4. DISCUSSION

4.1 Impact of aging

As we enter the third millenium, we are witnessing an unprecedented rapid expansion of the population of elderly people both in the developed and developing world (Diczfalusy 1996). Since 1840, life expectancy has increased at a rate of about three months per year (Oeppen and Vaupel 2002; Birg 2004), whereas the total worldwide aged population is expected to rise from 605 million in 2000 to 1.2 billion in 2025 and to nearly 2 billion in 2050 (Aleksandrova and Velkova 2003).

In Germany an industrialized country with a high population density, the median life expectancy increased during the last century by about 30 years. According to the World Health Organization's (WHO) estimates, only between 1990 and 2001, Germans gained 3.2 years in life expectancy, with men showing a greater gain than women: 3.6 years and 2.9 years, respectively. In parallel, Germany has the second lowest birth rate in Europe, which has dropped by 21% since 1990. Consequently, as the large birth cohorts of the late 1940s approach retirement age, the number of Germans aged 65 and over is expected to grow from about 17.5% of the population in 2003 (Council of Europe, 2003) to 26.4% in 2030 (Figs. 4.1.1, 2). In the year 2025 the median life expectancy will be about 83 years for women and 76 years for men (Diepgen 2003) and by 2030, one person out of every four is expected to be aged 65 or over.

Such a rapid and ubiquitous growth has never been seen in the history of civilization. Among multiple challenges, our society has to deal with important socio-economic, political and health-economic consequences and particularly with the maintenance of the quality of life of the aging people. It is well established that the prevalence of many chronic and degenerative diseases increases with advancing age, probably because of long-term exposure to exogenous factors (Diepgen 2003). Nowadays, physicians have to confront with age-associated diseases, which were almost unknown some centuries ago.

The applied research on aging aims among other issues the recognition of molecular pathways that may be associated with aging. Identifying the factors that contribute to long and healthy life can lead to improved intervention that can help delay or prevent the onset of major aging-related diseases and disabilities and increase the time that elderly people spend in good health. Hence, the aim of this study was to evaluate whether circulating hormones in sex- and age-

specific levels account for the process of cellular aging and if so which molecular pathways are involved.

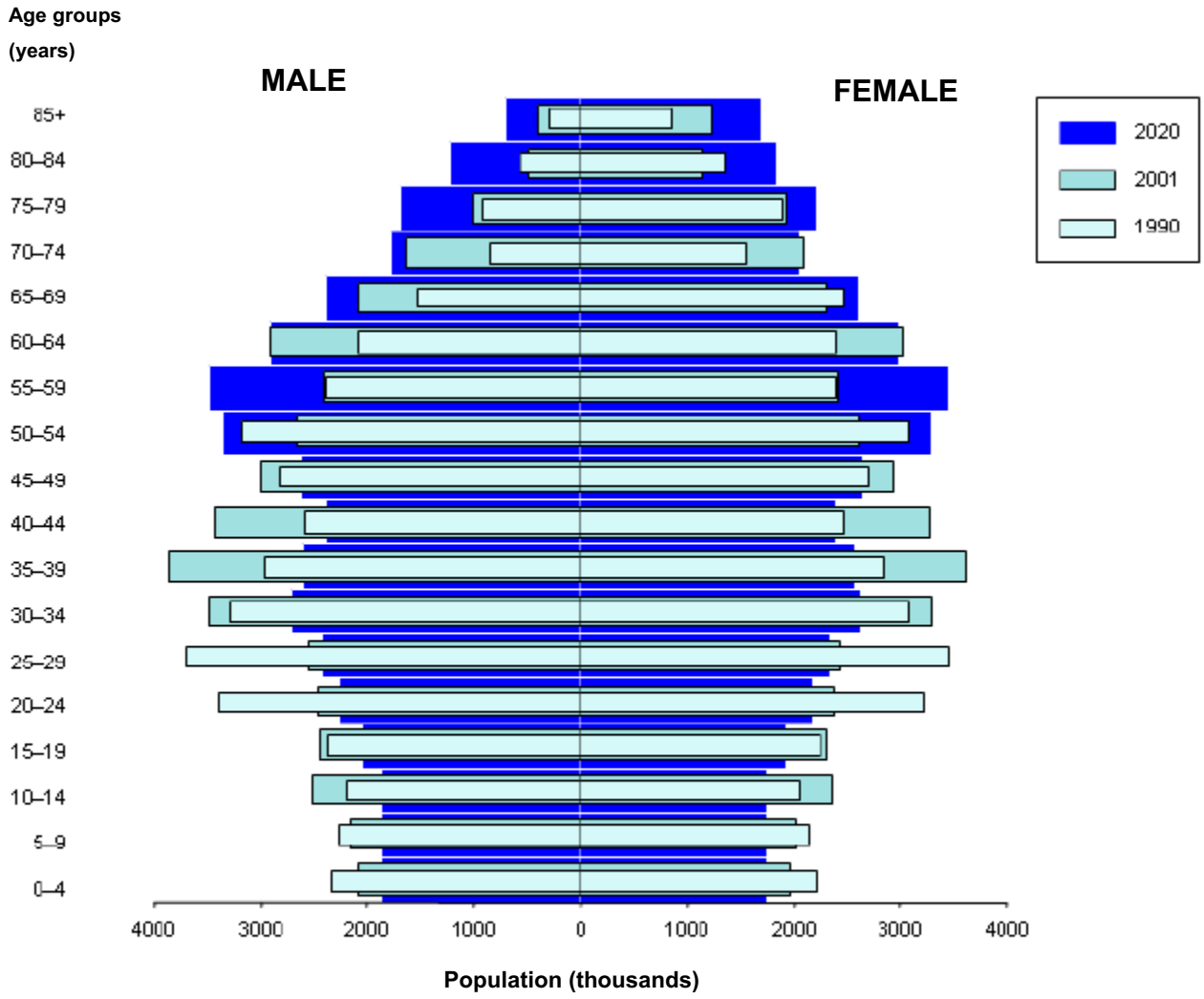

Fig. 4.1.1 Age pyramid for Germany [Sources: WHO Regional Office for Europe (2004) and United Nations (2002)].

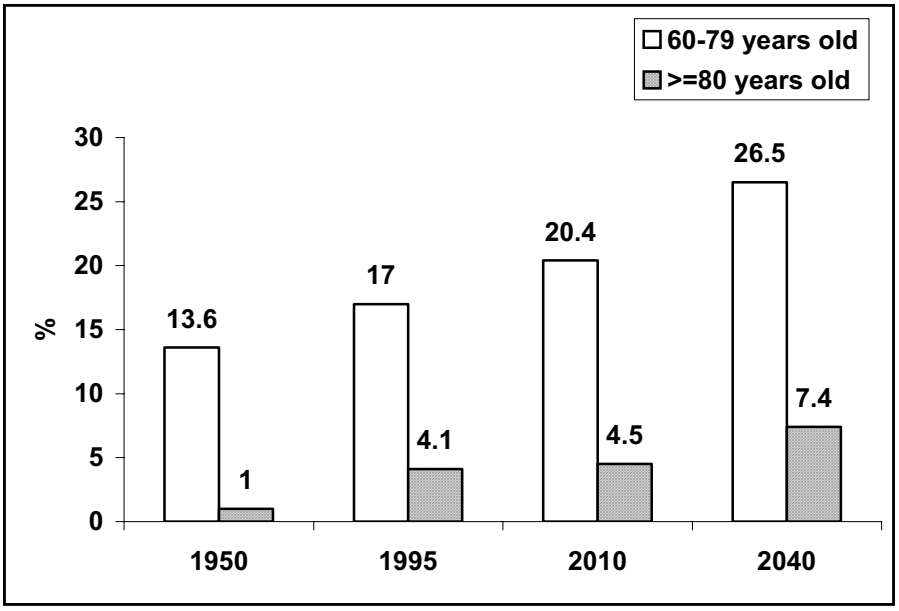

Fig. 4.1.2 Portion of 60-79-y-old and above 80-y-old persons in Germany. The prognosis is based on data provided by the German Federal Statistical Office (1998).

4.2 Expression of hormone receptors in human skin cells

These data suggest that growth factor receptors, such as GHR, IGF-IR and IR and steroid receptors, such as AR, ERα and ERß are expressed on mRNA and protein levels in human SZ95 sebocytes, epidermal keratinocytes and dermal fibroblasts, demonstrating that these skin cells, which were further used for the experiments, were susceptible to the corresponding hormones (Makrantonaki, Fimmel et al. 2002). Moreover, a general insight into the cellular localization of the receptors was given by means of immunocytochemistry. These findings are supported by several previous studies on the expression of these receptors in skin sections and cultured cells. For instance, GHR is known to be present in melanocytes, dermal fibroblasts, epidermal and follicular keratinocytes of the outer root sheath, sebocytes, cells of the eccrine sweat gland secretory duct, hair matrix cells of the dermal papillae, endothelial cells, Swann cells of peripheral nerve fascicles and adipocytes of the dermis (Lobie, Breipohl et al. 1990; Tavakkol, Elder et al. 1992; Simard, Manthos et al. 1996), whereas IGF-IR and IR have already been found to be expressed in human keratinocytes (Tavakkol, Elder et al. 1992; Tavakkol, Varani et al. 1999). AR is present in epidermal and follicular keratinocytes, sebocytes, sweat gland cells, dermal papilla cells, dermal fibroblasts, endothelial cells, and

genital melanocytes (Liang, Hoyer et al. 1993; Tadokoro, Itami et al. 1997; Lachgar, Charveron et al. 1999; Fritsch, Orfanos et al. 2001). Furthermore, ERα is also expressed in skin, especially in sebocytes (Pelletier and Ren 2004) and ERβ has been shown to be expressed in dermal papilla cells and dermal fibroblasts, sebocytes, adipocytes, melanocytes, and keratinocytes of the outer root sheath (Lachgar, Charveron et al. 1999; Thornton, Taylor et al. 2000). This information on the differential expression and localization of growth and sex steroid receptors in human skin cells supports investigations on the specific role of GH, IGF-I, estrogens and androgens on skin.

4.3 Effects of hormone-induced aging on the proliferation and lipid synthesis in SZ95 sebocytes and fibroblasts

The activity of sebaceous glands and fibroblasts including major functions such as proliferation and differentiation is affected by hormones (Zouboulis 2000). While cell proliferation was not affected, SZ95 sebocytes incubated with hormones at aged levels (60 y) showed lower content of neutral lipids compared with SZ95 sebocytes maintained in hormones at young levels (20 y) (Makrantonaki, Seltmann et al. 2002). These results correspond to *in vivo* observations, which have documented that aged skin is characterized by a decline in lipid production and lack of skin moisture (Ghadially, Brown et al. 1996; Elias and Ghadially 2002). Moreover, levels of free fatty acids (FFA), squalene, the precursor of cholesterol, linoleic acid, an exclusive polyunsaturated fatty acid, and monounsaturated acids such as palmitoleic and oleic acids were found to be decreased in methanol extracts from human aged skin surface in contrast to the skin surface of young donors (Hayashi, Togawa et al. 2003).

On the other hand, proliferation of foreskin fibroblasts, in contrast to SZ95 sebocytes, was affected by the treatment. Furthermore, not only neutral but also polar lipid synthesis was shown to be affected in fibroblasts after treatment with the hormone mixture and was also found to be dependent on the hormone levels. Neutral lipids may increase in fibroblasts as an indicator of ongoing differentiation and apoptosis. Polar lipids constitute the membrane and organelle lipids of the cells and are, therefore, used as markers of cell proliferation, as well. Consequently, the biological activity of fibroblasts is more globally influenced by hormones than that of sebocytes. These results indicate that hormones and their decrease occurring with

aging may play a dominant role in the regulation of cell proliferation and lipid biosynthesis of skin cells *in vivo*.

4.4 Expression of aging-associated genes in hormonally aged SZ95 sebocytes

After having treated SZ95 sebocytes with the hormone mixture of IGF-I, GH, 17β-estradiol, testosterone, DHEA and progesterone in concentrations corresponding to 20- and 60-y-old individuals, the expression of two aging-associated genes -c-Myc and fibronectin- was measured on RNA and protein level (Makrantonaki, Oeff et al. 2004). Furthermore, in order to identify pathways that are altered as a consequence of hormonal induction of aging, a cDNA microarray was performed.

The c-Myc proto-oncogene encodes a transcription factor, the cellular homologue to the viral oncogene (v-myc) of the avian myelocytomatosis retrovirus (Vennstrom, Sheiness et al. 1982) and has been shown to be a member of a family of proto-oncogenes comprising c-Myc, N-Myc, and L-Myc. Activated oncogenic c-Myc is instrumental in the progression of Burkitt's lymphoma (Spencer and Groudine 1991), its expression is elevated or deregulated in a wide range of other human cancers and is often associated with aggressive, poorly differentiated tumors (Nesbit, Nesbit et al. 1999; Schlagbauer-Wadl, Griffioen et al. 1999). In its physiological role, c-Myc is expressed ubiquitously during embryogenesis and in tissue compartments of the adult possessing high proliferative capacity.

C-Myc encodes a multifacetted protein and is implicated in cellular growth, proliferation, apoptosis and cellular metabolism (Boxer and Dang 2001). The Myc oncoprotein is a basic helix-loop-helix-leucine zipper (bHLHZIP) transcription factor, which activates a variety of known target genes as part of a heterodimeric complex with its partner protein, Max. Myc–Max heterodimers bind specific DNA sequences, such as the E-box sequence CACGTG (Watson, Oster et al. 2002). The proteins encoded by Myc transcriptional target genes appear to regulate cell-cycle progression and cell growth, while sensitizing cells to apoptotic stimuli (Evan, Wyllie et al. 1992). Myc may also be able to promote tumorigenesis by up-regulating the expression of genes such as hTERT that play a role in cellular immortalization or the escape of senescence (Greenberg, O'Hagan et al. 1999; Wu, Grandori et al. 1999).

In our results, c-Myc was found to be upregulated in hormonally aged sebocytes both on mRNA and protein level. This finding corresponds to results showing increased expression of c-Myc in fibroblasts derived from Werner syndrome patients (WRN) (Grandori, Robinson et

al. 2004). The WRN phenotype resembles premature aging and is characterized by loss of function of the WRN RecQ helicase protein, involved in DNA repair and mitotic recombination (Brosh and Bohr 2002). Moreover, it is known that the incidence of cancer increases with advancing age and c-Myc was found to be overexpressed in cancer cells derived from elderly persons (Tanaka, Nagaoka et al. 2002).

The expression of fibronectin, a gene encoding a glycoprotein of high molecular weight, was measured in hormonally aged SZ95 sebocytes. It is involved in many cellular processes, including tissue repair, embryogenesis, blood clotting, cell migration and adhesion. Fibronectin exists in two main forms: as an insoluble glycoprotein dimer that serves as a linker in the extracellular matrix, and as a soluble disulphide linked dimer found in the plasma (plasma fibronectin) (Kornblihtt, Umezawa et al. 1985; Odermatt, Tamkun et al. 1985; Umezawa, Kornblihtt et al. 1985). The plasma form is synthesized by hepatocytes, and the extracellular matrix form is made by fibroblasts, chondrocytes, endothelial cells, macrophages, as well as certain epithelial cells like sebocytes. Fibronectin sometimes serves as a general cell adhesion molecule by anchoring cells to collagen or proteoglycan substrates and can also serve to organize cellular interaction with the extracellular matrix by binding to different components of the extracellular matrix and to membrane-bound fibronectin receptors on cell surfaces. Its structure is rod-like, composed of three different types of homologous, repeating modules, types I, II, and III (Potts, Bright et al. 1999; Niimi, Osawa et al. 2001). These modules, though all part of the same amino acid chain, can be envisioned as "beads on a string," each one joined to its neighbors by short linkers. Twelve type I modules make up the amino-terminal and carboxy-terminal region of the molecule, and are involved mainly in fibrin and collagen binding. Only two type II modules are found in fibronectin. They are instrumental in binding collagen. The most abundant module in fibronectin is type III, which contains the RGD fibronectin receptor recognition sequence along with binding sites for other integrins and heparin. Depending on the tissue type and/or cellular conditions, the fibronectin molecule is made up of 15-17 type III modules. In addition, there is a module that does not fall into any of these categories, called IIICS. This module, along with EDB and EDA (both type III modules), is regulated through alternative splicing of fibronectin pre-mRNA. Fibronectin molecules can form two disulphide bridges at their carboxy-termini, producing a covalently-linked dimer.

Fibronectin was used in these experiments as a marker of induced aging as its enhanced expression has been already correlated with aging and senescence in several studies (Kang, Kameta et al. 2003). In the hormonally aged SZ95 sebocytes, fibronectin was upregulated on the protein level, a finding also observed in senescent fibroblasts (Labat-Robert 2003). Surprisingly, the gene was downregulated at the mRNA level, which may be explained by a negative feedback by molecules, which downregulate enhanced expression of fibronectin, such as caveolin-1 (Sottile and Chandler 2005).

Using cDNA microarrays, genes under male and female aging hormone conditions were identified to be regulated (Makrantonaki, Adjaye et al. 2005). The functional annotation of these genes according to the Gene Ontology (Makrantonaki, Adjaye et al. 2005) identified pathways related to mitochondrial function, oxidative stress, ubiquitine-mediated proteolysis, cell cycle, immune responses, organization of the extracellular matrix, steroid biosynthesis and phospholipid degradation, which are all hallmarks of the aging process (Smith and Pereira-Smith 1996; Sohal and Weindruch 1996; Michikawa, Mazzucchelli et al. 1999; Ly, Lockhart et al. 2000; Gems and McElwee 2003; Martin and Loeb 2004; Nemoto and Finkel 2004), underlining the importance of hormones in the aging process.

4.5 Biological activity of IGF-I and GH

The importance of IGF-I and GH for the aging process has been illustrated by several studies.

In humans, evidence is presented that isolated GH deficiency (IGHD), multiple pituitary hormone deficiency (MPHD) including GH, as well as primary IGF-I deficiency (GH resistance, Laron syndrome) present signs of early skin aging such as dry, thin and wrinkled skin, obesity, hyperglucemia, reduced body lean mass, osteopenia, lowered venous access, rise in serum cholesterol, tendency for cardiovascular diseases and subsequent premature mortality (Rosen and Bengtsson 1990; Carroll, Christ et al. 1998; Tomlinson, Holden et al. 2001; Laron 2005).

Treatment with GH of normal elderly males resulted in amelioration and reverse of the aging signs and symptoms (Rudman, Feller et al. 1990; Rudman, Feller et al. 1991; Veldhuis, Patrie et al. 2004). However, recent reports of an association of GH substitution and increased risk of prostate, lung, colon, breast cancer as well as possible decrease of insulin insensitivity make further investigations necessary on safety and efficacy of growth hormone therapy in the aging population (Riedl, Kotzmann et al. 2001).

On the other hand, some scientists support that GH/IGF-I deficiency can lead to prolonged life and that the decline of both hormones occurring with age may be a protective phenomenon (Laron 2005). Their theory is supported by several experiments performed in model organisms such as the yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the mouse *Mus musculus* (Gems and Partridge 2001). For example, Laron dwarf mice, which exhibit isolated GH insensitivity as a result of an engineered defect in the GH receptor (Zhou, Xu et al. 1997), show increased lifespans by 38% and 55% in females and males, respectively (Coschigano, Clemmons et al. 2000). However, whether these models are representative for humans remains controversial.

GH activity is mainly mediated by IGFs but GH has also direct effects on human skin cells (Deplewski and Rosenfield 2000). The increased serum level of GH in acromegaly is associated with high sebum secretion (Burton, Libman et al. 1972), correlating to the results obtained from SZ95 sebocytes. In Mini rats, in which the expression of growth hormone (GH) gene is suppressed by the presence of antisense transgene, thinner skin with less collagen, more abundant subcutaneous adipose tissue and small-sized sebaceous glands was observed, giving a better insight into the influence of GH deficiency on skin (Ikawa, Ishii et al. 2002).

Complementary, IGF-I has been shown to stimulate sebocyte proliferation and differentiation especially in combination with GH in *in vitro* experiments (Deplewski and Rosenfield 1999), while in human keratinocytes IGF-I acts as a mitogen (Tavakkol, Varani et al. 1999). A further evidence that IGF-I and GH regulate the biological activity of sebocytes is the fact that acne vulgaris, a disease associated with increased sebum production *in vivo,* peaks in mid-adolescence at a time that GH and IGF-I levels reach their highest levels (Deplewski and Rosenfield 1999). In our experiments, IGF-I also amplified lipid synthesis in human SZ95 sebocytes and fibroblasts in a dose-dependent manner, whereas only fibroblast proliferation was affected underlining the fact that the decline observed in IGF-I levels with increasing age may play a significant role in the reduction of skin moisture and thickness.

In addition, clinical studies have recently documented that sex-steroid hormones can regulate GH and IGF-I secretion. Supplementation of a high dose of testosterone in middle-aged and older men has been shown to attenuate IGF-I feedback-dependent inhibition of GH secretion (Veldhuis, 2005), whereas acute administration of estradiol accentuated IGF-I-induced

inhibition of GH secretion (Veldhuis, JD 2004). Lack of steroids occurring with advancing age can therefore lead to minor regulation of both growth factors.

4.6 Biological activity of androgens

DHEA may be related to the process of skin aging through the regulation of production and degradation of extracellular matrix. DHEA could increase procollagen synthesis and inhibit collagen degradation by decreasing matrix metalloproteinases (MMP)-1 and collagenase synthesis, and increasing tissue inhibitor of matrix metalloprotease (TIMP-1) and stromelysin-1 production in cultured dermal fibroblasts (Lee, Oh et al. 2000; Shin, Rhie et al. 2005). Moreover, improvement of the skin quality was observed after treatment with DHEA, particularly in women in terms of hydration, epidermal thickness, sebum production, and pigmentation (Baulieu, Thomas et al. 2000). Consequently, researchers have suggested the possibility of using DHEA as an anti-skin aging agent (Minghetti, Cilurzo et al. 2001; Shin, Rhie et al. 2005).

On the other hand, substitution of DHEA in elderly persons was accompanied with a small increase of testosterone and estradiol, which may be the explanation of the significantly demonstrated physiological clinical manifestations (Baulieu, Thomas et al. 2000). This could explain also the fact that in *in vitro* experiments DHEA showed no effect on lipid synthesis and cell proliferation of SZ95 sebocytes, suggesting that *in vivo* the action of DHEA may be implemented through indirect pathways.

Testosterone has been shown to enhance the keratinization of epidermal cells (Tammi 1982) and increase angiogenesis in human foreskin (Stern, Chen et al. 2004). In addition, high levels of testosterone *in vivo* have been implicated with enhanced sebaceous gland activity in humans (Pochi and Strauss 1969; Giltay and Gooren 2000) and consequently with diseases marked by hyperseborrhea such as in acne vulgaris. This is postulated to be the result of the generated production of the potent androgen 5α-DHT (Zouboulis 2004).

On the other hand, *in vitro* experiments, although they demonstrated a dose-dependent induction of sebocytes proliferation by testosterone (Akamatsu, Zouboulis et al. 1992; Zouboulis, Seltmann et al. 1999), showed no effect on lipid synthesis. Additional experiments by Rosenfield et al (Rosenfield, Deplewski et al. 1998) showed that the effect of testosterone *in vivo* is regulated by co-factors and peroxisome-proliferator activated receptor (PPAR) ligands were presented as such candidates. PPARs regulate multiple lipid metabolic genes in

mitochondria, peroxisomes and microsomes, all prominent in the cytoplama of sebocytes (Rosenfield, Deplewski et al. 1998; Chen, Yang et al. 2003, Makrantonaki and Zouboulis, 2005). In the experiments presented here, testosterone showed a significant increase of the polar lipid production and as polar lipids can be used as markers for cell proliferation, this result is compatible with the known effect of testosterone on sebocyte proliferation.

4.7 Biological activity of estrogens and progesterone

After menopause, which is marked by the sudden decline of estrogens, dermal cellular metabolism is influenced leading to changes in the skin thickness, collagen content, alterations in the concentration of glycosaminoglycans (Affinito, Palomba et al. 1999; Contet-Audonneau, Jeanmaire et al. 1999; Bosset, Barre et al. 2002; Youn, Kwon et al. 2003; Sumino, Ichikawa et al. 2004) and most importantly the water content (Pierard-Franchimont, Letawe et al. 1995) and the cutaneous vascular reactivity. Hair loss has been also associated with the beginning of menopause (Raine-Fenning, Brincat et al. 2003). Moreover, one of the most important consequences is delay in cutaneous wound healing, as reduced levels of estrogen have been associated with impaired cytokine signal transduction, unchecked inflammation and altered protein balance (Ashcroft and Ashworth 2003).

Administration of estrogen given both topically and systemically reversed the observed alterations and retarded the skin aging process (Maheux, Naud et al. 1994; Callens, Vaillant et al. 1996; Schmidt, Binder et al. 1996; Fuchs, Solis et al. 2003; Raine-Fenning, Brincat et al. 2003). Fibroblast and keratinocyte function was stimulated by estrogen application, and among other effects significant increase of collagen fibers were demonstrated six months after the onset of hormone replacement therapy (Schmidt 2005).

In vitro, 17β-estradiol has been shown to stimulate the proliferation of human keratinocytes by promoting the expression of cyclin D2 and to suppress oxidative stress-induced apoptosis in keratinocytes by promoting expression of the anti-apoptotic protein Bcl-2 after binding to the membrane GPR30 on keratinocytes (Kanda and Watanabe 2003; Kanda and Watanabe 2004). In addition, 17β-estradiol had a stimulative effect on collagen synthesis, increased the expression of both ER mRNA levels and also increased IGF-IR expression in cultured fibroblasts obtained from postmenopausal women (Surazynski, Jarzabek et al. 2003).

In our work, 17β-estradiol showed only an effect on the polar lipid production of SZ95 sebocytes, whereas neutral lipid production and proliferation of the cells was not affected.

This result corresponds to previous *in vitro* findings presenting estrogens not to have any influence on the biological activity of the sebaceous glands (Guy, Ridden et al. 1996). On the other hand, 17β-estradiol amplified lipid synthesis in fibroblasts affecting significantly both the production of neutral and polar lipids. The proliferation remained unchanged. This finding demonstrates that fibroblasts are more susceptible to estradiol in *in vitro* experiments than SZ95 sebocytes and corresponds to results from *in vivo* studies which consider fibroblasts as the main target of estrogens, as skin thickness and collagen content are significantly affected after estrogen substitution (Schmidt 2005).

Progesterone is known to enhance the keratinization of epidermal cells (Tammi 1982), increase keratinocyte proliferation (Urano, Sakabe et al. 1995), block action of 5α-reductase in genital fibroblasts, a key enzyme in normal male sexual differentiation (Dean and Winter 1984), and inhibit growth of fibroblasts *in vitro* (Comini Andrada, Hoschoian et al. 1985). In addition, progesterone suppresses the expression of members of the matrix metalloproteinase super family, stimulates the production of tissue inhibitor of metalloproteinases (TIMP) in fibroblasts (Kanda and Watanabe 2005) and reduces the collagenolytic activity (Huber and Gruber 2001).

Experiments in humans and animals studying possible effects of progesterone on the activity of the sebaceous gland remain controversial. A stimulation of sebum secretion was observed in female and castrated rats. The degree of stimulation was related to the time points of gonadectomy and was higher when this was carried out before puberty. In contrast, in intact males sebum secretion was inhibited by progesterone (Shuster, Hinks et al. 1977). These results of studies in the rat may lead to the conclusion that the effect of progesterone seems to be depending on the sex and on changes in the early endocrine environment. In SZ95 sebocytes, progesterone in concentrations similar to young and elderly men and women did not affect the biological activity of the cells.

4.8 Interaction of estrogen and IGF-I signaling pathway

As shown above, after treatment with 17β-estradiol, although the lipid synthesis of human fibroblasts was affected, this was not the case in treated SZ95 sebocytes. On the other hand, IGF-I was the only hormone that could significantly increase the lipid production of both cell types (Makrantonaki, Oeff et al. 2004). Estrogens *in vivo* play a significant role in ameliorating skin surface and moisture after topical or systemical application in

postmenopausal women (Schmidt, Binder et al. 1996). Since human sebocytes account for the maintenance of skin moisture, one should expect that 17β-estradiol would increase the lipid production in the *in vitro* experiments presented here. However, the expected effect was not observed. This contradiction led us to the assumption that IGF-I might be the key hormone, which had as result all benefits seen after estrogen substitution *in vivo.* This would mean that estrogen activity could be mediated in aged skin cells through interaction with the IGF-I signaling pathway.

In order to investigate this hypothesis the effects of IGF-I and 17β-estradiol were measured on the expression of IGF-IR and ERα and the synthesis of IGF-I and 17β-estradiol in SZ95 sebocytes and human fibroblasts. Our findings indeed indicate an interaction between IGF-I and 17β-estradiol signaling pathways in both cell types.

The interplay between both pathways has been already documented in several studies. *In vivo*, IGF-I is probably required for estradiol-induced mitosis in the uterine epithelium (Richards, DiAugustine et al. 1996). Oral estradiol administration resulted in a significant fall of mean IGF-I levels and transdermal administration of 17β-estradiol resulted in a slight increase in serum IGF-I (Ho, O'Sullivan et al. 2003). The antiestrogen ICI 182780 decreased IGF-IR mRNA levels (Huynh, Nickerson et al. 1996) and the antiestrogen tamoxifen reduced serum IGF-I levels (Pollak, Huynh et al. 1992). In the CNS, estradiol and IGF-I interacted to regulate neuronal differentiation, gonadotrophin secretion, sexual behavior and synaptic remodeling (Cardona-Gomez, Mendez et al. 2002). In the mouse uterus, IGF-IR was activated after administration of estradiol to ovariectomized adult female mice (Richards, Walker et al. 1998). *In vitro*, 17β-estradiol increased the number of IGF-I receptors of basal, undifferentiated epidermal keratinocytes and indirectly the number of IGF-I/IGF-I receptor complexes, whereas IGF-I was found to be produced in fibroblasts (Rudman, Philpott et al. 1997). Estradiol responsive strains of breast epithelial cells could activate their own IGF-IR by secreting an IGF-IR activating factor (Hamelers and Steenbergh 2003). In osteoblasts, estradiol could increase the synthesis of IGF-I, which appeared to act as an autocrine growth factor (Westley and May 1994). In uterine tissue, estradiol was shown to increase the synthesis of IGF-I in the stroma, which then could modulate the proliferation of epithelial cells (Westley and May 1994). Insulin receptor substrate-I, the principal substrate for the tyrosine kinase of the insulin-like growth factor I receptor (IGF-IR), was regulated by

estradiol in MCF-7 breast cancer cells (Westley, Clayton et al. 1998). Finally, the induction of DNA synthesis in breast cells by estradiol could be blocked completely with the antibody alpha IR3, which binds to the IGF-IR (Thorsen, Lahooti et al. 1992).

In the results presented here, while 17β-estradiol alone was not so effective in inducing the IGF-I effects after activation of the IGF-IR in human sebocytes, in human fibroblasts showed IGF-I-like effects such as increased lipid production. This led to the assumption, that through the enhanced IGF-I production in fibroblasts and through a resulting autocrine mechanism, already reported in osteoblasts (Westley and May 1994), estradiol may unfold its action. The above observations could also explain why estradiol is so effective in ameliorating skin moisture *in vivo*. As sebocytes are in narrow neighborhood with dermal fibroblasts, the induction of IGF-I in fibroblasts through estradiol could induce in a paracrine way the lipid production in sebocytes. In conclusion, one should not just speak for an interaction between IGF-I and estradiol signaling pathways, but also for a paracrine interaction between sebocytes and fibroblasts *in vivo.*

Moreover, as shown above, IGF-I and estradiol acted through negative feedback on the downregulation of IGF-IR and both hormones could be considered as controllers of IGF-IR. With increasing age the IGF-I and estradiol levels decrease, which could mean uncontrolled expression and activation of IGF-IR. The insulin/insulin-like growth factor (IGF-I) signaling, which is involved in many functions that are necessary for metabolism, growth, and fertility in animal models, like flies, nematodes, and mammalians has been already implicated in aging. It has been shown that disruption of the insulin/IGF-I receptor in nematodes and flies increases lifespan significantly (Rincon, Muzumdar et al. 2004). The question which arises is how important is the IGF-I signaling pathway for the aging process in humans, and if increased and uncontrolled expression of IGF-IR occurring during aging plays a significant role.

4.9 Concluding remarks

The data presented here illustrate that hormones at age- and sex-specific levels can alter the development of cells regulating their transcriptome. Moreover, an *in vitro* model on endogenous skin aging was developed, which can be applied to all skin cell types and may serve to enhance our understanding on skin aging. By now, in models of animal aging, such as in organisms as diverse as the nematode *Caenorrhabditis elegans*, the fly *Drosophila*

melanogaster, and the mouse *Mus musculus*, the importance of hormonal signals on the aging phenotype has been already documented. Suppression of hormones such as insulin-like peptides, GH and sterols (Tatar, Bartke et al. 2003) or their receptors can increase lifespan and delay age-dependent functional decline. Conboy et al. (2005) showed that the age-related decline of progenitor cell activity of mice could be reversed by exposure to young serum and that the cells could retain much of their intrinsic proliferative potential even when old, underlining the great importance of the systemic environment. Hence, the importance of the endocrine system and the circulating hormones has begun to gain more attention.

Our model has allowed a better view into the world of the hormonally affected genes and pathways in human skin cells. Not only the effects of one hormone as a single agent were observed but also the effects of a mixture of hormones containing GH, IGF-I, DHEA, testosterone, 17β-estradiol and progesterone in an effort to resemble as far as possible the *in vivo* conditions and to give an insight into the resulting cellular and molecular processes, also altered by interactions between the corresponding hormonal signaling pathways.

The identification and molecular characterization of specific genes, which are affected by hormonally induced aging is a promising approach. In the future it will certainly provide further clues about the complex network involved in aging. Furthermore, understanding of the underlying mechanisms can be expected to open new strategies to deal with the various age-related diseases.