,36]. However, few clinical studies have specifically examined HRT and facial wrinkling, probably due to technical challenges in quantitation [17].

Blood flow

The dermis and epidermis are nourished by arterioles and capillaries that pass upwards from the subcutaneous layer. The integrity in the structure and function of capillary blood vessels is important in healthy skin. There is a rich capillary network in the dermal papillae, which is responsible for the menopausal flush [37]. Core temperature haemostasis is maintained by the cutaneous circulation. Peripheral microcirculation at the level of the nail-fold capillaries decreases significantly at menopause [38]. Six to twelve months of HRT increases capillary blood flow in the nail-fold by as much as 20%–30% [38]. The endothelium-dependent and endothelium-independent vascular reactivity in the cutaneous microcirculation improves substantially in postmenopausal women receiving oestrogen [39].

Wound healing

With increasing age, the skin becomes more fragile and susceptible to trauma, leading to more lacerations and bruising. Older women have been shown to heal less well, possibly due to low levels of transforming growth factor (TGF)-β (12). Venous ulcers and pressure sore are among the chronic wounds commonly

suffered by the elderly. These cause significant suffering and cost, thus imposing a burden for patients and physicians alike [30]. Cutaneous wound healing involves vascularization, granulation, collagen deposition and re-epithelialization [31]. The effect of oestrogen levels on the stages of wound healing is still unclear, because of contradictory findings in animal studies [17]. In humans, oestrogen has been shown to induce TGF-β secretion by dermal fibroblasts, and this can enhance the rate and quality of wound healing [32].

Impact on sexuality

Skin changes can affect psychosexual function [40,41]. Altered physical appearance with the appearance of wrinkles and skin sagging can cause lowering of self esteem. Altered peripheral nerve function, especially in the genital area, lead to reduced libido and dyspareunia [33].

Effects of menopause on structural components and physical characteristics of other connective tissue

Bone

There is a high incidence of thin skin in osteoporotic women [35,42]. In fact, there is a strong correlation between skin thickness, collagen content and bone density in postmenopausal women [5,7,43].

At the menopause, bone turnover increases, and may remain high for up to 25 years after the last menstrual period [36]. Bone turnover is controlled by a complex interrelationship of a number of factors, including oestrogen, progesterone, testosterone, Vitamin D, corticosteroids, thyroid hormones and retinoids [44]. Oestrogen alone has a known beneficial effect on reducing bone fractures and limiting bone loss. A number of studies have shown the positive effect of progesterone on bone proliferation and inhibition of bone resorption [45–48]. However, another study showed no difference between progesterone and placebo in terms of any difference in markers of bone resorption [49]. Consequently, large-scale randomized controlled trials are necessary to determine the role of progesterone alone in the prevention or treatment of osteoporosis [40]. Oestrogen and progesterone alone could have distinct yet complimentary roles in the maintenance of bone [41,50,51].

Intervertebral discs

Each intervertebral disc is composed of high collagen content and glycosaminoglycans. Intervertebral discs are responsible for 20% of the spinal column height and allow flexion and extension of the back and also act as "shock absorbers" of the spinal column. This may have an important role on osteoporotic compression fractures [52].

With the ageing process, there is a change in collagen type [53], with a more profound difference with increasing years since the menopause [54]. The collagen Types I, III and VI predominate at the expense of collagen Types II, IV and IX. There is also a significant decrease in glycosaminoglycans and elastin in the aged intervertebral disc [55].

The lumbar intervertebral disc height has been shown to be significantly higher in the premenopausal group (height of three lumbar discs 2.16 +/− 0.1 cm) and hormone-treated group (disc height 2.2 +/− 0.12 cm) compared to the untreated postmenopausal women (disc height 1.86 +/− 0.06 cm) ($p < 0.0001$) [54]. This has been confirmed by another study on a bigger cohort. The premenopausal women and hormone-treated women had disc heights of 2.01 +/− 0.09 cm and 2.15 +/− 0.08 cm, respectively, the latter results being significantly higher than the untreated postmenopausal group (height of three lumbar discs 1.82 +/− 0.06 cm) and the osteoporotic fracture group (1.58 +/− 0.1 cm) ($p = < 0.0001$) [54].

These results may be due to the effect that the menopause has on the connective tissue components of intervertebral discs. This may lead to loss of the shock-absorbing properties of the intervertebral disc and an altered discoid shape, influencing the occurrence of osteoporotic vertebral body fractures [56]. After the menopause, intervertebral disc space shows a progressive decrease that almost entirely occurs in the first 5 to 10 years since the menopause, suggesting that the decline in oestrogen level may rapidly change connective tissue metabolism in the intervertebral discs [57].

Carotid artery

It is thought that the arteries, including the carotids, could undergo postmenopausal connective tissue changes. Each artery is made up of three layers: externa, media and intima. The media layer has the highest connective tissue component, including collagen Types I and III and elastin fibres.

Hormone replacement has a morphological effect on the carotid arteries in postmenopausal women. It has been suggested that hormone replacement given to postmenopausal women differentially influences the layers of the carotid artery. Hormone replacement seems to encourage thickening of the layers with the highest connective tissue component (externa and media) and to delay thickening of the atheromatous intima layer [58].

A recent study carried out on young hypogonadal women showed that increasing doses of HRT result in a reduction of carotid intima-media thickness, along with increased serum high density lipoprotein (HDL) and decreased plasma glucose. Therefore, this study raises the possibility that exogenous oestrogen may be cardioprotective in young women. However, this observation needs to be balanced against a prothrombotic effect, which is predominant in postmenopausal women [59].

HRT– where do we stand?

In the wake of the Women's Health Initiative (WHI) trial, many dilemmas have yet to be resolved regarding the use of HRT in postmenopausal women. Several factors may have contributed to the widely different conclusions of the WHI trials in comparison to the observational studies.

The WHI included two randomized double-blind, placebo-controlled investigations of unopposed oestrogen (0.625 mg of conjugated equine oestrogen, CEE) alone for women with a prior hysterectomy [60] and of combined oestrogen–progestin (the progestin component consisted of 2.5 mg of medroxyprogesterone acetate, MPA) for women with a uterus [61]. The combined HRT arm of the WHI was stopped on May 2002 after a mean of 5.2 years follow-up, because the test statistic for invasive breast cancer exceeded the stopping boundary and the global index statistic supported risks exceeding benefits [62]. The conclusions from the study at that time were that the risks of coronary heart disease, stroke and pulmonary embolism were significantly increased in the intervention group, the risks of hip fracture and colorectal cancer were reduced and the mortality risk was unchanged [61].

The oestrogen-alone arm of the WHI study was stopped in February 2004, one year earlier than planned, due to excess stroke. It was concluded that CEE alone increases the risk of stroke, reduces the risk of hip fractures and does not affect the risk of cardiovascular heart disease in postmenopausal women with prior hysterectomy over an average of 6.8 years. There was a possible reduction of breast cancer risk, but this required further investigation [60].

The potential side effects and risks involved in taking HRT may be reduced by using lower HRT doses, minimizing or eliminating systemic progestogens, using non-oral routes in some women, and initiating HRT in symptomatic women near the menopause. When HRT is initiated near the menopause for symptom control, there may be additional benefits including reduced fracture and cardiovascular risk. These benefits outweigh the risks, which are not significantly raised in women under the age of 60 years. As long as their therapy and risks are assessed on an individual basis and each patient is aware of the risks, older women with continuing symptoms should not be denied HRT [63].

References

1 Castelo-Branco C. Skin collagen changes related to age, menopause and hormone replacement therapy. In: Brincat MP, editor. Hormone Replacement Therapy and the Skin. UK: Parthenon Publishing; 2001.
2. Lovell CR, Smolenski KA, Duance VC, Light ND, Young S, Dyson M. Type I and III collagen content and fibre distribution in normal human skin during ageing. Br J Dermatol 1987;117:419–428.
3. Uitto J. Connective tissue biochemistry of the aging dermis. Age-related alterations in collagen and elastin. Dermatol Clin 1986;4:433–446.
4. Bolognia JL, Braverman IM, Rousseau ME, Sarrel PM. Skin changes in menopause. Maturitas 1989;11:295–304.
5. Brincat M, Kabalan S, Studd JW, Moniz CF, de Trafford J, Montgomery J. A study of the decrease of skin collagen content, skin thickness, and bone mass in the postmenopausal woman. Obstet Gynecol 1987;70:840–845.
6. Brincat M, Moniz CJ, Studd JW, Darby A, Magos A, Emburey G, Versi E. Long-term effects of the menopause and sex hormones on skin thickness. Br J Obstet Gynaecol 1985;92:256–259.
7. Brincat M, Versi E, Moniz CF, Magos A, de Trafford J, Studd JW. Skin collagen changes in postmenopausal women receiving different regimens of estrogen therapy. Obstet Gynecol 1987;70:123–127.
8. Affinito P, Palomba S, Sorrentino C, Di Carlo C, Bifulco G, Arienzo MP, Nappi C. Effects of postmenopausal hypoestrogenism on skin collagen. Maturitas 1999;33:239–247.
9. Sauerbronn AV, Fonseca AM, Bagnoli VR, Saldiva PH, Pinotti JA. The effects of systemic hormonal replacement therapy on the skin of postmenopausal women. Int J Gynaecol Obstet 2000;68:35–41.
10. Castelo-Branco C, Duran M, González-Merlo J. Skin collagen changes related to age and hormone replacement therapy. Maturitas 1992;15:113–119.
11. Shah MG, Maibach HI. Estrogen and skin. An overview. Am J Clin Dermatol 2001;2:143–150.
12. Brincat M, Moniz CF, Studd JW, Darby AJ, Magos A, Cooper D. Sex hormones and skin collagen content in postmenopausal women. Br Med J (Clin Res Ed) 1983;287:1337–1338.
13. Kalogirou D, Aroni K, Kalogirou O, Antoniou G, Botsis D, Kontoravdis A. Histological changes induced by tibolone and estrogen/glucocorticoid on aging skin. Int J Fertil Womens Med 2000;45:273–278.
14. Beauregard S, Gilchrest BA. A survey of skin problems and skin care regimens in the elderly. Arch Dermatol 1987;123:1638–1643.
15. Dunn LB, Damesyn M, Moore AA, Reuben DB, Greendale GA. Does estrogen prevent skin aging? Results from the First National Health and Nutrition Examination Survey (NHANES I). Arch Dermatol 1997;133:339–342.
16. Callens A, Vaillant L, Lecomte P, Berson M, Gall Y, Lorette G. Does hormonal skin aging exist? A study of the influence of different hormone therapy regimens on the skin of postmenopausal women using non-invasive measurement techniques. Dermatology (Basel) 1996;193:289–294.
17. Brincat MP, Baron YM, Galea R. Estrogens and the skin. Climacteric 2005;8:110–123.
18. Haapasaari KM, Raudaskoski T, Kallioinen M, Suvanto-Luukkonen E, Kauppila A, Läärä E, Risteli J, Oikarinen A. Systemic therapy with estrogen or estrogen with progestin has no effect on skin collagen in postmenopausal women. Maturitas 1997;27:153–162.
19. Oikarinen A. Systemic estrogens have no conclusive beneficial effect on human skin connective tissue. Acta Obstet Gynecol Scand 2000;79:250–254.
20. Brincat M, Moniz CF, Kabalan S, Versi E, O'Dowd T, Magos AL, Montgomery J, Studd JW. Decline in skin collagen content and metacarpal index after the menopause and its prevention with sex hormone replacement. Br J Obstet Gynaecol 1987;94:126–129.
21. Punnonen R, Vaajalahti P, Teisala K. Local oestriol treatment improves the structure of elastic fibers in the skin of postmenopausal women. Ann Chir Gynaecol Suppl 1987;202:39–41.
22. Rebora A. Pathogenesis of androgenetic alopecia. J Am Acad Dermatol 2004;50:777–779.
23. Hoffmann R, Niiyama S, Huth A, Kissling S, Happle R. 17alpha-estradiol induces aromatase activity in intact human anagen hair follicles ex vivo. Exp Dermatol 2002;11:376–380.
24. Wallace ML, Smoller BR. Estrogen and progesterone receptors in androgenic alopecia versus alopecia areata. Am J Dermatopathol 1998;20:160–163.
25. Kossard S, Lee MS, Wilkinson B. Postmenopausal frontal fibrosing alopecia: a frontal variant of lichen planopilaris. J Am Acad Dermatol 1997;36:59–66.
26. Anttinen H, Orava S, Ryhänen L, Kivirikko KI. Assay of protocollagen lysyl hydroxylase activity in the skin of human subjects and changes in the activity with age. Clin Chim Acta 1973;47:289–294.
27. Henry F, Piérard-Franchimont C, Cauwenbergh G, Piérard GE. Age-related changes in facial skin contours and rheology. J Am Geriatr Soc 1997;45:220–222.
28. Piérard GE, Letawe C, Dowlati A, Piérard-Franchimont C. Effect of hormone replacement therapy for menopause on the mechanical properties of skin. J Am Geriatr Soc 1995;43:662–665.
29. Kafantari H, Kounadi E, Fatouros M, Milonakis M, Tzaphlidou M. Structural alterations in rat skin and bone collagen fibrils induced by ovariectomy. Bone 2000;26:349–353.
30. Ashcroft GS, Greenwell-Wild T, Horan MA, Wahl SM, Ferguson MW. Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. Am J Pathol 1999;155:1137–1146.
31. Calvin M. Oestrogens and wound healing. Maturitas 2000;34:195–210.
32. Schmidt JB, Binder M, Demschik G, Bieglmayer C, Reiner A. Treatment of skin aging with topical estrogens. Int J Dermatol 1996;35:669–674.
33. Graziottin A. Libido: the biologic scenario. Maturitas 2000;34 Suppl 1:S9–16.
34. Sumino H, Ichikawa S, Abe M, Endo Y, Ishikawa O, Kurabayashi M. Effects of aging, menopause, and hormone replacement therapy on forearm skin elasticity in women. J Am Geriatr Soc 2004;52:945–949.
35. Black MM, Shuster S, Bottoms E. Osteoporosis, skin collagen, and androgen. Br Med J 1970;4:773–774.
36. Takahashi M, Kushida K, Hoshino H, Ohishi T, Inoue T. Biochemical markers of bone turnover do not decline after menopause in healthy women. Br J Obstet Gynaecol 1999;106:427–431.
37. Brincat MP, Baron YM, Galea R. Skin and connective tissues. In: Studd J, Lauritzen C, editors. Current Management of the Menopause.London: Taylor & Francis; 2001.
38. Haenggi W, Linder HR, Birkhaeuser MH, Schneider H. Microscopic findings of the nail-fold capillaries–dependence on menopausal status and hormone replacement therapy. Maturitas 1995;22:37–46.
39. Arora S, Veves A, Caballaro AE, Smakowski P, LoGerfo FW. Estrogen improves endothelial function. J Vasc Surg 1998;27:1141–6; discussion 1147.
40. Moskowitz D. A comprehensive review of the safety and efficacy of bioidentical hormones for the management of menopause and related health risks. Altern Med Rev 2006;11:208–223.
41. Schmidt IU, Wakley GK, Turner RT. Effects of estrogen and progesterone on tibia histomorphometry in growing rats. Calcif Tissue Int 2000;67:47–52.
42. Jacobsen E, Billings JK, Frantz RA, Kinney CK, Stewart ME, Downing DT. Age-related changes in sebaceous wax ester secretion rates in men and women. J Invest Dermatol 1985;85:483–485.
43. Baron YM, Brincat M, Galea R. Increased reduction in bone density and skin thickness in postmenopausal women on long-term corticosteroid therapy. A suggested role for estrogen add back therapy. Adv Exp Med Biol 1999;455:429–436.
44. Bland R. Steroid hormone receptor expression and action in bone. Clin Sci 2000;98:217–240.
45. Liang M, Liao EY, Xu X, Luo XH, Xiao XH. Effects of progesterone and 18-methyl levonorgestrel on osteoblastic cells. Endocr Res 2003;29:483–501.
46. Barengolts EI, Gajardo HF, Rosol TJ, D'Anza JJ, Pena M, Botsis J, Kukreja SC. Effects of progesterone on postovariectomy bone loss in aged rats. J Bone Miner Res 1990;5:1143–1147.
47. Bowman BM, Miller SC. Elevated progesterone during pseudopregnancy may prevent bone loss associated with low estrogen. J Bone Miner Res 1996;11:15–21.
48. Fujimaki T, Kurabayashi T, Yamamoto Y, Yasuda M, Tojo Y, Yahata T, Tanaka K. Effects of progesterone on the metabolism of cancellous

bone in young oophorectomized rats. J Obstet Gynaecol (Tokyo 1995) 1995;21:31–36.
49. Ikram Z, Dulipsingh L, Prestwood KM. Lack of effect of short-term micronized progesterone on bone turnover in postmenopausal women. J Womens Health Gend Based Med 1999;8:973–978.
50. Burnett CC, Reddi AH. Influence of estrogen and progesterone on matrix-induced endochondral bone formation. Calcif Tissue Int 1983;35:609–614.
51. Yamamoto Y, Kurabayashi T, Tojo Y, Yahata T, Honda A, Tomita M, Tanaka K. Effects of progestins on the metabolism of cancellous bone in aged oophorectomized rats. Bone 1998;22:533–537.
52 Brincat MP, Baron YM, Galea R. Connective tissue changes in the menopause and with HRT. In: Lobo RA, editor. Treatment of the postmenopausal woman: basic and clinical aspects. 2007.
53. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. Spine 2002;27:2631–2644.
54. Baron YM, Brincat MP, Galea R, Calleja N. Intervertebral disc height in treated and untreated overweight post-menopausal women. Hum Reprod 2005;20:3566–3570.
55. Brincat MP, Calleja-Agius J, Baron YM. The skin, carotid and intervertebral disc: making the connection! Climacteric 2007;10 Suppl 2:83–87.
56. Muscat Baron Y, Brincat MP, Galea R, Calleja N. Low intervertebral disc height in postmenopausal women with osteoporotic vertebral fractures compared to hormone-treated and untreated postmenopausal women and premenopausal women without fractures. Climacteric 2007;10:314–319.
57. Gambacciani M, Pepe A, Cappagli B, Palmieri E, Genazzani AR. The relative contributions of menopause and aging to postmenopausal reduction in intervertebral disk height. Climacteric 2007;10:298–305.
58. Baron YM, Galea R, Brincat M. Carotid artery wall changes in estrogen-treated and -untreated postmenopausal women. Obstet Gynecol 1998;91: 982–986.
59. Ostberg JE, Storry C, Donald AE, Attar MJ, Halcox JP, Conway GS. A dose-response study of hormone replacement in young hypogonadal women: effects on intima media thickness and metabolism. Clin Endocrinol (Oxf) 2007;66:557–564.
60. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, et al.; Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 2004;291:1701–1712.
61. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, et al.; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288: 321–333.
62. Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. The Women's Health Initiative recruitment methods and results. Ann Epidemiol 2003;13:S18–S77.
63. MacLennan AH. HRT: a reappraisal of the risks and benefits. Med J Aust 2007;186:643–646.