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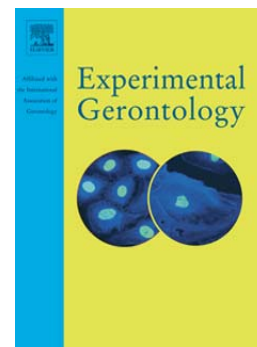
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## Skin and brain age together: The role of hormones in the ageing process

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**ABSTRACT**

The importance of the endocrine environment in the initiation of the ageing process has been elucidated in several in vivo and in vitro studies. Changes in endocrine pathways accompany healthy ageing, these include the growth hormone /insulin like growth factor-I axis (somatopause) and that of sexual hormones, namely estradiol (menopause), testosterone (andropause), and dehydroepiandrosterone and its sulphate (adrenopause). The clinical significance of these changes is variable and results in morphological and functional alterations of all organ systems including the skin and the central nervous system. Moreover, the pathogenesis of age-associated diseases such as epithelial skin cancer and neurodegenerative diseases has been partly attributed to the lack of hormones. Several studies have been conducted in an attempt to reverse the ageing process and clinical signs by substitution of the serum hormone levels in older individuals, however the benefits of hormone replacement therapy, if any, are still controversial. On the other hand, recent data suggest that skin is a window to the human organism and represents an adequate model for ageing research, also implying the use of skin samples for evaluating the ageing status of the central nervous system.

## 1. Introduction

Despite the progress in elucidating the genetic basis of longevity and ageing in many model organisms ranging from yeast and nematodes to mice, a large-scale, systematic approach for the exploration of ageing in humans is still at its infancy. So far, a variation in the gene apolipoprotein E (*APOE*) (Blanche et al., 2001; Christensen et al., 2006) and forkhead box O3A gene (*FOXO3A*) have been found to be consistently associated with longevity in various populations (Flachsbarth et al., 2009; Willcox et al., 2008). Recently, stress response genes, particularly the heat shock protein HSP70 genes which are present within the MHC-III region on the short arm of chromosome 6 (Singh et al., 2007) and a functional EXO1 promoter variant have gained a lot of attention and have been shown to be significantly associated with human longevity and survival (Nebel et al., 2009).

With healthy ageing there are changes in endocrine pathways, including the growth hormone (GH) / insulin like growth factor-I (IGF-I) axis (somatopause) and that of sexual hormones, namely estradiol (menopause), testosterone (andropause), and dehydroepiandrosterone (DHEA) and its sulphate (DHEA-S) (adrenopause). GH is produced by somatotroph cells in the anterior pituitary. GH secretion is pulsatile, principally under the stimulatory influence of the hypothalamus via GH-releasing hormone, and is inhibited by somatostatin also produced in hypothalamus. GH secretion is relatively stable during childhood, increases during adolescence and decreases gradually during adulthood. This age-related decline in GH secretion involves a number of changes in the GH axis, including decreased serum levels of IGF-I and decreased secretion of GH-releasing hormone from the hypothalamus. The cause of the normal age-related decrease in GH secretion is not well understood, but is thought to result in part from increased secretion of somatostatin, the GH-inhibiting hormone, or from an age-related decrease in the number and size of somatotrophs. Moreover, the GH response to GH-releasing hormone is attenuated with advancing age (Corpas et al., 1993).

IGF-I is produced primarily by hepatocytes, but also by many other cells including chondrocytes, osteoblasts, epithelial breast cells, granulosa cells, melanocytes and dermal fibroblasts (Adashi et al., 1985; D'Ercole et al., 1984; Han et al., 1987; Tavakkol et al., 1992), where it may act in an autocrine or paracrine manner. Serum levels of IGF-I have been reported to increase from birth to puberty, followed by a slow decline through adulthood. This reduction is correlated with the progressive decline of GH with advancing age (Bennett et al., 1984)

Steroidogenic organs include the adrenal cortex, the gonads and the placenta. All these tissues use cholesterol as the precursor for the formation of characteristic steroid hormones. Cholesterol synthesis and steroid hormone synthesis from cholesterol has been confirmed that also occur in the epidermis and the sebaceous glands (Menon et al., 1985; Smythe et al., 1998). Sex steroid secretion is under the stimulatory influence of luteinizing hormone (LH) and follicle stimulating hormone (FSH), both derived from the pituitary and regulated by a decapeptide, the gonadotropin-releasing hormone (GnRH), synthesised in the hypothalamus.

DHEA and DHEA-S are the most abundant steroids in the human plasma having serum concentrations in the order of  $10^{-8}$  and  $10^{-6}$  M, respectively. In adult men and women, serum DHEA-S levels are 100 to 500 times higher than that of testosterone and are 1,000 to 10,000 times higher than that of estradiol (Labrie et al., 1998). The DHEA /DHEA-S concentrations in serum reach a peak between the ages of 25 and 30 years and thereafter decline steadily, so that by the age of 60, serum concentrations are only 5-10% of corresponding values in young adults (Orentreich et al., 1984). This decline in women appears to be primarily a function of age and seems to be unrelated to menopause status (Burger et al., 2000).

In males, over 95% of the testosterone is secreted by the testicular Leydig cells; the remainder is derived from the adrenals. In addition to testosterone, the testes secrete small amounts of the potent androgen  $5\alpha$ -dihydrotestosterone ( $5\alpha$ -DHT). In females, small amounts of testosterone are also produced by the ovaries and are released into the circulation as precursors of estrogen synthesis as well as to act on peripheral tissues. The secretion of testosterone displays a diurnal rhythm (Faiman and Winter, 1971). Serum testosterone levels in men decrease with age (Gray et al., 1991; Harman et al., 2001). It has been shown that 20% of healthy men in their sixties and 30% of men in their seventies have lower testosterone levels than 97.5% of healthy 20- to 45-year old men (Harman et al., 2001). Feldman *et al.* found that total testosterone levels decreased by 0.8% per year while bioavailable testosterone fell by 2% per year and sex hormone binding globulin levels increased by 1.6% per year (Feldman et al., 2002). These changes are due in part to a reduction in the number of Leydig cells in the testes that produce testosterone.

The ovary is normally the major source of estrogens, although the conversion of androgen precursors to estrogens in other tissues such as skin is clinically important after the menopause. In males, small quantities of estrogens such as estradiol, estrone and progesterone are produced by the Leydig cells of the testes. However, estradiol is not only derived by direct

secretion from the testes but also by conversion in peripheral tissues of estrogen precursors secreted both by the testes and the adrenals. Thus, about 80% of the circulating concentrations of estradiol is derived from peripheral conversion. In women, estrogen levels decline rapidly at menopause as a result of the loss of ovarian follicles (Smyth et al., 1994), whereas in men the levels of estrogens remain unchanged.

The effects of the observed hormone decline are gradually reflected on our skin, however internal organs such as the central nervous system (CNS) are also affected by the changing endocrine conditions (Bishop and Simpkins, 1995; Fernandez-Tresguerres Hernandez, 2004; Hollis et al., 2009; Lustig, 1994). Dementia – a hallmark of the ageing CNS has been partially attributed in several studies to the lack of circulating hormones. It is estimated that worldwide 24 million people suffer from dementia. By 2040, this number is projected to increase to 81 million people (Ferri et al., 2005). The most frequent cause of dementia is Alzheimer's disease (AD) (more than 60%) (Qiu et al., 2007). Associated with these numbers is not only human suffering but also enormous costs which go towards the maintenance of a certain level of life quality and the treatment of comorbidities.

This review article attempts to delineate some aspects of the interaction between the regulation of endocrine function and the ageing process and explores the age-related changes in hormone metabolism and production, with their clinical consequences on the physiology of two tissues with common embryology, the skin and the CNS. The pathogenesis of age-associated disorders such as skin and neurodegenerative diseases, particularly with a focus on AD, and their connection with hormones will be discussed. In addition, evidence will be provided that skin may be used as a valuable *in vitro* model for studies on AD and for development of potential therapies.

## **2. Skin ageing**

Regarding the pathogenesis of aged skin, several theories have been proposed including the theory of cellular senescence (Dimri et al., 1995; Gilchrest, 1983; Hayflick, 1965; Schneider and Mitsui, 1976; Tresini et al., 2007), decrease in cellular DNA repair capacity and loss of telomeres (Allsopp et al., 1992; Bodnar et al., 1998; Kosmadaki and Gilchrest, 2004; Smith and Pereira-Smith, 1996), point mutations of extranuclear mtDNA (Michikawa et al., 1999), oxidative stress (Miquel, 1998), increased frequency of chromosomal abnormalities (Benn, 1976; Ly et al., 2000), single gene mutations

(Makrantonaki and Zouboulis, 2007a) and hormones (Makrantonaki et al., 2008; Makrantonaki and Zouboulis, 2009; Zouboulis et al., 2007; Zouboulis Ch, 2003).

In contrast to extrinsic skin ageing, which is an organ specific event influenced by environmental factors [e.g. ultraviolet (UV)/infrared radiation] mostly affecting sun-exposed areas of the body, such as face, neck, arms and hands, intrinsic skin ageing is influenced by the individual genetic predisposition and affects both exposed and non-exposed areas of the body (e.g. inner side of the upper arm). Intrinsically aged skin is characterised by smoothness, dryness, pale color and fine wrinkling (Gilchrest, 1982), whereas extrinsically aged skin shows specific histological changes and is clinically characterised by deep wrinkles and multiple pigmentary changes [reviewed in (Makrantonaki and Zouboulis, 2007c)].

During the last decade, the rapid development of genomics, proteomics, metabolomics and bioinformatics has enabled a comprehensive assessment of skin ageing at the fundamental levels of gene and protein expression as well as their downstream metabolic processes has helped us to identify important biomarkers of skin ageing. Gene expression patterns compatible with mitotic dysregulation and alterations in intracellular transport and metabolism were identified in fibroblasts of ageing humans and afflicted with progeria (Ly et al., 2000). Lener *et al.* investigated the gene expression profiles of young and aged human foreskin via cDNA microarrays. The mechanisms proposed to be involved in the induction of skin ageing comprise disturbed lipid metabolism, altered insulin and STAT3 signalling, upregulation of apoptotic genes partially due to the dysregulation of FOXO1, downregulation of members of the jun and fos family, differential expression of cytoskeletal proteins (e.g., keratin 2A, 6A, and 16A), extracellular matrix components (e.g., PI3, S100A2, A7, A9, SPRR2B), and proteins involved in cell-cycle control (e.g., CDKs, GOS2) (Lener et al., 2006). Gene expression profiling was also examined in sun-protected (buttocks) and sun-exposed skin (extensor forearm) from 10 young (age 19 to 20 years) and 10 aged women (age 63 to 67 years) in order to examine gene expression profiles associated with chronological skin ageing and photoageing. Both chronologic ageing and photoageing were associated with downregulation of the biological process of lipid synthesis. In particular, genes were downregulated involved in the cholesterol and fatty acid synthesis. A global reduction in skin surface lipids and a profound abnormality in cholesterol synthesis have been already described in aged skin and correspond to the above results (Elias and Ghadially, 2002). In addition, genes associated with epidermal differentiation, including keratin filaments and cornified envelop components were downregulated. An upregulation of the biological processes of inflammatory response and wound healing, the molecular functions of cytokine



activity and protease activity and the cellular component theme of extracellular matrix was also observed in both skin ageing types. The elastin gene expression was upregulated with ageing only in the photodamaged arm and remained unchanged in the sun-protected buttock. This finding corresponds to the histopathological findings which show typical elastotic changes the so called 'solar elastosis' in photoaged skin (Robinson et al., 2009).

Advanced age is associated with a hyper-inflammatory state, referred to as inflamm-ageing (Franceschi et al., 2000). The immune system of aged animals and humans undergoes alterations that may account for an increased susceptibility to certain infections, autoimmune diseases, and malignancies (Linton and Dorshkind, 2004; Plackett et al., 2004; Plowden et al., 2004). The number or function of T cells and B cells deteriorate (Effros, 2001; Gardner and Murasko, 2002; Grubeck-Loebenstein and Wick, 2002) while the effects of age on innate immune cells, such as macrophages have been widely investigated (Plackett et al., 2004). In human skin, cytokine dysregulation, particularly of the interleukin (IL)-1 family has been described to contribute to ageing (Ye et al., 2002) and disturbed skin barrier function. The microinflammatory model of skin ageing postulates that every stimulus able to induce the synthesis of intercellular adhesion molecule-1 (ICAM-1) in the endothelium is a factor of skin ageing. ICAM-1 favours the diapedesis and the recruitment in the dermis of circulating immune cells, which in turn release reactive oxygen species and proteolytic enzymes which digest structural fibers and provoke damages to resident cells (Giacomoni and Rein, 2004; Goyarts et al., 2007).

## **2.1 Effects of hormones on skin ageing**

Ageing is associated with physiological changes of the endocrine glands as a result of programmed cell death, autoimmune-mediated destruction of the gland or neoplastic transformation of glandular tissue. Consequently, hormone secretion is reduced and secondary physiological changes in circadian and seasonal rhythms or in the frequency or the height of hormone pulses are observed (Mooradian, 1993b). In a recent study, the age-related decline of progenitor cell activity of mice could be reversed by exposure to young serum and the cells could retain much of their intrinsic proliferative potential even when they become old, underlining the great importance of the systemic environment (Conboy et al., 2005). Hence, the importance of the endocrine system and the circulating hormones has currently begun to gain more attention.

### 2.1.1 Sex steroids

Among the multiple functions of the skin, synthesis of sex steroids has been elucidated in several studies *in vitro*. The production of sex steroids depends upon the expression of the androgen- and estrogen-synthesising enzymes in each cell type, with sebaceous glands and sweat glands being the major contributors. Skin has the ability to use cholesterol for the formation of epidermal barrier and, in extreme hormone insufficiency, also sebum. One of the key enzymes, cytochrome P450c17, which is necessary for synthesis of dehydroepiandrosterone and androstenedione, has been documented to be expressed in human sebocytes. These androgenic pro-hormones can be further converted by sebocytes and sweat glands, and probably also by dermal papilla hair cells, into more potent androgens, such as testosterone and 5 $\alpha$ -DHT (Makrantonaki and Zouboulis, 2009; Zouboulis et al., 2007). In addition, the enzyme aromatase may convert testosterone and androstenedione to estrogens in sebaceous glands, outer as well as inner root sheath cells of anagen terminal hair follicles, and dermal papilla cells (Fritsch et al., 2001; Thornton et al., 2006).

During the decline of the circulating hormones with age, the skin partially overtakes the function of the gonads, regarding the synthesis of sex hormones, however, for a definite period of time only. The imbalances between androgen and estrogen levels that occur may be responsible for the observed skin alterations and indirectly for several age-associated diseases (Zouboulis, 2000) [Figure 1]. The typical skin phenotype accompanying menopause has been attributed to hypoestrogenism in several studies. Brincaat *et al.* demonstrated a significant decrease in skin thickness and amount of dermal collagen in the initial postmenopausal years and this reduction correlated to a reduction in bone mineral density (Brincaat, 2000). This observation has been confirmed in further studies showing that collagen content in the dermis and especially the production of type I and III collagen as well as the type III/I ratio decrease with age (Affinito et al., 1999). In addition, postmenopausal women not receiving replacement therapy are more likely to experience dry skin and develop wrinkles in comparison with those under estrogen substitution (Dunn et al., 1997). Skin elasticity is also decreased and changes in the number and morphology of elastic fibers have been observed in women after menopause (Punnonen et al., 1987; Sumino et al., 2004).

In an animal study, the skin phenotypes of aromatase-knockout hairless (ArKO) mice and wild-type hairless (WT) mice were documented and they were compared with those of ovariectomised (OVX) and control (Sham) mice. ArKO and OVX mice similarly exhibited a deterioration of skin properties as compared to their respective controls illustrating the importance of estrogens for maintaining skin homeostasis. This deterioration was amplified

by chronic UVB irradiation. Furthermore, while the skin of ArKO mice showed no reaction after an acute UVB irradiation at dose intensities which could cause sunburn, a follow-up observation revealed delayed reactions associated with brownish skin colour and swelling only in this type of mice. This finding demonstrates the role of extragonadal estrogens in the protection of skin (Tsukahara et al., 2008).

In *in vivo* studies, topical or oral application of estrogens have been correlated with beneficial effects on skin parameters in postmenopausal women. Amongst them improvement of skin dryness, wrinkles and increase of epidermal thickness have been described (Schmidt, 2005). Interestingly, local  $17\beta$ -estradiol can only induce procollagen I and III and collagen I in light-protected but not in light exposed skin (Rittie et al., 2008). In *in vitro* experiments,  $17\beta$ -estradiol can significantly induce the proliferation of human dermal fibroblasts (Makrantonaki et al., 2008) and the production of collagen and this effect has been attributed to regulation of transforming growth factor (TGF)- $\beta$  (Ashcroft et al., 1997; Chau et al., 1998). Furthermore, estradiol induces the proliferation and DNA synthesis of keratinocytes. Apart from the known estrogen receptors (ER)  $\alpha$  and  $\beta$ , new described estrogen-binding entities, e.g. in the plasma membrane or the endoplasmic reticulum may contribute to this action (Kanda and Watanabe, 2005). Even the existence of a third ER, ER $\gamma$ , is being discussed (Hawkins et al., 2000). Recently, we demonstrated that fibroblasts are more susceptible to  $17\beta$ -estradiol in contrast to human sebocytes and the observed effects of estrogens *in vivo* may be implemented through an interaction with the IGF-I signalling pathway (Makrantonaki et al., 2008) in human skin cells.

Furthermore, estrogens have been shown to act as anti-inflammatory agents. Reduced levels of estrogens observed in postmenopausal women have been associated with disturbed effects on tissue regeneration through impaired cytokine transduction, altered protein balance and uncontrolled inflammation. In *in vitro* studies estrogens downregulate the production of the neutrophil, type 1 T cell and macrophage-attracting chemokines, CXCL8, CXCL10, CCL5, by keratinocytes, and suppresses IL-12, TNF $\alpha$  production and antigen-presenting capacity while enhancing anti-inflammatory IL-10 production by dendritic cells reviewed in (Kanda and Watanabe, 2005). In ovariectomised young female rodents, topical application of estrogens on skin can significantly accelerate the wound healing process and is accompanied by an activation of the TGF $\beta$  signalling pathway (Ashcroft et al., 1997). Estrogens inhibit the expression of the macrophage migration inhibitory factor influencing the initiated local inflammation (Ashcroft and Ashworth, 2003) and they preferentially induce dermal cellular expression of ER $\beta$  within the wound indicating the importance of ER $\beta$  in wound healing

(Gilliver and Ashcroft, 2007). Systemic treatment of 10-week old ovariectomised C57/Bl6 mice and of 10-week C57/Bl6 mice with intact ovaries with selective estrogen receptor modulators (SERMs), e.g. tamoxifen or raloxifene, after injury may result in a statistically significant acceleration of healing, reduced wound areas, and accelerated reepithelialisation (Hardman et al., 2007). Both SERMs inhibit wound expression of the pro-inflammatory cytokines MIF, IL6 and TNF in wounds from ovariectomised mice, indicating anti-inflammatory activity. In healthy elderly men and women topical estrogens significantly induce the expression of fibronectin on cutaneous ulcerations and decrease elastase levels secondary to reduced neutrophil numbers resulting in a rapid progress of wound healing. In contrast, male sex hormones especially endogenous testosterone inhibit skin wound healing response in males (Gilliver et al., 2007). Smad3 plays a crucial role in mediating androgen signalling (Ashcroft et al., 2003) and is associated with an enhanced inflammatory response. It is not by coincidence that elderly males show a slower healing process in comparison with their age-matched female counterparts (Makrantonaki and Zouboulis, 2009).

Dehydroepiandrosterone (DHEA) seems to play a key role in the synthesis of extracellular matrix and its decline with age may lead to a decrease in procollagen synthesis and enhancement of collagen degradation by increasing matrix metalloproteinases (MMP)-1 and collagenase synthesis whilst decreasing tissue inhibitor of matrix metalloproteinase (TIMP)-1 and stromelysin-1 production (Lee et al., 2000; Shin et al., 2005). In a double-blind, placebo-controlled study conducted with 280 elderly healthy individuals, 50 mg DHEA was given orally daily over one year. Apart from several benefits described, e.g. in bone turnover and increase in libido parameters, the skin status was significantly improved particularly in women, in terms of hydration, epidermal thickness, sebum production, and pigmentation (Baulieu et al., 2000). However, this study did not include an effective long-term evaluation.

Androgens are a major stimulus of sebaceous gland differentiation and sebum production, while estrogens directly suppress human sebum secretion (Guy et al., 1996; Pochi et al., 1979). However, despite clinical evidence that androgens stimulate sebaceous lipid synthesis, experiments in vitro could not confirm a direct androgen effect on sebocyte lipogenesis. Current studies could elucidate this discrepancy: the lipogenic effect of testosterone in vivo is regulated by co-factors such as peroxisome-proliferator activated receptor (PPAR) ligands. PPARs regulate multiple lipid metabolic genes in mitochondria, peroxisomes and microsomes, all prominent in the cytoplasm of sebocytes (Chen et al., 2003; Rosenfield et al., 1998). In human sebocytes the combination of testosterone and the natural PPAR- $\alpha/\delta$  ligand linoleic acid, an essential fatty acid, exhibits a synergistic effect on lipid synthesis and

5 $\alpha$ -DHT levels in cultured sebocytes. Furthermore, the 5 $\alpha$ -reductase type-I inhibitor LY191704 may only unfold its action under concurrent cell exposure to both compounds. These data suggest a catalytic PPAR effect on cellular testosterone activation and discover a new mode of androgen involvement in sebum production (Makrantonaki and Zouboulis, 2007b). Further studies are needed to elucidate whether the expression of PPARs is altered in advanced age.

Hormone replacement therapy has been correlated with enhancement of collagen production, increase in skin thickness, surface lipids, hydration, vascularisation and increase of the hair follicle life cycle (Brincaat, 2000; Callens et al., 1996; Fuchs et al., 2003; Patriarca et al., 2007; Sator et al., 2007; Sator et al., 2001) leading to an improvement of skin tone and appearance. Hormone replacement may only improve the skin quality in female patients treated during the first 24 months after menopause (Phillips et al., 2008) (Table I).

However, further investigations are warranted to determine the appropriate timing of treatment and the effective concentrations of estrogens that can be used without the reported systemic risks (Beral, 2003): In a study consisting of two parallel randomised, double-blind, placebo-controlled clinical trials of hormone therapy to determine whether conjugated equine estrogen alone or in combination with progestin would have any beneficial effects, increased risks of breast cancer, coronary heart disease, stroke and pulmonary embolism were documented in the estrogen / progestin group (Rossouw et al., 2002).

### **2.2.2 Growth factors**

The GH / IGF-I axis is considered to be one of the most important signalling pathways associated with ageing. Its relevance to the skin can be observed in patients with isolated GH deficiency, multiple pituitary hormone deficiency including GH, as well as primary IGF-I deficiency (GH resistance, Laron syndrome) which present signs of early skin ageing, such as dry, thin and wrinkled skin. Other resulting characteristics of GH / IGF-I deficiency are obesity, hyperglycemia, reduced body lean mass, osteopenia, lowered venous access, hypercholesterolemia, cardiovascular diseases and, subsequently, premature mortality (Carroll et al., 1998; Laron, 2005; Tomlinson et al., 2001; Zouboulis et al., 2007) (Table II). These features can be reversed by long-term hormone replacement therapy with recombinant human GH (Carroll et al., 1998). This has led to the suggestion that the elderly with low levels of GH would benefit from GH treatment.

On the other hand, the increased serum GH levels in acromegaly has been associated with enhanced sebum secretion (Burton et al., 1972) an observation that could be confirmed by GH

treatment of human SZ95 sebocytes in vitro (Makrantonaki et al., 2008). In Mini rats, suppression of GH gene expression by an antisense transgene resulted in thinner skin with less collagen, increase of subcutaneous adipose tissue and small-sized sebaceous glands (Ikawa et al., 2002).

Moreover, IGF-I has been shown to stimulate sebocyte differentiation in vitro especially in combination with GH (Deplewski and Rosenfield, 1999), while in human keratinocytes it acts as a mitogen (Tavakkol et al., 1999). In acne vulgaris, increased sebum production peaks in mid-adolescence at a time that GH and IGF-I reach their highest serum levels (Deplewski and Rosenfield, 1999). IGF-I amplified lipid synthesis in SZ95 sebocytes and fibroblasts as well as fibroblast proliferation, all in a dose-dependent manner underlining the fact that the declining IGF-I levels with increasing age may play a significant role in the reduction of skin surface lipids and thickness (Makrantonaki et al., 2008). Furthermore, it has been shown that there is diminished IGF-I expression in aged skin and this correlates with an inappropriate UVB response in elderly volunteers. The appropriate UVB response can be restored in aged skin in vivo through pre-treatment with exogenous IGF-I. These results imply that reduced expression of IGF-I in aged skin could be an important component in the development of aging-related non-melanoma skin cancer (Lewis et al., 2009).

Treatment of normal elderly males with GH has been documented to ameliorate and reverse the ageing signs and symptoms. After 6 months treatment of recombinant GH in 12 healthy 61- to 81-year-old men who exhibited serum IGF-I concentrations below those of young men resulted in increased lean body mass, decreased adipose-tissue mass, increased average lumbar vertebral bone density and increased skin thickness (Rudman et al., 1990). However, the study was not double-blind and lacked assessment of muscle strength, exercise endurance or quality of life (Vance, 2003). In addition, the observed increase in lean body mass and skin thickness may be due to increased body water (Marcus and Hoffman, 1998). On the other hand, GH supplementation did not result in the improvement of functional parameters (Papadakis et al., 1996). Co-administration of low dose GH with testosterone resulted in more beneficial changes amongst them increased lean body mass, midthigh muscle, muscle strength, aerobic capacity and decreased body fat (Giannoulis et al., 2006). Recent reports of an association of GH substitution with increased risk of prostate, lung, colon and breast cancer as well as a possible improvement of insulin insensitivity make further investigations necessary regarding safety and efficacy of GH substitution in the ageing population (Riedl et al., 2001).

Injectable recombinant human IGF-I (mecasermin) has been available for nearly 20 years for treatment of the rare instances of GH insensitivity caused by GH receptor defects or GH-inhibiting antibodies. Other applications that have been considered or are under investigation are based on the beneficial effects of IGF-I on burns, wound healing, Werner syndrome, Alzheimer's disease (Carro et al., 2006), bone mass, muscular dystrophy (Boonen et al., 2002), amyotrophic lateral sclerosis, spinal cord injury (Hollis et al., 2009), cardiovascular protection and diabetes (Rosenbloom, 2009). However, side effects such as hypoglycemia, hyperplasia of lymphoid tissue accumulation of body fat and coarsening of the face have been described after IGF-I application (Chernausk et al., 2007; Guevara-Aguirre et al., 1997; Guevara-Aguirre et al., 1995). In addition, the anti-apoptotic effects of IGF-I are also a concern for long-term therapy as it may result in cancer induction (Samani et al., 2007).

Recently, clinical studies have documented that sex-steroid hormones can regulate GH and IGF-I secretion. Supplementation of middle-aged and older men with high-dose testosterone attenuated IGF-I feedback-dependent inhibition of GH secretion (Veldhuis et al., 2004), whereas acute administration of estradiol accentuated IGF-I-induced inhibition of GH secretion (Veldhuis et al., 2004). The reduction of circulating sex steroids, which occurs with advancing age, can therefore lead to a defective regulation of both growth factors.

### **3. CNS and ageing - Cerebral and molecular changes in neurodegenerative diseases**

AD is a progressive neurodegenerative disorder with gradual onset and deterioration of cognitive functions such as memory, language and visio-spatial skills (Schonknecht et al., 2005). The clinical diagnosis of AD necessitates exclusion of other forms of dementia such as vascular dementia or dementia due to cerebral neoplasm, inflammation or other pathologies. Histopathologically, AD is associated with neurofibrillary tangle formation and deposition of amyloid plaques in susceptible brain regions. Tau protein, a microtubule-associated protein, seems to be released into the cerebrospinal fluid (CSF) during neurofibrillary tangle formation and has been found to be increased in patients with manifest AD (Schonknecht et al., 2003) as well as in mild cognitive impairment or incipient AD (Schonknecht et al., 2007).

A major feature of AD is the presence of amyloid plaques in the brain. Amyloid plaques are extracellular deposits of fibrillar aggregates mostly composed of a 4 kDa peptide,  $\beta$ -amyloid 1-42 (A $\beta$ 42), which is derived along with the peptide  $\beta$ -amyloid 1-40 (A $\beta$ 40) from the larger amyloid precursor protein (APP) by proteolytic cleavage (Jensen et al., 1999). Several studies have indicated that the deposition of A $\beta$ 42 constitutes an important process in

the aetiology of neuronal degeneration, and CSF A $\beta$ 42 concentrations have been shown to be a sensitive marker of AD pathology (Ida et al., 1996).

Neuroimaging studies have demonstrated that cerebral changes characteristic of AD primarily strike the temporal lobe with a particular focus on medial temporal substructures. These cerebral changes may be reliably assessed using quantitative magnetic resonance imaging (Pantel et al., 1999).

Several studies have indicated that both genetically determined disturbances in APP metabolism with consecutive overexpression of the protein and a pathological enzymatic processing of APP to A $\beta$  are essentially involved in the production of amyloid plaques. The A $\beta$  release, however, has been proposed to be modified by several co-factors such as metals (copper, zinc), apolipoprotein E, cholesterol and oestrogens.

In order to identify age-associated changes of the cholesterol metabolism, healthy elderly were compared with patients with AD with respect to serum and CSF levels of cholesterol and its major metabolites (Schonknecht et al., 2002). Experiments in cell cultures indicated earlier, that accumulation of cholesterol in hippocampal neurons results in an accelerated cleavage of amyloid precursor protein into amyloidogenic components which leads to formation of amyloid plaques in susceptible brain regions of AD patients. Cholesterol is converted to 24S-hydroxycholesterol by cholesterol 24S-hydroxylase prior to be eliminated from the brain. Since plasma 24S-hydroxycholesterol levels are strongly influenced by hepatic clearance it was hypothesized that only CSF 24S-hydroxycholesterol may be an appropriate indicator of cholesterol turnover of the brain than its plasma concentration, differentiating it from age-associated changes. Since epidemiological evidence indicated that serum cholesterol levels are altered in AD and in studies on 24S-hydroxycholesterol this presents a potential bias, CSF 24S-hydroxycholesterol levels in AD patients and controls were investigated in subjects with plasma cholesterol levels in a normal range (Schonknecht et al., 2002). CSF and plasma samples were obtained from twenty-five patients with probable AD as part of the routine diagnostic procedure and nineteen patients without cognitive deficits nor psychiatric or neurological disorders who received lumbar anesthesia for surgery. 14 samples from AD patients and 10 from controls with plasma cholesterol levels in the normal range between 150 – 230 mg/dl were selected. CSF samples were drawn in the morning before noon, immediately aliquoted and frozen at  $-80^{\circ}\text{C}$ . Light microscopy was performed to exclude any probes contaminated with erythrocytes. CSF and plasma 24S-hydroxycholesterol and CSF cholesterol levels were measured using combined gas chromatography and mass spectrometry. In the AD patients, CSF 24S-hydroxycholesterol - but not plasma levels - were



significantly increased compared to the controls. Neither total CSF cholesterol nor total plasma cholesterol levels differed significantly between groups. Repeated analyses with BMI entered as a covariate revealed consistent results. Within the AD group, no significant correlation between 24S-hydroxycholesterol CSF levels and age, age of onset, severity of dementia, BMI nor plasma cholesterol nor significant differences with respect to medication status arose. To address potential effects of the ApoE genotype, a two-way analysis of variance with diagnosis and ApoE genotype as main factors was calculated. Again, a significant effect of diagnosis with significantly increased CSF 24S-hydroxycholesterol values in the AD patients emerged. Based on these findings it may be summarized that an increase of CSF 24S-hydroxycholesterol levels is rather due to AD pathology and that this effect is not triggered by plasma cholesterol which may be normal in the patients as in the healthy elderly. However, a significant correlation between CSF tau protein concentrations and CSF 24S-hydroxycholesterol levels did not arise indicating that changes in the cholesterol metabolism in AD may not fully parallel AD pathology.

As in skin ageing, inflammatory processes have been also discussed in the pathophysiology of AD (Tan and Seshadri, 2010). This has recently been investigated in a study by van Exel et al. (2009) who used known risk factors of AD to identify high- and low-risk samples in whom they compared levels of inflammatory risk factors (van Exel et al., 2009). In contrast to blood-borne ions, molecules, and cells, only peripheral blood monocytes and macrophages may actively cross the blood-brain barrier and hence better reflect CNS inflammation. This might explain why large population-based studies failed to relate circulating levels of inflammatory markers such as CRP to risk of AD. Nevertheless, the Framingham Study reported an association between increased cytokines (IL-1 and tumor necrosis factor- $\alpha$ ) in peripheral blood monocytes and an approximately twofold increase in risk of incident AD (Tan et al., 2007). This finding is in accordance with van Exel et al. (2009) who demonstrated that middle-aged persons with a parental history of late onset AD show adverse levels of vascular risk factors such as higher blood pressures and a lower ankle-brachial index, as well as an increase of proinflammatory cytokines in lipopolysaccharide-stimulated whole blood samples when compared with middle-aged subjects without a parental history of AD. Interestingly, this association was independent of the APOE genotype.

The mechanisms how cytokine dysregulation may injure neurons include an altered neurotransmission, apoptosis, and activation of microglia and astrocytes, followed by an increase of free radicals, complement factors, glutamate, or nitric oxide (Wilson et al., 2002). This has been emphasized by postmortem studies of AD brains which demonstrated CRP,

proinflammatory cytokines, and activated complement cascade proteins in plaques and neurofibrillary tangles (Duong et al., 1997; Iwamoto et al., 1994). In addition to  $\beta$ -amyloid, IL-1 has been demonstrated to increase neuronal tau phosphorylation (Li et al., 2003). To-date, it remains unclear whether these proinflammatory cytokines are etiopathologically involved in or merely are a result of the AD cascade. The present findings, however, emphasize, at least, that inflammation may amplify the disease process of AD.

Based on this assumption, therapeutic effects of anti-inflammatory drugs have been expected in AD. However, a recent meta-analysis failed to observe any association of either the serum-amyloid lowering (SALA) or the non-SALA NSAIDs with a lower risk of AD as did randomized placebo-controlled clinical trials (for review see Tan and Seshadri, 2010). Again, the recent finding by van Exel et al. (2009) indicate the possibility of an therapeutic impact of any longer anti-inflammatory intervention than those investigated (van Exel et al., 2009). This is in accordance with the observation that higher blood pressures may act together with increased inflammation to enhance the risk for AD. One may conclude by the present findings that future studies of anti-inflammatory interventions should be undertaken in high risk populations of AD, such as positive family history, at least one APOE  $\epsilon$ 4 allele, or progressive MCI (Schonknecht et al., 2005).

### **3.1 Effects of hormones on CNS and $\beta$ -amyloid metabolism**

Although the fundamental mechanisms are still poorly understood, a growing body of evidence points towards age-related hormone decline as one of the primary determinants of the initiation of AD. Among other potential benefits of long-term hormone replacement therapy such as the prevention of osteoporosis, atherosclerotic cardiovascular diseases and amelioration of the skin phenotype, there has been considerable interest in the potential of estrogens to be protective against the risk of developing neurodegenerative diseases, e.g. AD (Tang et al., 1996; Yaffe et al., 1998) (Table III).

#### **3.1.1 Sex steroids**

Estrogens can readily pass the blood-brain barrier and have several effects on the CNS (Henderson, 1997). They enhance the outgrowth of neurites and promote the formation of dendrite spines and synapses (Lustig, 1994; Woolley and McEwen, 1994). In the brain, estrogens increase cerebral blood flow and glucose metabolism and modulate acetylcholine metabolism (Bishop and Simpkins, 1995; Luine, 1985; Ohkura et al., 1995). Furthermore, the beneficial effect of estrogens on the CNS may be mediated by cholesterol and apo E

metabolism since estrogens have been found to decrease serum cholesterol and apo E levels (Urabe et al., 1996) in postmenopausal women. In addition, oestrogens have been characterised as having an important impact on A $\beta$  formation in the CNS, a hallmark of AD. Experiments in cell cultures revealed that physiological concentrations of 17 $\beta$ -estradiol increases the production of soluble APP at the expense of A $\beta$ 40 and A $\beta$ 42 production. Jaffe *et al.* demonstrated in-vitro that physiological concentrations of 17 $\beta$ -estradiol may modulate APP metabolism by increasing the cellular release of the soluble, non-amyloidogenic components (Jaffe et al., 1994). Xu *et al.* investigated the effect of physiological concentrations of 17 $\beta$ -estradiol on the APP metabolism of neurites and not only found an increase of soluble APP components but also significantly reduced A $\beta$ 42 and A $\beta$ 40 concentrations (Xu et al., 1998).

Moreover, animal studies have shown that prolonged ovariectomy results in uterine atrophy and decreased serum 17 $\beta$ -estradiol levels, and is associated with increased cerebral A $\beta$  levels (Petanceska et al., 2000). In the same study, 17 $\beta$ -estradiol treatment significantly reversed the ovariectomy-induced increase in brain A $\beta$  levels.

The effect of estrogens on A $\beta$  metabolism may be mediated by a morphological modification of the intracellular trans-Golgi compartments where matured APP is processed to A $\beta$ . Furthermore, estrogens support neuronal APP transport and act against the apparent dysregulation which leads to increased A $\beta$  production (Xu et al., 1998). The protective effects of estrogens may also be mediated by interactions not directly related to APP processing such as modulation of neural functioning or prevention of oxidative toxicity due to glutamate, free radicals, and A $\beta$ 40 or A $\beta$ 42 (Mooradian, 1993a). Behl *et al.* showed that 17 $\beta$ -estradiol can prevent intracellular peroxide accumulation and, ultimately, the degeneration of hippocampal neurons (Behl et al., 1997). To date, little is known about potential differential effects of the various estrogens on the CNS. The majority of studies have focused on 17 $\beta$ -estradiol, which is generally considered to be the most potent estrogen. While some studies also addressed estrone, other estrogens, such as estriol or progestagens, such as progesterone have not been studied.

Taken together, data from studies investigating the potential role of estrogens as a beneficial agent for the cause and progression of AD in women appear rather conflicting. The Women's Health Initiative study for instance showed that estrogen therapy alone or in combination with progestin treatment increased the risk of both dementia and mild cognitive impairment (Shumaker et al., 2004). Since basic studies of the mechanisms of neurodegenerative disorders suggest at least two phases in their pathogenesis namely as

initiation and propagation it was hypothesised that estrogens may interact differentially in each phase (Johnson et al., 1998). Dosage form, or doses required and specific type of estrogen may influence hormone substitution treatment effects on initiation and propagation of AD. Nevertheless, the impact of age, early onset and severity of dementia, and of longer-term treatment on the potential beneficial effects of hormone substitution treatment has still to be addressed.

### 3.1.2 Growth factors

It has been speculated that neuropeptides also play an important role in the development of AD due to their action as neuromodulators in the CNS (Guroff, 1993). Among them insulin-like growth factors (IGFs) are produced in the brain and act as trophic factors in the CNS. More specifically, IGF-I and IGF-II are known to play an important role in the regulation, differentiation and proliferation of numerous peripheral and central tissues (Masters and Raizada, 1993). Secretion of IGF-I is mainly regulated by GH but also by other hormones, such as glucocorticoids (Tham et al., 1988).

Interestingly, in AD, neurofibrillary tangles accumulate in hypothalamic regions which are associated with the regulation of pituitary hormones (Thienhaus et al., 1987). A number of authors have proposed that neurocognitive decline might be promoted by a dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis with menopause and andropause (Atwood et al., 2005), since dysregulation of the axis leads to alterations in the concentrations of all serum HPG hormones (decreased neuronal sex steroid signalling, but increased neuronal gonadotropin releasing hormone, luteinizing hormone, and activin signalling).

Mustafa *et al.* determined plasma IGF-I levels in family members carrying the Swedish amyloid precursor protein (APP) 670/671 mutation with or without AD and in age-matched controls from the same family. They only found a significant reduction in the family members with AD compared to age-matched controls. Levels of GH and prolactin did not differ significantly between the mutation carriers with or without AD and their respective age-matched controls (Mullan et al., 1992; Mustafa et al., 1999).

High levels of  $\beta$ -amyloid have been found at an early stage in mutant mice with low circulating IGF-I, and  $\beta$ -amyloid burden can be reduced in ageing rats by increasing serum IGF-I. IGF-I seems to induce clearance of brain  $\beta$ -amyloid, probably by enhancing transport of  $\beta$ -amyloid carrier proteins like albumin and transthyretin into the brain (Carro et al., 2002). Tumour necrosis factor- $\alpha$ , a proinflammatory cytokine possibly related to neurodegeneration

and ageing, can antagonise this effect. In another important study, endocrine, liver-derived IGF-I improved spatial learning and memory functions in old mice (Svensson et al., 2006).

Since clearance of CNS  $\beta$ -amyloid by IGF-I has been shown to be effective in a mouse model, clinical trials in humans seemed promising (Carro and Torres-Aleman, 2004, , 2006). However, application of the GH secretagogue MK-677, which is a potent inducer of IGF-I secretion, did not show a clinical effect on the disease progression in patients with AD in a randomised trial (Sevigny et al., 2008). More and detailed data is warranted from ongoing trials, which plan to finally evaluate the efficacy of IGF-I variants as potential AD modifying agents.

#### **4. Skin and CNS - Is there a connection?**

##### **4.1 Embryology of skin and CNS**

Neural and epidermal tissues are both ectodermal derivatives. In several organisms, like amphibians and more particular in *Xenopus laevis*, enormous progress has been made in understanding the way the choice of neural or epidermal fate is determined. During gastrulation the cells of the embryonic ectoderm give rise to epidermal progenitors in the ventral side and neural progenitors in the dorsal side. In 1924, Spemann and Mangold demonstrated that the dorsal mesoderm is able to induce nervous system from the adjacent ectoderm by secreting a source of inducing signal. Recently, signalling molecules, which participate in this embryonic decision have been identified. Induction of epidermis occurs through a signalling cascade involving Bone Morphogenetic Proteins (BMP) 2, 4, 7 and receptor-regulated Smad proteins, which translocate into the nucleus to form active transcriptional complexes. These molecules mediate BMP activation of epidermal-specific gene expression and repression of neural-specific gene transcription. On the other hand, neural fate is induced by factors secreted by the dorsal mesoderm (e.g. noggin, chordin and follistatin), which act by blocking BMP signalling. In addition, it has been shown that calcium plays a key role and it is required for neural determination [Figure 2] (Moreau and Leclerc, 2004).

##### **4.2 Skin models of ageing as tools for understanding molecular ageing mechanisms of CNS**

Recent data suggest that skin represents an adequate model for ageing research and imply the use of skin samples for evaluating the ageing status of the nervous system. Within the scope of the Explorative Project 'Genetic aetiology of human longevity' supported by the

German National Genome Research Network 2 (NGFN-2) an in vitro model of human hormonal ageing was developed. Human epithelial cells - SZ95 sebocytes - were maintained under a hormone-substituted environment consisting of growth factors and sexual steroids in concentrations corresponding to those circulating in 20- and in 60-year-old women. Expression profiling employing a cDNA microarray composed of 15,529 cDNA sequences identified 899 genes with altered expression levels in SZ95 sebocytes maintained under the 20- and 60-year-old hormone mixture, respectively. The functional annotation of these genes according to the Gene Ontology identified pathways related to mitochondrial function, oxidative stress, ubiquitine-mediated proteolysis, cell cycle, immune responses, steroid biosynthesis and phospholipid degradation - biological processes which are all hallmarks of the ageing process.

According to the KEGG database, the most significantly altered signalling pathway identified was that of the transforming growth factor- $\beta$  (TGF $\beta$ ). The TGF $\beta$  family consists of multifunctional factors, which regulate many cellular processes, such as differentiation and proliferation of keratinocytes and fibroblasts and the synthesis of extracellular matrix proteins (Massague, 1998). The family members include TGF $\beta$  isoforms, activins and BMPs and propagate the signal through binding to cell surface receptors and phosphorylating Smad proteins. A differential expression of TGF $\beta$  isoforms, activins, BMPs, MADHs / SMADs, and other components of the TGF $\beta$  signalling cascade has been documented in the SZ95 sebocytes under hormone-substituted conditions. As already mentioned above, activation of this pathway represses neural-specific gene transcription in ectodermal cells leading them to epidermal differentiation. The question that arises is to what extent hormones may play a role in the decision of epidermal or neural fate.

In addition, key genes operating in neurodegenerative diseases such as AD and Parkinson's disease, Huntington's disease, dentatorubral-pallidoluyisian atrophy and amyotrophic lateral sclerosis (ALS) have been documented to be expressed in the SZ95 sebocytes and their expression was influenced by the hormone treatment. In particular 24 genes involved in the pathogenesis of AD, among them APP and presenilin-1, were regulated under treatment with growth factors and sex steroids in concentrations corresponding to those circulating in 20 and 60-year-old women, respectively. Though not proven, these results might imply that a disturbed hormone status might induce the regulation of these genes and the generation of neurodegenerative diseases (Makrantonaki et al., 2006).

APP which was initially detected in cells of the CNS has been found also in the epidermis where it fulfils a variety of distinct biological roles. It facilitates keratinocyte adhesion due to

its ability to interact with the extracellular matrix, it serves as an adapter protein for binding the motor protein kinesin, thereby mediating the centripetal transport of melanosomes in epidermal melanocytes and the soluble N-terminal portion of APP has been shown to be a potent epidermal growth factor thus stimulating proliferation and migration of keratinocytes as well as the exocytic release of melanin by melanocytes (Herzog et al., 2004). The expression of  $\beta$ -amyloid and tau-protein has been documented in the mast cells of skin in AD patients, which supports the hypothesis that AD is a systemic disease involving different organs and tissues (Kvetnoi et al., 2003).

Cutaneous melanocytes have provided a valuable *in vitro* model for studies of AD and for development of potential therapies. It has been shown that under the presence of  $\beta$ -amyloid, melanocytes undergo apoptosis, like neurons and the nerve growth factor, which has been reported to attenuate the loss of cholinergic neurons in AD, exerts a protective effect (Yaar and Gilchrest, 1997).

In cultured skin fibroblasts derived from sporadic AD patients and from age-matched controls the differences in intracellular free calcium levels ( $[Ca^{2+}]_i$ ) have been investigated. AD cells exhibited lower  $[Ca^{2+}]_i$  as compared to the control cultures. Exposure of fibroblasts to  $\beta$ -amyloid resulted in increased  $[Ca^{2+}]_i$  of the control cells, but not of AD fibroblasts (Palotas et al., 2001). Further studies have shown the presence of inflammatory and immunorelated markers in cultured skin fibroblasts from familial (FAD) and sporadic AD patients (Scali et al., 2002). Additionally, fibroblasts from AD patients were more resistant than those from control subjects to  $H_2O_2$  treatment, although the extent of DNA damage induced by the oxidative injury was similar in both experimental groups. The protective mechanism of AD fibroblasts was related to an impairment of  $H_2O_2$ -induced cell cycle arrest and characterised by an accelerated re-entry into the cell cycle and a diminished induction of apoptosis which may be due to a profound impairment in the  $H_2O_2$ -activated, p53-dependent pathway (Uberti et al., 2002; Uberti et al., 2006).

Compared to controls, the fibroblasts carrying APP and presenilin-1 gene mutations showed a clear increase in lipoperoxidation products, malondialdehyde, and 4-hydroxynonenal. The antioxidant defenses of cells from FAD patients were lower than those from normal subjects. Furthermore, ADP ribosylation levels of poly(ADP-ribose) polymerase (PARP) nuclear substrates were significantly raised, whereas the PARP content did not differ significantly between fibroblasts carrying gene mutations and control cells (Cecchi et al., 2002).

A characteristic feature of AD is the increased Ser phosphorylation of tau microtubule-associated protein in the brain. In skin fibroblasts derived from persons who have or will develop AD due to presenilin 1 mutations or trisomy 21, bradykinin (BK) B2 receptor activation leads to selective Ser phosphorylation of tau. This is not the case in skin fibroblasts from normal individuals at any age. This reflects modification of the G protein-coupled BK B2 receptors themselves suggesting that BK receptor pathway dysfunction may be a molecular signature yielding information about the pathogenesis of AD (Jong et al., 2003). Finally, a disturbed degradation of proteins in lysosomes has been demonstrated not only in CNS but also in peripheral level, in skin fibroblasts from patients affected either by sporadic or familial forms of AD (Urbanelli et al., 2008).

### **Conclusion**

Skin mirrors the first signs of human ageing due to the accompanying hormone decline. Recent studies have revealed that skin can be used as a model for a better understanding of the global pathogenesis of ageing. The fact that skin shares the same embryologic origin with the nervous system makes it an attractive alternative for exploring the pathways involved in the pathogenesis of neurodegenerative diseases, in particular since the collection of specimens from human brain throughout life for experimental research purposes is associated with major practical and ethical obstacles. The skin and neural tissue have also in common strong influences and dependencies on the endocrine environment. Elucidating the molecular mechanisms involved in the initiation of ageing of these two tissues and the magnitude of hormone contribution would offer immense opportunities for the development of future treatments.



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**Table I: Effects of hormone replacement therapy (HRT) in skin**

Hormone treatment	Skin effects	Authors
<ul style="list-style-type: none"> <li>21 men 61 to 81 years old with serum IGF-I concentrations less than 350 U/lit participated in a vehicle-controlled clinical study with ca. 0.03 mg/kg BW recombinant biosynthetic human growth hormone per kilogram subcutaneously three times a week treatment.</li> </ul>	<ul style="list-style-type: none"> <li>After 12 months treatment period skin thickness increased.</li> </ul>	(Rudman et al., 1990)
<ul style="list-style-type: none"> <li>65 patients applied a creme containing 0,01% estradiol or 15% glycolic acid alone or in combination to one side of the face and a vehicle creme to the opposite side.</li> </ul>	<ul style="list-style-type: none"> <li>After 6 months estradiol treatment 23% increase of epidermal skin thickness was documented in contrast to control. The combination of estradiol and glycolic acid treatment resulted in 38% increase of epidermal skin thickness.</li> </ul>	(Fuchs et al., 2003)
<ul style="list-style-type: none"> <li>40 postmenopausal women and 30 age-matched men (mean age of both 75 years) with photodamaged skin underwent estradiol and vehicle extern treatment on light-protected hip or photodamaged forearm skin (0,01%-2,5%, 3 times, every other day).</li> <li>5 males and 5 females were treated with 0,2% estradiol creme 2 times per day on the face.</li> </ul>	<ul style="list-style-type: none"> <li>After 2 weeks treatment induction of procollagen I and III mRNA and collagen I protein only in light-protected hip skin, but not in photoaged forearm and face skin.</li> </ul>	(Rittie et al., 2008)
<ul style="list-style-type: none"> <li>Postmenopausal women (mean age 51.3 years) with no menses for at least 12 months using oral or transdermal estrogen therapy for at least 1 year were treated with topical 0.01% micronized 17<math>\beta</math>-estradiol gel on the facial skin once a day for 16 weeks.</li> </ul>	<ul style="list-style-type: none"> <li>After a 16-week treatment a significant increase in the epithelial and dermal thickness and in the amount of collagen was documented.</li> </ul>	(Patriarca et al., 2007)
<ul style="list-style-type: none"> <li>In a pilot study, the effects of three different HRT regimes</li> </ul>	<ul style="list-style-type: none"> <li>After 6-months hormone treatment</li> </ul>	(Sator et al., 2001)

<p>were compared on different parameters of skin ageing in postmenopausal women (mean age 54.9 years) over a period of 6 months. The patients were assigned to four groups: group 1, estrogen (estradiol) alone (Estraderm TTS® 50); group 2, estrogen (estradiol) transdermally and progesterone (Estraderm TTS® 50 and 0.4 mg progesterone vaginal suppository); group 3, estrogen (estradiol) orally and progesterone (2 mg Progynova® and 0.4 mg progesterone vaginal suppository); and group 4, without therapy, as a control group.</p>	<p>parameters of skin ageing were significantly improved.</p>	
<ul style="list-style-type: none"> <li>• In a prospective, randomized, double-blind, placebo-controlled study forty non-hysterectomised, postmenopausal women received an oral sequential treatment with a combination of 2 mg 17<math>\beta</math>-estradiol/10 mg dydrogesterone (Femoston®) for seven 28-day cycles.</li> </ul>	<ul style="list-style-type: none"> <li>• After 7 months of HRT, skin ageing signs were reversed and skin elasticity, hydration and thickness showed to be significantly increased.</li> </ul>	(Sator et al., 2007)
<ul style="list-style-type: none"> <li>• 17<math>\beta</math>-estradiol treatment at age-specific levels of human fibroblasts in vitro</li> <li>• GH and IGF-I treatment at age-specific levels of human sebocytes in vitro</li> </ul>	<ul style="list-style-type: none"> <li>• Dose-dependent induction of cell proliferation</li> <li>• Dose-dependent induction of lipid synthesis</li> </ul>	(Makrantonaki et al., 2008)

**Table II: Effects of growth factors decline**

- Reduction of skin thickness
- Loss of dermal collagen, wrinkles
- Increase of subcutaneous adipose tissue
- Small-sized sebaceous glands, reduction of sebum production, skin dryness
- Development of ageing-related non-melanoma skin cancer
- Obesity
- Osteopenia
- Decrease of muscle strength
- Lowered venous access
- Cardiovascular diseases
- Hypercholesterolemia
- $\beta$ -amyloid accumulation in animal models
- AD phenotype in patients with Swedish amyloid precursor protein (APP) mutation
- Premature mortality

Table III: Effects of hormone-replacement therapy in CNS

Hormone treatment	CNS effects	Authors
<ul style="list-style-type: none"> <li>Postmenopausal women were treated with ERT (estrogen replacement therapy), which was arranged as follows: day 0 to 21, 0.625 to 1.25 mg/day conjugated estrogen (CE); day 22 to 28, no hormone treatment. 2.5 mg/day medroxyprogesterone acetate (MPA) was also given or not on days 11 to 21 of ERT.</li> <li>Serum samples were measured within 6 days after finished ERT.</li> </ul>	<ul style="list-style-type: none"> <li>Reduction of hepatic triglyceride lipase, total cholesterol, LDL-cholesterol, apolipoprotein B, and especially apolipoprotein E serum levels.</li> <li>Apolipoprotein E serum level were higher in cases with &lt; 20 pg/ml of serum 17 <math>\beta</math>-estradiol</li> <li>No significant effect on post heparin plasma lipases and lipid levels after MPA addition.</li> </ul>	(Urabe et al., 1996)
<ul style="list-style-type: none"> <li>Women's Health Initiative Memory Study (WHIMS): Randomized, double-blind and placebo-controlled clinical trials in 65 to 79 years old postmenopausal women treated with conjugated equine estrogens 0,626 mg/d (CEE) with medroxyprogesterone acetate 2,5 mg/d (MPA) for 7 years or CEE alone for 9 years.</li> </ul>	<ul style="list-style-type: none"> <li>Increased risk of both dementia and mild cognitive impairment</li> </ul>	(Shumaker et al., 2004)
<ul style="list-style-type: none"> <li>In a double-blind, multicenter, placebo-controlled study 563 male and female patients with mild to moderate AD at age of at least 50 years received 25 mg of the growth hormone secretagogue MK-677 (ibutamoren mesylate) for 12 months.</li> </ul>	<ul style="list-style-type: none"> <li>Treatment did not show a significant clinical effect on the AD progression</li> <li>IGF-I serum levels were strongly increased.</li> </ul>	(Sevigny et al., 2008)
<ul style="list-style-type: none"> <li>ZR-75-1 cells (human breast carcinoma cell line) were</li> </ul>	<ul style="list-style-type: none"> <li>Induction of soluble Alzheimer amyloid precursor</li> </ul>	(Jaffe et al., 1994)

<p>preincubated with complete medium containing <math>1,8 \times 10^{-9}</math> M <math>17\beta</math>-estradiol (comparable concentration with that expressed at the peak of the human menstrual cycle).</p> <ul style="list-style-type: none"> <li>• After 9 days the medium was replaced with serum-free medium containing <math>1,8 \times 10^{-9}</math> M <math>17\beta</math>-estradiol and the cells were incubated for additional 40 h.</li> </ul>	<p>protein (sAPP).</p> <ul style="list-style-type: none"> <li>• No significant effect on intracellular immature or mature APP.</li> </ul>	
<ul style="list-style-type: none"> <li>• N2a cells (mouse neuroblastoma cell line) were treated with <math>17\beta</math>-estradiol in various concentrations for 7 days.</li> <li>• <math>17\beta</math>-estradiol treatment in rat and mouse (200nM for 7-10 and 5 days) and human (several concentrations for 7-10 days) embryonic primary neuronal cells.</li> </ul>	<ul style="list-style-type: none"> <li>• Reduction of <math>A\beta</math>-amyloid release (f.e. <math>A\beta</math>1-40 , 1-42 , 11-40)</li> </ul>	(Xu et al., 1998)
<ul style="list-style-type: none"> <li>• Ovariectomised female guinea pigs received soy-free food supplemented with either 1 or 5 mg/1 kg BW/day <math>17\beta</math>-estradiol for 10 days</li> </ul>	<ul style="list-style-type: none"> <li>• Increase of total brain <math>A\beta</math> levels in non treated ovariectomised animals and reversal after <math>17\beta</math>-estradiol treatment.</li> <li>• No significant differences between low and high concentration.</li> </ul>	(Petanceska et al., 2000)
<ul style="list-style-type: none"> <li>• Rat primary hippocampal neurons and mouse clonal hippocampal HT22 cells incubated as follows: Pretreatment for 20 h with <math>10 \mu\text{M}</math> <math>17\beta</math>-estradiol, estriol, or estrone and <math>17\alpha</math>-estradiol and treatment with <math>A\beta_{25-35}</math> (<math>2 \mu\text{M}</math>) for 24h or <math>\text{H}_2\text{O}_2</math> (<math>30 \mu\text{M}</math> or <math>60 \mu\text{M}</math>) and BSO (<math>500 \mu\text{M}</math>)</li> <li>• Mouse primary cortical neurons and HT22 cells were pretreated for 20 h with <math>1 \mu\text{M}</math> and <math>10 \mu\text{M}</math> <math>17\alpha</math>-Estradiol,</li> </ul>	<ul style="list-style-type: none"> <li>• Estrogens and estrogen derivatives with an intact 3-OH group on the A ring elucidated a neuroprotective antioxidant role in an estrogen receptor independent way.</li> </ul>	(Behl et al., 1997)



<p>ethinyl estradiol, quinestrol, mestranol, 2-OH-estradiol and 4-OH-estradiol and then treated with 500 <math>\mu\text{M}</math> BSO and 30 <math>\mu\text{M}</math> <math>\text{H}_2\text{O}_2</math> or 1000 <math>\mu\text{M}</math> glutamate and 60 <math>\mu\text{M}</math> <math>\text{H}_2\text{O}_2</math> respectively.</p> <ul style="list-style-type: none"> <li>• Postnatal rats organotypic hippocampal slice cultures were pretreated with either 10 <math>\mu\text{M}</math> mestranol, quinestrol, or ethinyl estradiol. After 20 h followed a challenge with 250 <math>\mu\text{M}</math> <math>\text{H}_2\text{O}_2</math> for 24h.</li> </ul>		
<ul style="list-style-type: none"> <li>• Aging Wistar rats were chronic subcutaneously injected with IGF-I (50mg/kg).</li> <li>• Liver-specific IGF-I-deficient (LID) mice with decreased serum IGF-I levels of 60%.</li> <li>• Tg2576 mice overexpressing human APP695 were treated with IGF-I for a month.</li> <li>• Biotinylated <math>\text{A}\beta_{1-40}</math> (60nM) was injected in the cerebral cortex of young adult rats two days after pretreated with IGF-I (10 <math>\mu\text{g}</math>/ rat) intracarotid injection.</li> <li>• IGF-I (<math>1 \times 10^{-7}</math> M) was added in rat choroid plexus cultures, which is a doubled chamber, imitates the blood-cerebrospinal fluid interface.</li> </ul>	<ul style="list-style-type: none"> <li>• Reduced <math>\text{A}\beta</math> burdens in the brain tissue after IGF-I treatment.</li> <li>• IGF-I stimulated clearance of brain <math>\text{A}\beta</math> probably via carrier proteins (albumin and transthyretin). TNF-<math>\alpha</math> blockaded this induction.</li> </ul>	(Carro et al., 2002)
<ul style="list-style-type: none"> <li>• Young (6 months old) and old (18 months old) LI-IGF-I <math>^{-/-}</math> mice, with inactivation of the liver-specific, inducible production of IGF-I, via the Cre-LoxP conditional knockout system.</li> </ul>	<ul style="list-style-type: none"> <li>• Decline of about 80% of serum IGF-I levels in LI-IGF-I <math>^{-/-}</math> mice compared to control mice.</li> <li>• Reduction of spatial working and reference memory in old LI-IGF-I <math>^{-/-}</math> mice.</li> <li>• Increased enkephalin and dynorphin</li> </ul>	(Svensson et al., 2006)

	immunoreactivity and decreased mRNA levels in hippocampus, elevated astrogliosis and metabotropic glutamate receptor 7a-immunoreactivity in old LI-IGF-1 <sup>-/-</sup> mice compared to control mice.	
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ACCEPTED MANUSCRIPT

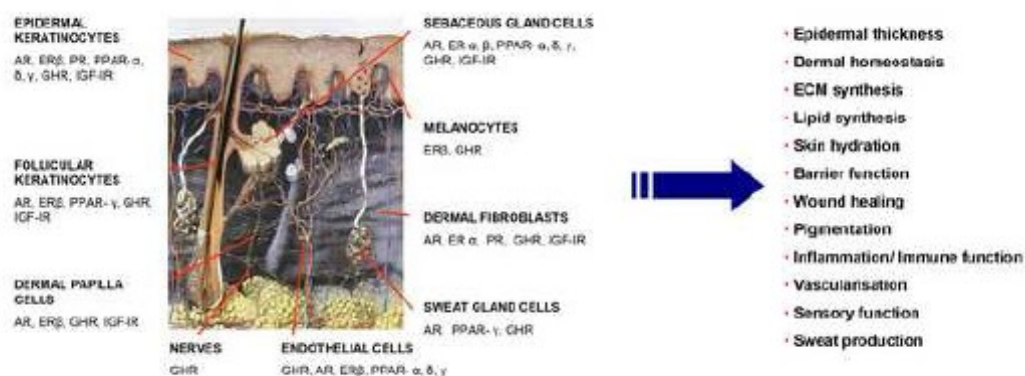
## Figure Legends

### Figure 1

#### Expression of growth factors and sex steroid receptors in human skin.

Human skin cells possess a great number of growth factors and sex steroid hormone receptors. Imbalances of the microendocrine environment may lead to various morphological and physiological disturbances in skin with age (summarised on the right).

[GHR: growth hormone receptor, IGF-IR: insulin-like growth factor I, AR: androgen receptor, ER: estrogen receptor, PR: progesterone receptor, PPAR: peroxisome-proliferation activated receptor, ECM: extracellular matrix]



**Figure 2****A schematic overview of epidermal and neural induction in *Xenopus laevis* embryo.**

Epidermal and neural tissue share the same embryological origin. The activation of BMP pathways leads to the formation of a complex between phosphorylated Smad1 and Smad4. After translocation of the complex into the nucleus DNA binding sites such as Msx1, Vent and Gata1 are activated, which in turn induce epidermal gene expression and repress neural gene transcription (green dots). The neural fate is determined by inducers such as noggin, chordin and follistatin (red dots), which prevent binding of BMPs to its receptors and block the BMP signalling pathway.

[BMP: bone morphogenetic proteins, BMPR: bone morphogenetic protein receptors]

