3. RESULTS

3.1 Expression of receptors in human skin cells

In order to assure that hormone treatment would affect the cultured cells, the expression of hormone receptors was first investigated by means of RT-PCR, Western blotting and immunocytochemistry (Figs. 3.1.1-8). IR, IGF-IR, GHR, AR, ERα, and ERβ were found to be expressed on mRNA and protein level in SZ95 sebocytes, keratinocytes and fibroblasts, whereas IGF-IR and AR showed a weaker expression in fibroblasts. By means of immunocytochemistry, the localization of the receptors was also documented. Cells which were not incubated with the primary antibody were set as negative controls. While IR, IGF-IR and GHR were membrane bound, AR, ERα and ERβ were localized mostly on the cytosol and in the nucleus of the cells (Figs. 3.1.3-8).

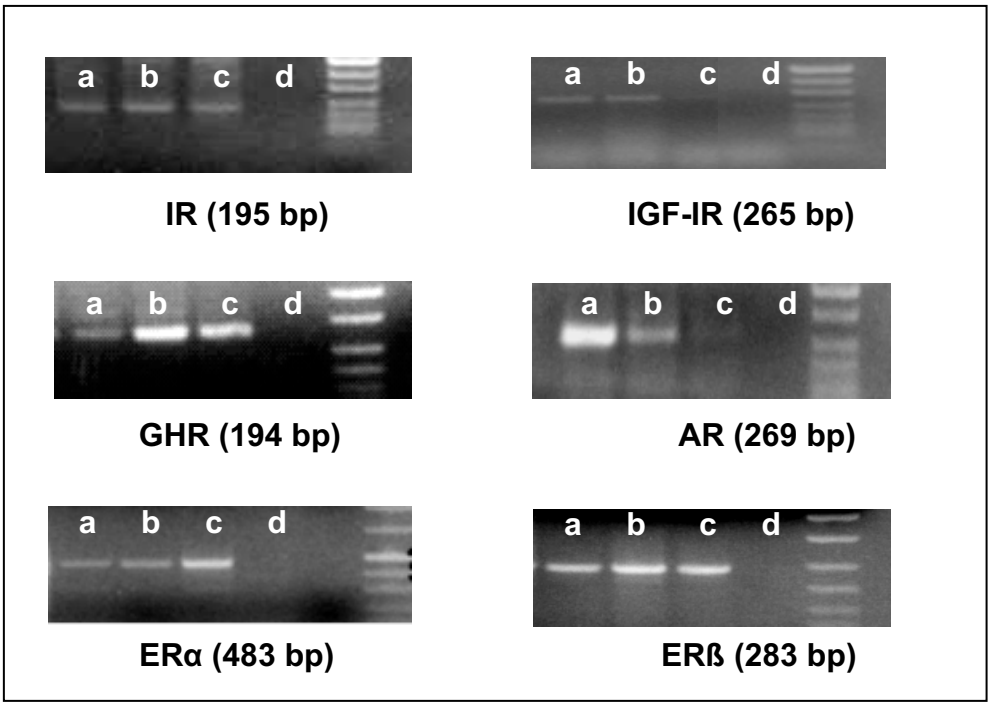


Fig. 3.1.1 Expression of IR, IGF-IR, GHR, ERα, ERβ and AR on mRNA level by means of *RT-PCR* (a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= H_2O)

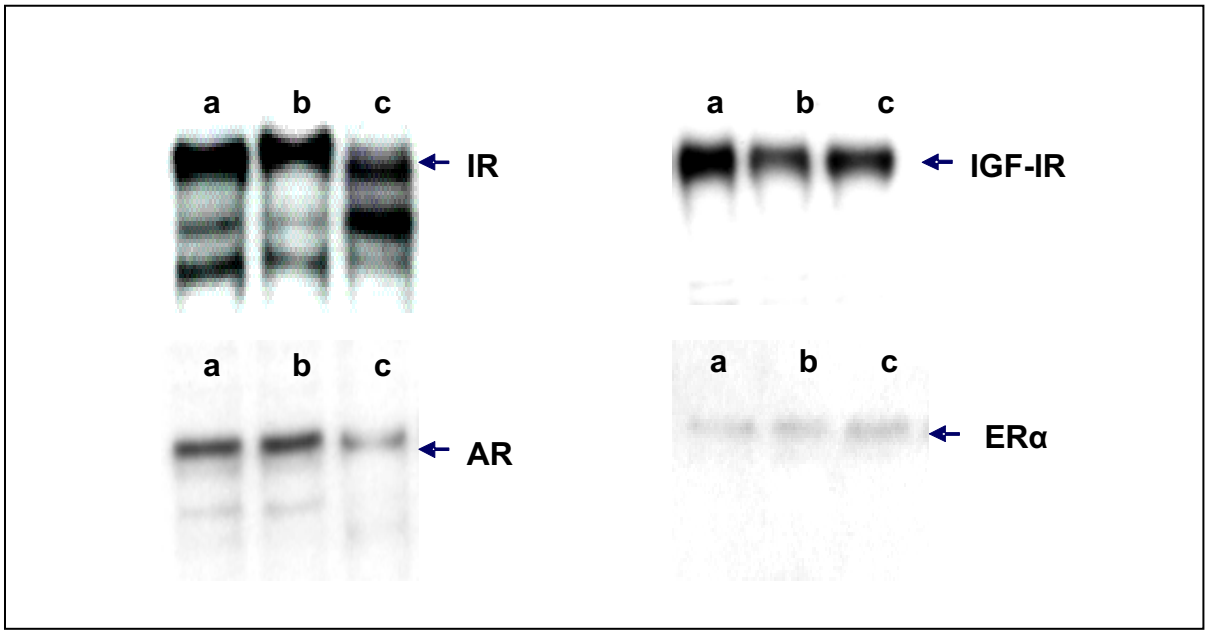


Fig. 3.1.2 Expression of IR, IGF-IR, AR and ERα on protein level by means of *Western Blotting*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts)

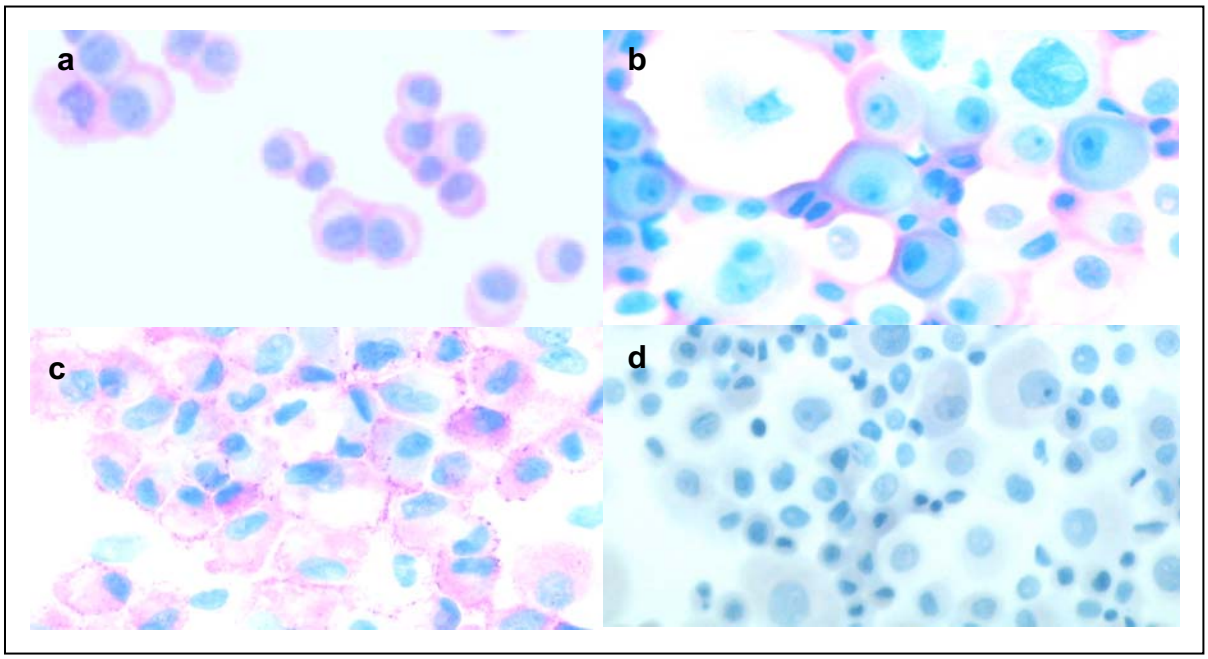


Fig. 3.1.3 Expression of IR on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, SZ95 sebocytes)

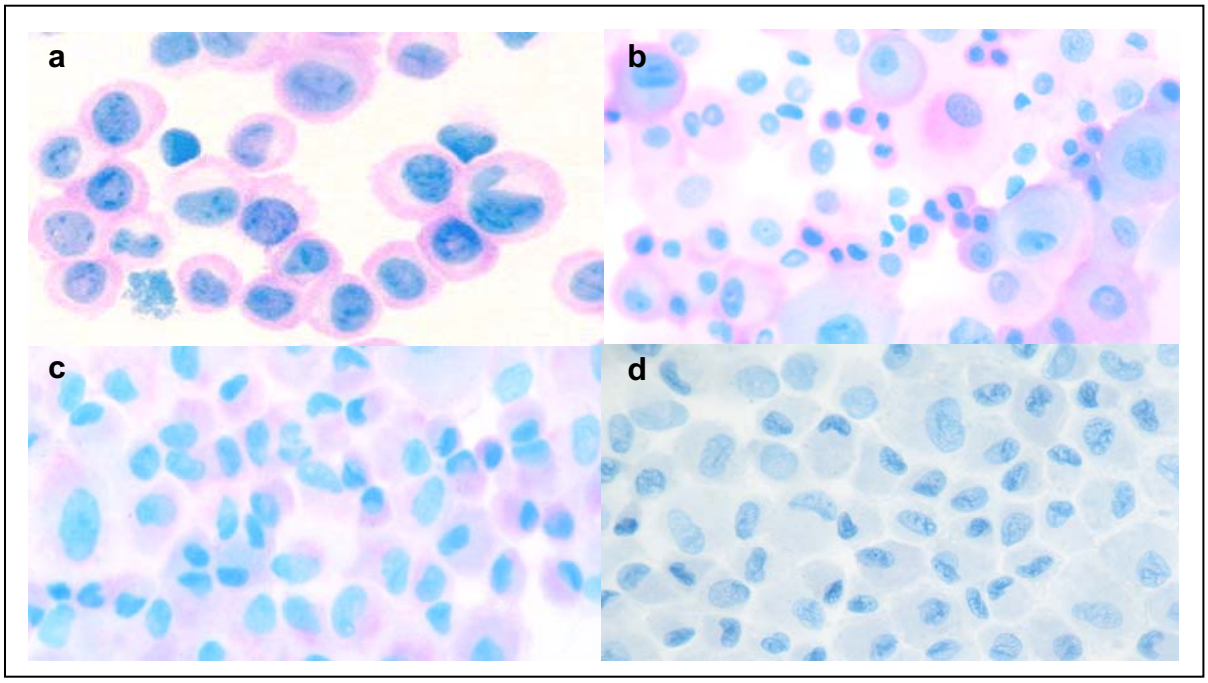


Fig. 3.1.4 Expression of IGF-IR on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, fibroblasts)

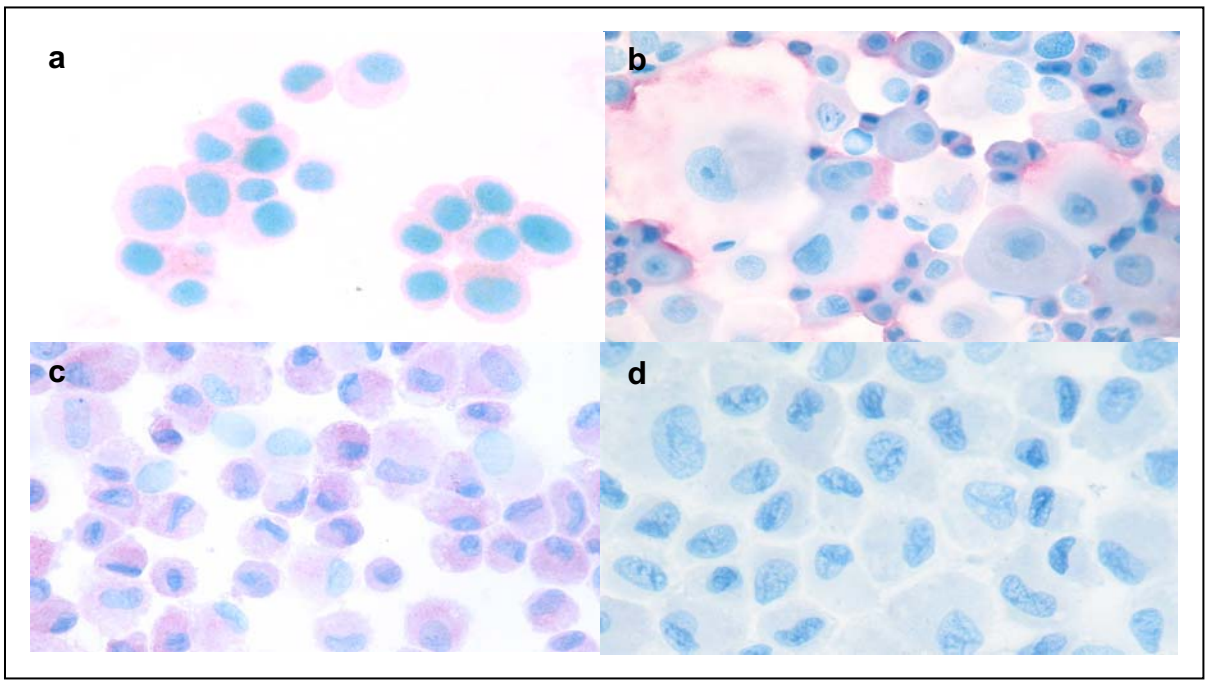


Fig. 3.1.5 Expression of GHR on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, fibroblasts)

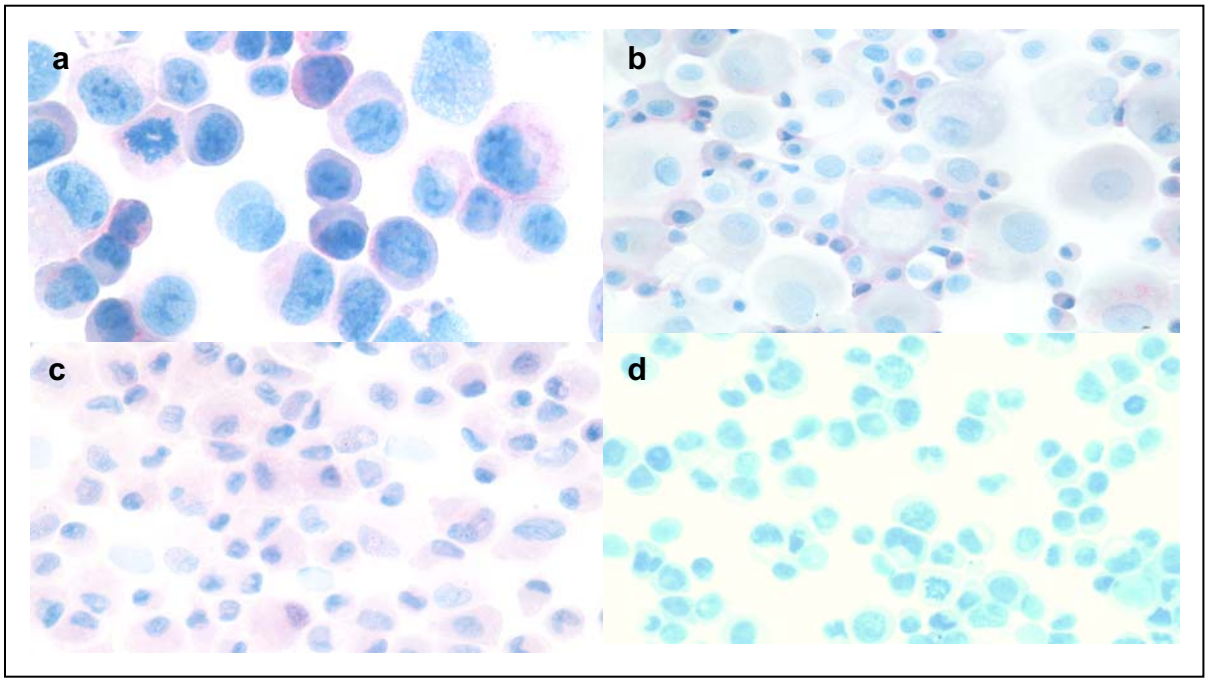


Fig. 3.1.6 Expression of AR on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, SZ95 sebocytes)

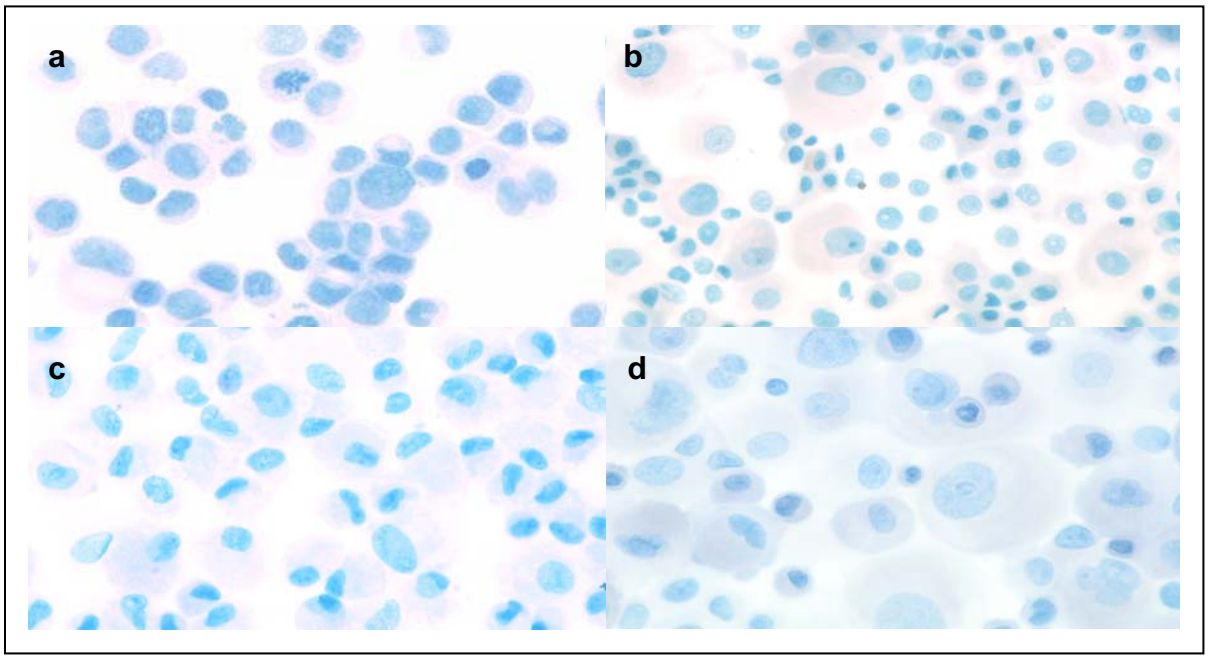


Fig. 3.1.7 Expression of ERα on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, keratinocytes)

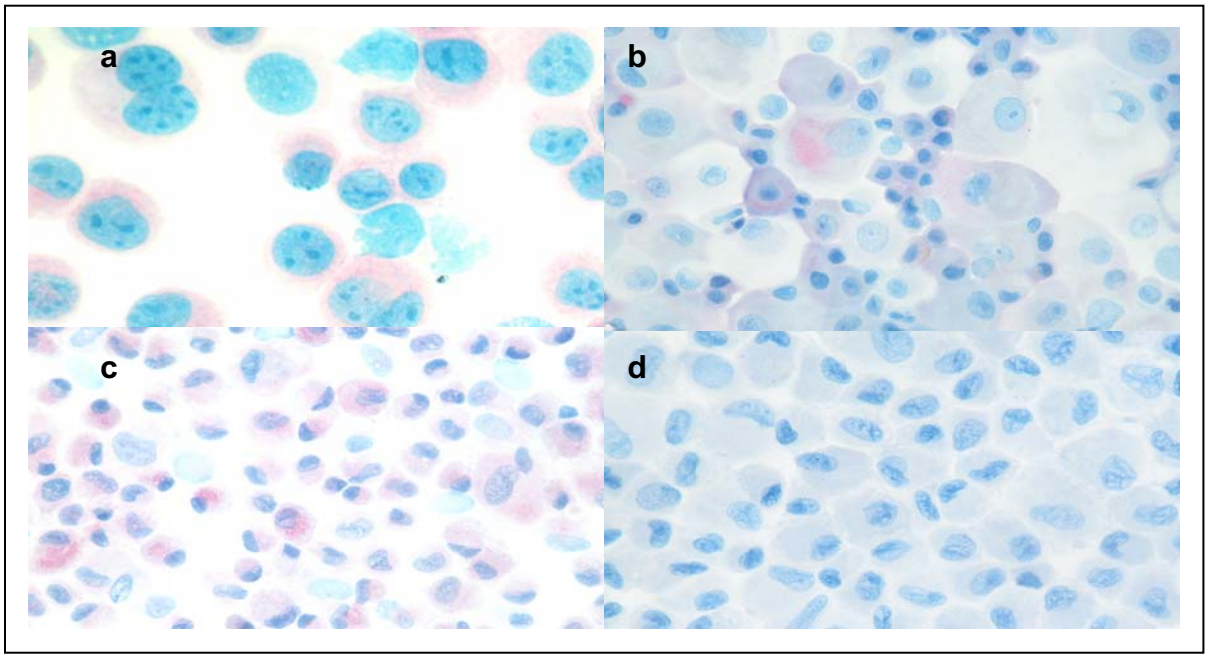


Fig. 3.1.8 Expression of ERβ on protein level by means of *immunocytochemistry*
(a= SZ95 sebocytes, b= keratinocytes, c= fibroblasts, d= negative control, fibroblasts)

3.2 Proliferation of SZ95 sebocytes incubated with hormones mixture

No significant change was observed in the proliferation of SZ95 sebocytes treated for 4 d with the mixture of IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those circulating in 20- and 60-y-old men (m20 and m60, respectively) and women (f20 and f60, respectively) (Fig. 3.2).

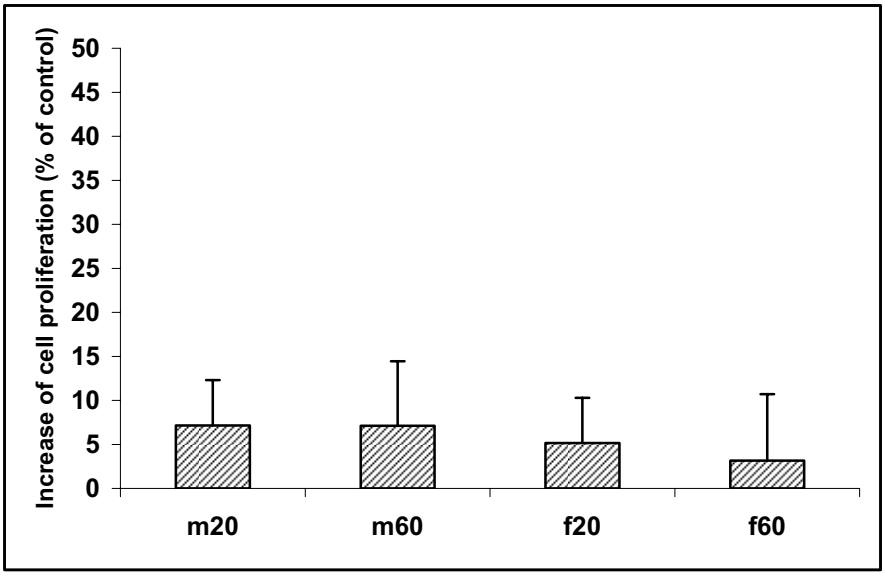


Fig. 3.2 Proliferation of SZ95 sebocytes incubated with hormones at levels similar to young and elderly men and women by means of MUH fluorescence assay. SZ95 sebocytes were seeded at a density of 1,500 cells/well in a 96-well plate and were left 48 h to adhere. On day 0 and 2 they were treated with IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those in 20- and 60-y-old men (m20, m60) and women (f20, f60) and on day 4 cell proliferation was measured by means of MUH fluorescence assay. Proliferation of control cells with no treatment was set at 0% and proliferation of the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD.

3.3 Content of lipids in SZ95 sebocytes incubated with hormones mixture

SZ95 sebocytes incubated with hormones at aged female levels (60 y) showed significantly lower content of neutral lipids ($p<0.001$) in contrast to SZ95 sebocytes with hormones at young female levels (20 y), whereas in contrast to the control (untreated SZ95 sebocytes) the content of neutral lipids of SZ95 sebocytes with hormones at young and aged female levels was significantly increased ($p<0.001$ and $p<0.01$, respectively) (Fig. 3.3, light columns). On the other hand, in contrast to the control the content of neutral lipids of SZ95 sebocytes with

hormones at young and aged male levels was significantly increased ($p<0.001$ and $p<0.05$, respectively), but there was no significant difference in the production of neutral lipids between SZ95 sebocytes with hormones at young and aged male levels. SZ95 sebocytes incubated with hormones at aged female levels (60 y) showed a slight but not significant decrease of polar lipids in contrast to 20-y-old SZ95 sebocytes, whereas the content of polar lipids of SZ95 sebocytes with hormones at young and aged male levels remained unchanged (Fig. 3.3, dark columns). The lipid production was detected after 2 d treatment by means of nile red microassay.

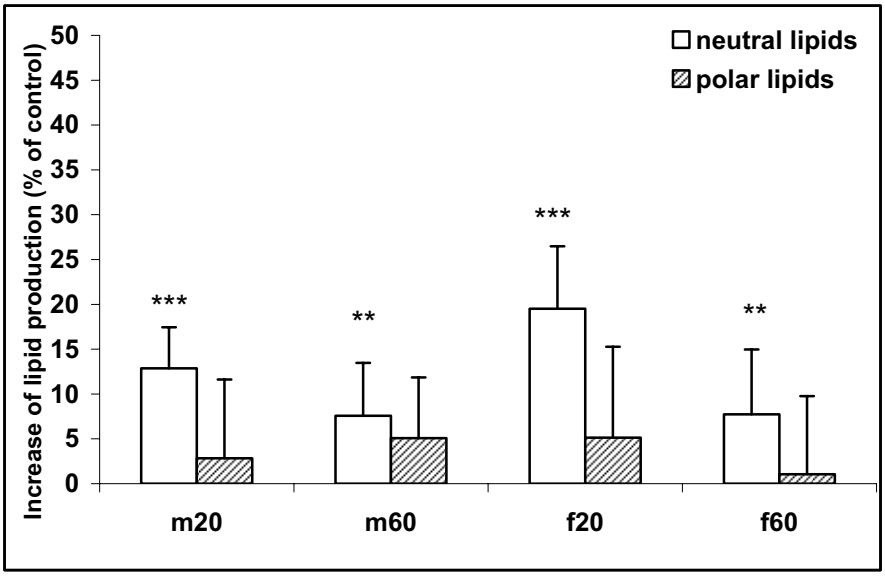


Fig. 3.3 Content of lipids in SZ95 sebocytes incubated with hormones at levels similar to young and elderly men and women by means of nile red microassay. SZ95 sebocytes were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. On day 0 they were treated with IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those in 20- and 60-y-old men (m20, m60) and women (f20, f60) and after 48 h lipid production was measured by means of nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production of the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (** $p<0.01$, *** $p<0.001$).

3.4 Expression of age-associated genes in SZ95 sebocytes incubated with hormones mixture

3.4.1 Expression of c-Myc and fibronectin on mRNA level via *Northern Blotting*

mRNA levels of c-Myc showed a tendency to increase in SZ95 sebocytes maintained at 60-y-old male and female hormone levels compared to those detected in cells at 20-y-old hormone levels after a 5 d treatment (+18% and +20%, respectively) (Fig. 3.4.1). Not significant decreased mRNA levels of fibronectin (-22% and -33%, respectively) were detected in cells at 60-y-old male and female hormone levels compared to those detected at 20-y-old hormone levels after a 5 d treatment (Fig. 3.4.1).

3.4.2 Expression of c-Myc and fibronectin on protein level via *Western Blotting*

Protein levels of c-Myc increased [+ 6% and +64% ($p<0.05$), respectively] in SZ95 sebocytes maintained at 60-y-old male and female hormone levels compared to those detected in cells at 20-y-old hormone levels after a 5 d treatment (Fig. 3.4.2), while protein levels of fibronectin showed a tendency to increase in SZ95 sebocytes at 60-y-old male and female hormone levels compared to those detected in cells at 20-y-old hormone levels after a 5 d treatment [+25% and +47%, respectively] (Fig. 3.4.2). In contrast to the control (untreated cells), cells treated with 60-y-old female hormone levels showed a significant increase [+99%, ($p<0.01$)] of c-Myc protein expression.

3.5 cDNA microarray

Using cDNA microarrays increasing regulation of one gene under female (inhibin, beta A) and no genes under male aging hormone conditions was identified; 3 genes (angiopoetin-like factor and two novel) showed decreasing regulation under female and 16 genes under male hormone aging conditions. There were genes regulating inflammatory signaling (e.g. HLA-C, MAP3K3, CDT6), cell cycle control (e.g. INHBA, HNRPU, BRUNOL5), cytoskeleton (e.g. ACTN1), protein synthesis and processing (e.g. EEF2, NXN), chaperone/transport activity (e.g. VCP) and metabolism/hormone activity (INHBA, DHCR7, MAP3K3) (Fig.3.5).

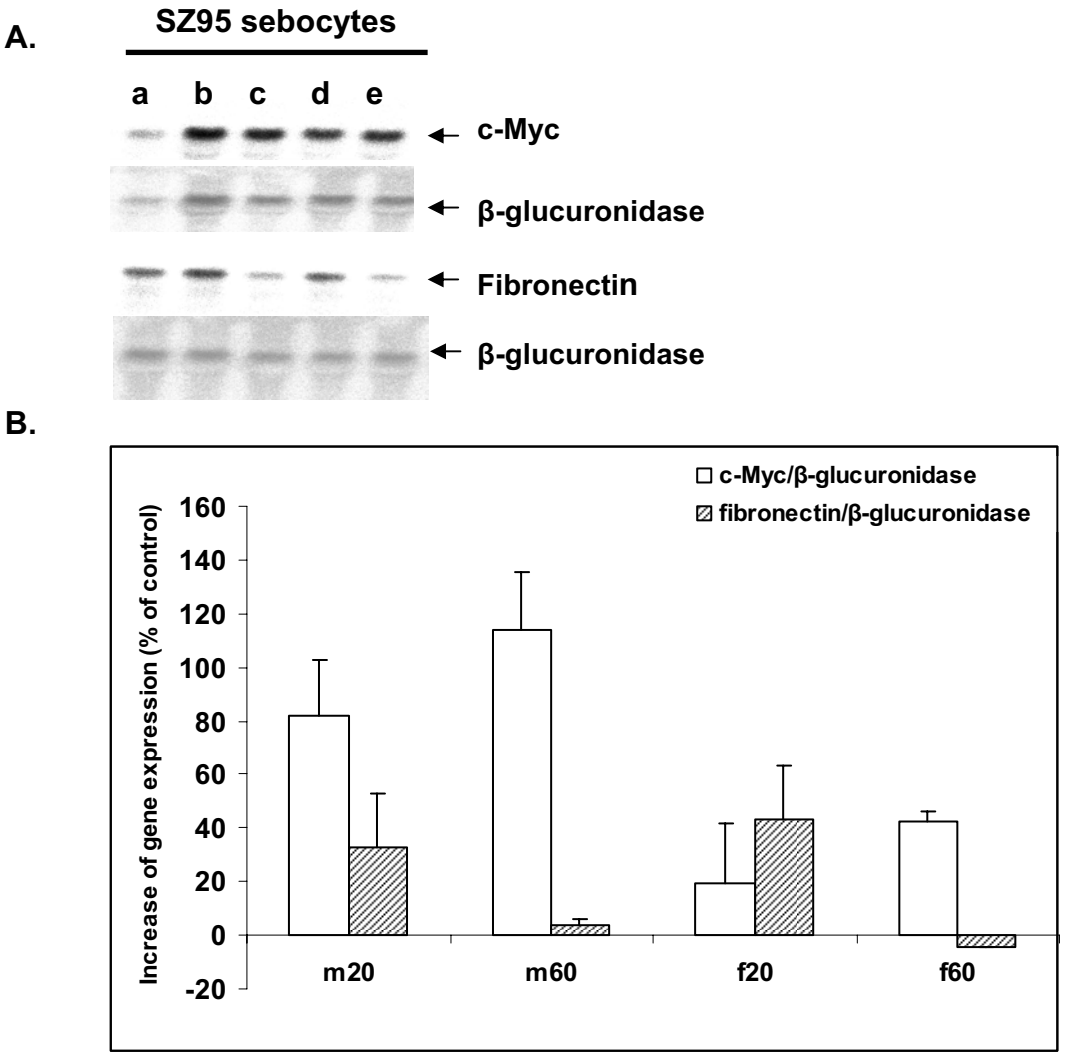


Fig. 3.4.1 Expression of c-Myc and fibronectin in SZ95 sebocytes incubated with hormones at levels similar to young and elderly men and women by means of Northern blotting. Cells were seeded at a density of 300,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. On day 0 cells were treated with two different mixtures of hormones, respectively, containing IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels corresponding to 20-y-old males and females. On day 2 and 4 half of the cells already treated with the 20-y-old male (m20) , female (f20) mixture were treated again with the 20-y-old male, female hormone mixtures, respectively, whereas the other half of the cells was incubated with the same hormone mixture, but at levels similar to 60-y-old males (m60) and females (f60), accordingly. 20 µg RNA was used for the detection of c-Myc and fibronectin via Northern blotting (A). (B) Diagram showing the expression of c-Myc and fibronectin relating to the house-keeping gene β-glucuronidase (a: untreated SZ95 sebocytes, b: m20, c: m60, d: f20, e: f60)**.** As control were used untreated cells and was set at 0%. Gene expression in the treated cells was calculated as percentage of control.

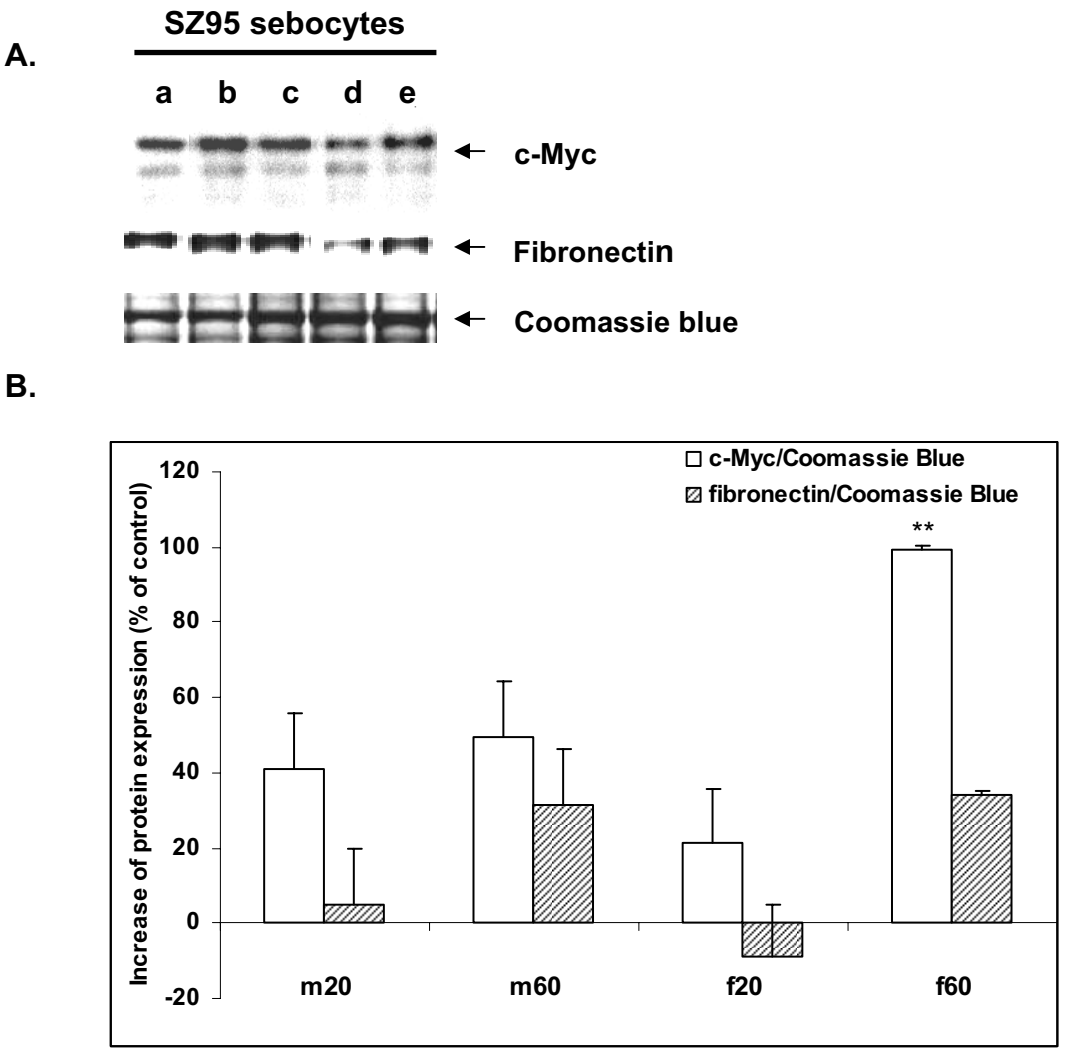


Fig.3.4.2 Expression of c-Myc and fibronectin in SZ95 sebocytes incubated with hormones at levels similar to young and elderly men and women by means of Western blotting. Cells were seeded at a density of 300,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. On day 0 cells were treated with two different mixtures of hormones, respectively, containing IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels corresponding to 20-y-old males and females. On day 2 and 4 half of the cells already treated with the 20-y-old male, female mixture were treated again with the 20-y-old male (m20), female (f20) hormone mixtures, respectively, whereas the other half of the cells was incubated with the same hormone mixture, but at levels similar to 60-y-old males (m60) and females (f60), accordingly. 40 µg of protein was used for the detection of c-Myc and fibronectin via Western blotting (A). (B) Diagram showing the expression of c-Myc and fibronectin relating to the Coomassie Blue gel (a: untreated SZ95 sebocytes, b: m20, c: m60, d: f20, e: f60). As control were used untreated cells and was set at 0%. Protein expression in the treated cells was calculated as percentage of control (** $p<0.01$).

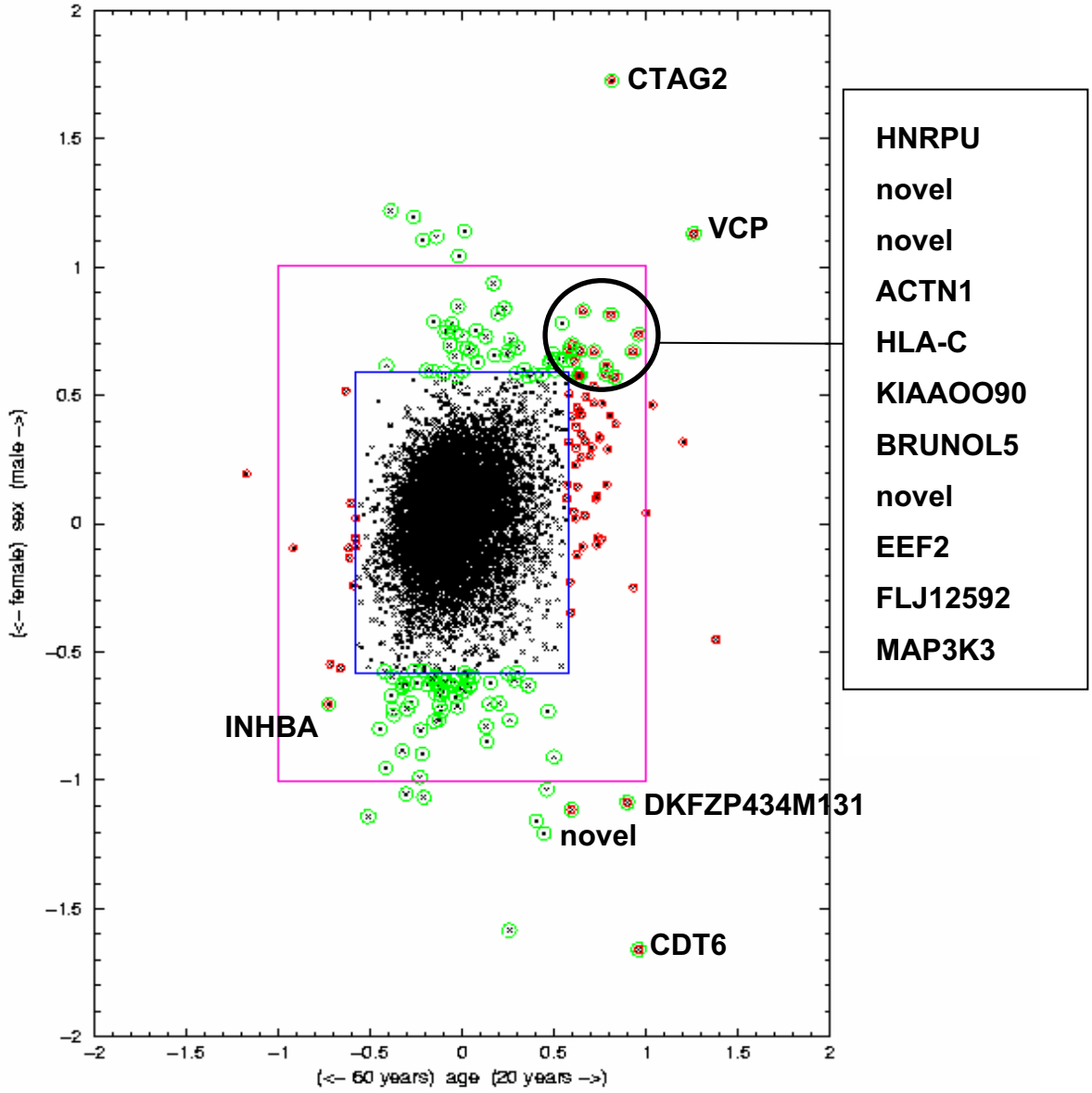


Fig. 3.5 cDNA gene microarray detection of 15,529 genes in SZ95 sebocytes incubated with hormones at levels similar to young and elderly men and women. Black spots: 7846 selected genes that were present in at least one treatment (f20 or f60 or m20 or m60, BG-tag > 0.95). Red circles: 77 targets selected after age (at least 1.5 fold change of the factor A). Green circles: 134 targets selected after sex (at least 1.5 fold change of the factor B). Magenta square marks the 2-fold change area, blue square marks the 1.5-fold change area. Factor A = 0.5 * [log(m20/m60) + log(f20/f60)] (X-axis). Factor B = 0.5 * [log (m20/f20) + log (m60/f60)] (Y-axis). The log is to the base 2, i.e. a „1“ means 2-fold.

3.6 Effects of hormones on SZ95 sebocytes as single agents

3.6.1 IGF-I amplified lipid synthesis in SZ95 sebocytes.

After treatment of the SZ95 sebocytes with IGF-I for 48 h in concentrations similar to the median serum concentration in 20-y-old and 60-y-old individuals (400 ng/ml and 84 ng/ml, respectively), a significant increase of neutral ($p<0.001$) and polar lipids ($p<0.001$) was observed at both concentrations tested (Fig. 3.6.1). The production of neutral lipids was stronger stimulated than that of polar lipids. Moreover, the lipids produced by the cells cultured with IGF-I in concentrations similar to those in elderly persons showed a slight but not significant decline in comparison to the lipids produced by the cells cultured with IGF-I in concentrations similar to those in young individuals.

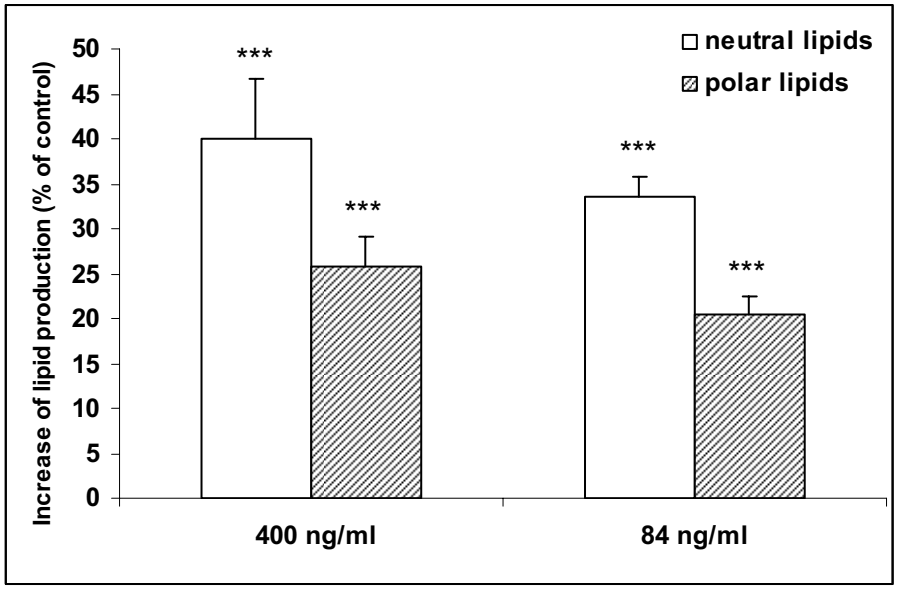


Fig. 3.6.1 Content of lipids in SZ95 sebocytes incubated with IGF-I by means of nile red microassay. SZ95 sebocytes were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. The following day they were treated with IGF-I for 48 h in levels similar to young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (*** $p<0.001$).

3.6.2 GH amplified lipid synthesis in SZ95 sebocytes.

On the other hand, GH in concentrations similar to those found in 20- and 60-y-old individuals (8 ng/ml and 2 ng/ml, respectively) also enhanced significant the production of neutral ($p<0.01$ at 20 y and $p<0.05$ at 60 y) and polar lipids ($p<0.05$ at 20 y and $p<0.01$ at 60 y) in SZ95 sebocytes (Fig. 3.6.2). The production of neutral lipids was stronger stimulated than that of polar lipids. The lipids produced by the cells cultured with GH in concentrations similar to those in aged individuals showed a slight but not significant increase in comparison to the lipids produced by the cells cultured with GH in concentrations similar to those in young individuals.

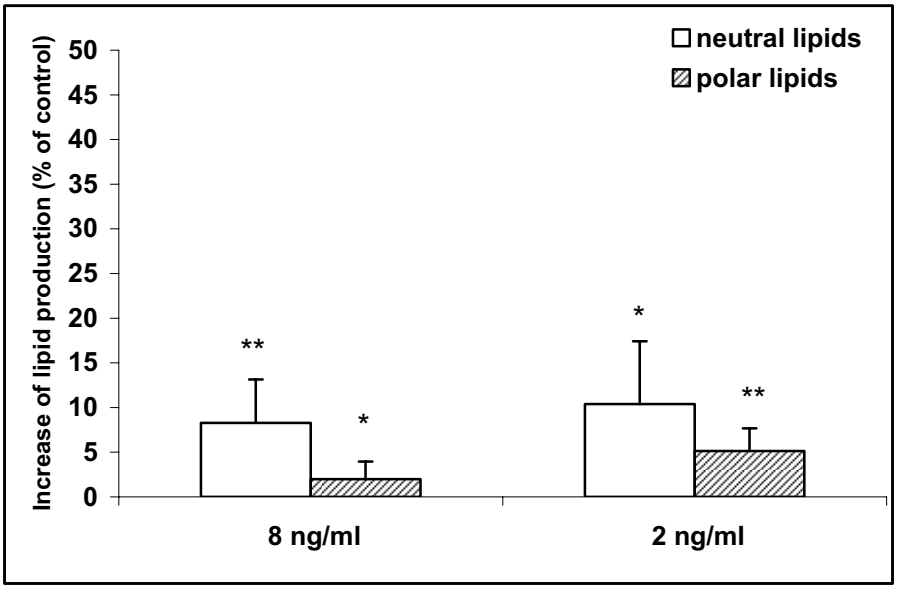


Fig. 3.6.2 Content of lipids in SZ95 sebocytes incubated with GH by means of nile red microassay. SZ95 sebocytes were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. The following day they were treated with GH for 48 h in levels similar to young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (* $p<0.05$, ** $p<0.01$).

3.6.3 17β-estradiol showed an effect on polar lipid synthesis in SZ95 sebocytes.

After having treated SZ95 sebocytes with 17β-estradiol in concentrations similar to those circulating in 20- and 60-y-old males (100 pmol/l) and in 20- and 60-y-old females (1000 pmol/l and 74 pmol/l, respectively) there was no significant change shown in the production of the neutral lipids (Fig. 3.6.3). However, the polar lipid production was significantly increased in contrast to untreated cells in SZ95 sebocytes incubated with 17β-estradiol at levels: 100 pmol/l -corresponding to 20- and 60-y-old men- ($p<0.01$), 1000 pmol/l ($p<0.01$) and 74 pmol/l ($p<0.01$) -corresponding to 20- and 60-y-old women, respectively (Fig.3.6.3).

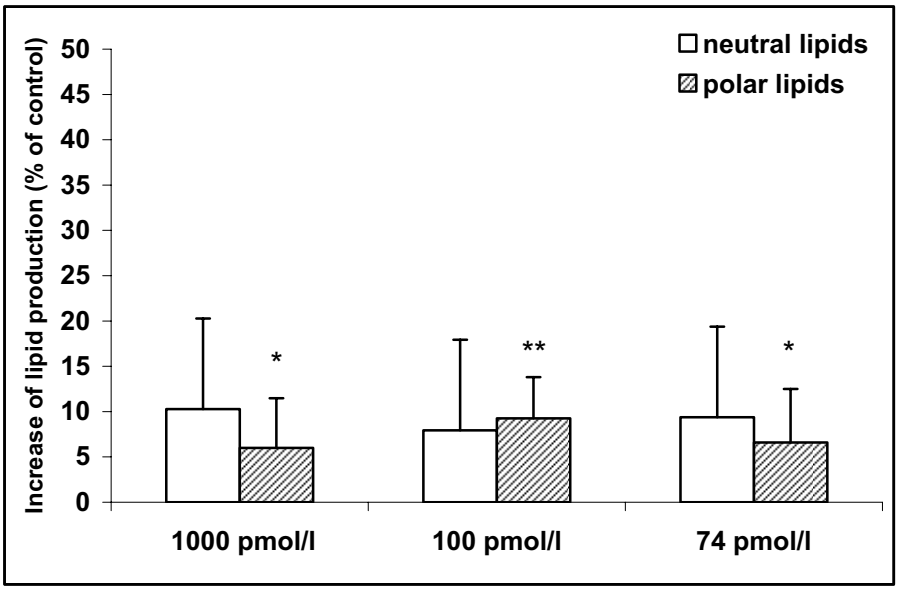


Fig. 3.6.3 Content of lipids in SZ95 sebocytes incubated with 17β-estradiol by means of nile red microassay. SZ95 sebocytes were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. The following day they were treated with 17β-estradiol for 48 h in levels similar to young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (* $p<0.05$, ** $p<0.01$).

3.6.4 Testosterone showed an effect on lipid synthesis in SZ95 sebocytes.

After having treated SZ95 sebocytes with testosterone in concentrations similar to those found in 20- and 60-y-old males (20 nmol/l and 10 nmol/l, respectively) and in 20- and 60-y-old females (2 nmol/l and 0.7 nmol/l, respectively), there was a significant change shown in the

production of neutral lipids in SZ95 sebocytes treated with 20-y-old female levels ($p<0.01$, Fig. 3.6.4). On the other hand, the polar lipid production was significantly increased in cells incubated with testosterone at 20 nmol/l and 10 nmol/l -corresponding to 20- and 60-y-old men- ($p<0.01$ and $p< 0.05$, respectively), and at 2 nmol/l ($p<0.05$) -corresponding to 20-y-old females (Fig. 3.6.4).

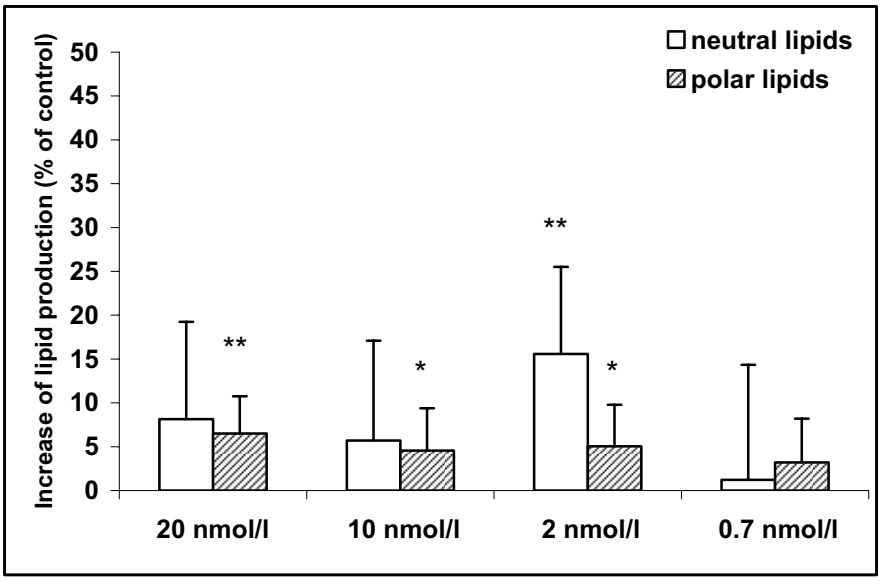


Fig.3.6.4 Content of lipids in SZ95 sebocytes incubated with testosterone by means of nile red microassay. SZ95 sebocytes were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. The following day they were treated with testosterone for 48 h in levels similar to young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (* $p<0.05$, ** $p<0.01$).

3.6.5 Progesterone and DHEA showed no effect on lipid synthesis in SZ95 sebocytes.

After having treated SZ95 sebocytes with progesterone in concentrations similar to those found in 20- and 60-y-old males (0.5 nmol/l) and in 20- and 60-y-old females (50 nmol/l and 1 nmol/l, respectively) and with DHEA at levels corresponding to 20- and 60-y-old males and females (20 nmol/l and 5 nmol/l, accordingly), there was no significant change shown in the production of neutral and polar lipids.

3.6.6 IGF-I, GH, 17β-estradiol, testosterone, progesterone and DHEA showed no effect on proliferation of SZ95 sebocytes.

After having treated SZ95 sebocytes with IGF-I, GH, 17β-estradiol, testosterone, progesterone and DHEA as single agents in concentrations similar to those found in 20- and 60-y-old males and females, no significant effect was observed on cell proliferation. Cell proliferation was measured after 4 d treatment by means of MUH fluorescence assay.

3.7 IGF-I amplified lipid synthesis in SZ95 sebocytes.

After treatment of SZ95 sebocytes with IGF-I and 17β-estradiol for 48 h in concentrations similar to the median serum concentration in 20-y-old females (400 ng/ml and 1000 pmol/l, respectively), the morphology of the cells and the neutral lipid production was documented by means of fluorescence microscopy (Fig. 3.7.1-3). In contrast to untreated SZ95 sebocytes (Fig.3.7.1), cell size of treated SZ95 sebocytes with IGF-I or 17β-estradiol showed to be increased. Furthermore, lipid accumulation was enhanced in IGF-I treated cells, illustrating the induce of cell differentiation and corresponding to the results coming from the nile red microassay.

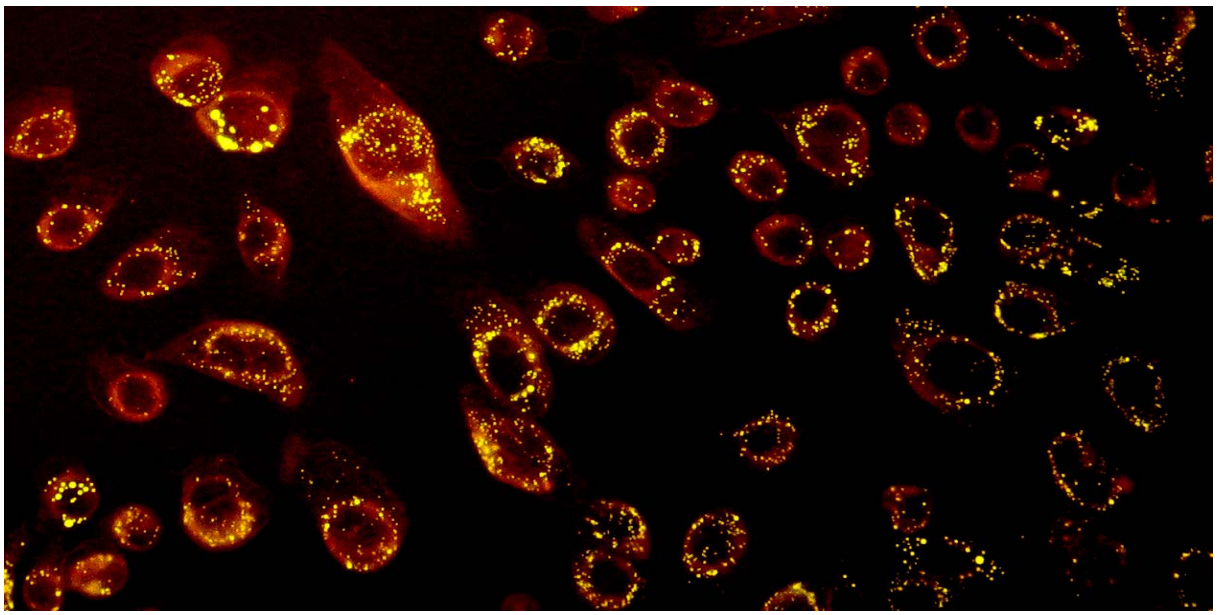


Fig.3.7.1 Content of neutral lipids in untreated SZ95 sebocytes by means of fluorescence microscopy after nile red staining

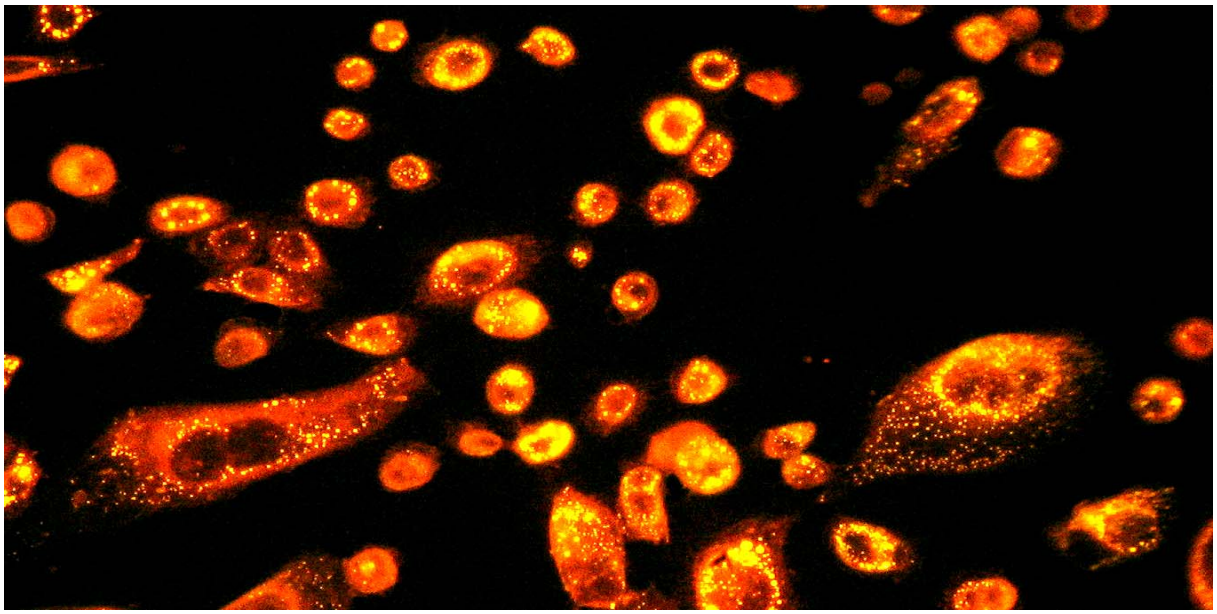


Fig.3.7.2 Content of neutral lipids in SZ95 sebocytes after treatment with IGF-I by means of fluorescence microscopy after nile red staining

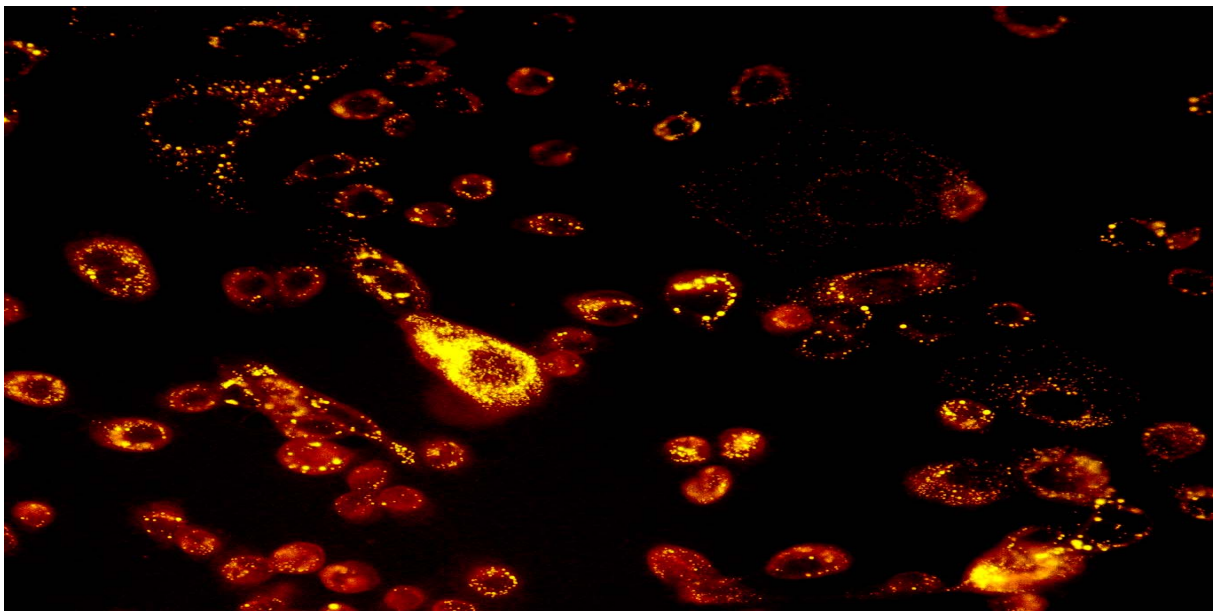


Fig.3.7.3 Content of neutral lipids in SZ95 sebocytes after treatment with 17β-estradiol by means of fluorescence microscopy after nile red staining

3.8 17β-estradiol and IGF-I synthesis in SZ95 sebocytes after treatment with IGF-I and 17β-estradiol

3.8.1 17β-estradiol synthesis in SZ95 sebocytes was enhanced after IGF-I treatment.

The 17β-estradiol synthesis in SZ95 sebocytes treated with IGF-I in a concentration similar to the median serum concentration in young individuals (400 ng/ml) showed a tendency to increase after 48 and 72 h of treatment [+37.5% and +14.5%, respectively] (Fig. 3.8.1).

3.8.2 IGF-I synthesis in SZ95 sebocytes showed no effect after 17β-estradiol treatment.

The IGF-I synthesis in SZ95 sebocytes treated with 17β-estradiol in a concentration similar to the median serum concentration in young women showed no change after 48 and 72 h of treatment, respectively.

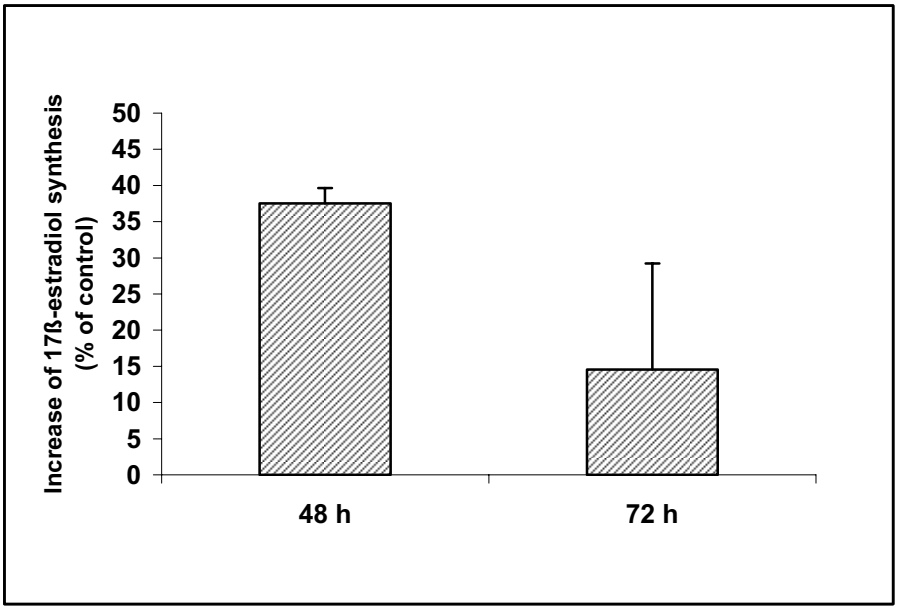


Fig 3.8.1 17ß-estradiol synthesis in SZ95 sebocytes incubated with IGF-I by means of ELISA.
SZ95 sebocytes were seeded at a density of 700,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. The following day they were treated with 400 ng/ml IGF-I for 48 h and 72 h at levels similar to those circulating in young individuals. The production of 17ß-estradiol was measured by means of ELISA. The 17ß-estradiol synthesis of control cells with no treatment was set at 0% and the 17ß-estradiol production in the treated cells was calculated as percentage of control. Values represent the mean of three experiments ± SD.

3.9 Proliferation of fibroblasts incubated with hormone mixture

A significant change was observed in cell proliferation of human foreskin fibroblasts treated with IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those circulating in 20- and 60-y-old men and women (Fig. 3.9) in contrast to cells receiving no treatment ($p<0.001$). Fibroblasts treated with aged male hormone levels showed a slight but not significant decrease of the proliferation in contrast to cells incubated with young hormone levels. The cell proliferation was detected after 4 d treatment by means of MUH fluorescence assay.

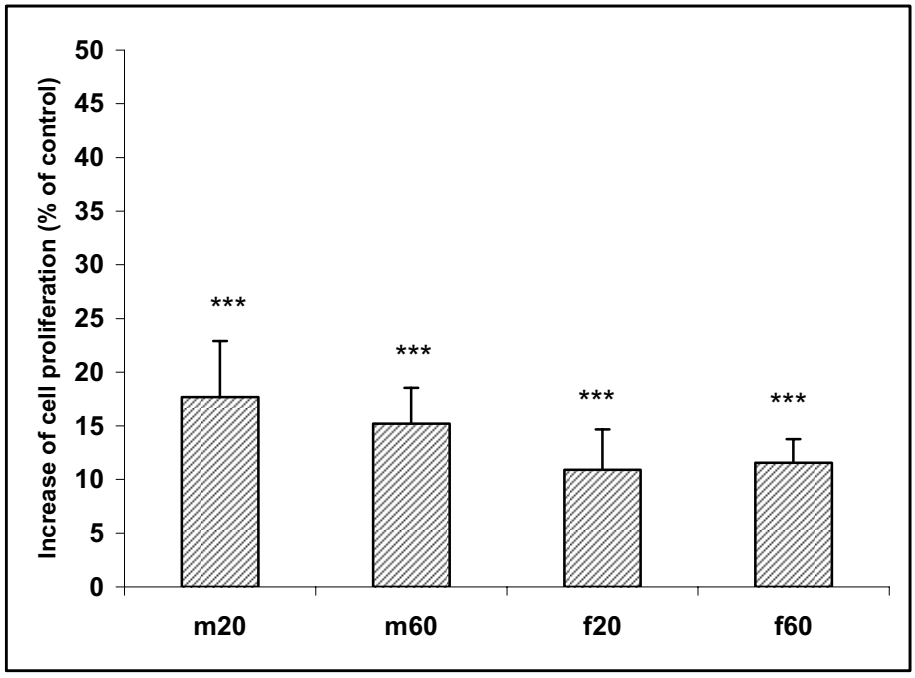


Fig. 3.9 Proliferation of human fibroblasts with hormones at levels similar to young and elderly men and women by means of MUH fluorescence assay. Fibroblasts were seeded at a density of 1,500 cells/well in a 96-well plate and were left 48 h to adhere. On day 0 and 2 they were treated with IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those circulating in 20- and 60-y-old men (m20, m60) and women (f20, f60) and on day 4 proliferation was measured by means of MUH fluorescence assay. Proliferation of control cells with no treatment was set at 0% and proliferation of the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (*** $p<0.001$).

3.10 Content of lipids in fibroblasts incubated with hormones mixture

Fibroblasts incubated with hormones at aged male and female levels (60 y) showed significantly lower content of neutral lipids ($p<0.01$) in contrast to fibroblasts with hormones at young male and female levels (20 y), whereas in contrast to the control the content of neutral lipids of cells with hormones at young and aged male and female levels was significantly increased [$p<0.001$ and $p<0.01$, respectively] (Fig. 3.10, light columns). The same changes were also observed in the polar lipid production (Fig.3.10, dark columns). The lipid production was detected after 2 d treatment by means of nile red microassay.

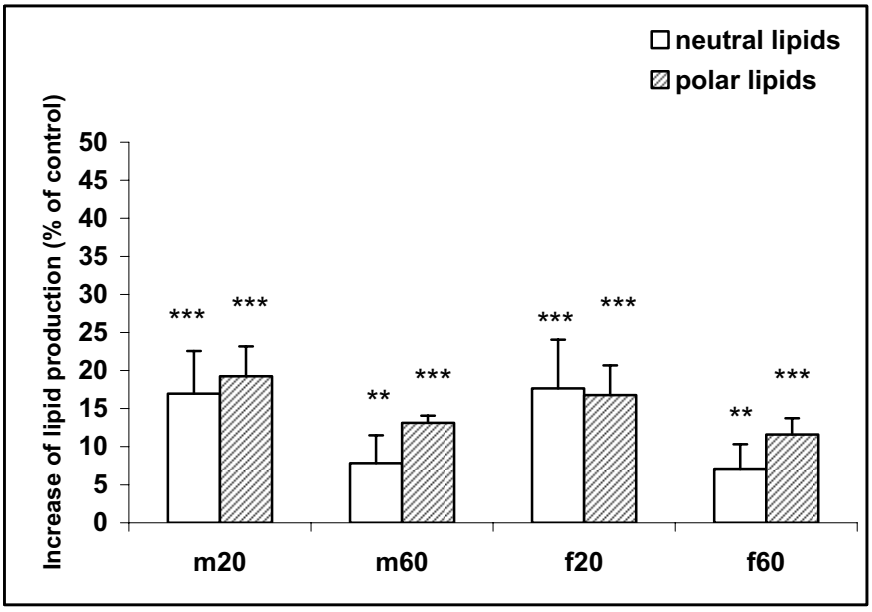


Fig. 3.10 Content of lipids in fibroblasts incubated with hormones at levels similar to young and elderly men and women by means of nile red microassay. Fibroblasts were seeded at a density of 10,000 cells/well in a 96-well plate and were left 48 h to adhere. On day 0 they were treated with IGF-I, GH, 17β-estradiol, progesterone, testosterone and DHEA at levels similar to those circulating in 20- and 60-y-old men (m20, m60) and women (f20, f60) and after 48 h lipid production was measured by means of nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production of the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (** $p<0.01$, *** $p<0.001$).

3.11 Effects of IGF-I and 17ß-estradiol on fibroblasts as single agents

3.11.1 IGF-I but not 17ß-estradiol enhanced fibroblast proliferation.

After treatment of fibroblasts coming from the breast of a 60-y-old woman undergoing surgery with IGF-I or 17ß-estradiol for 96 h in concentrations similar to the median serum concentration in 20- and 60-y-old women, there was only a significant increase of fibroblast proliferation after treatment with IGF-I ($p<0.001$) (Fig. 3.11.1).

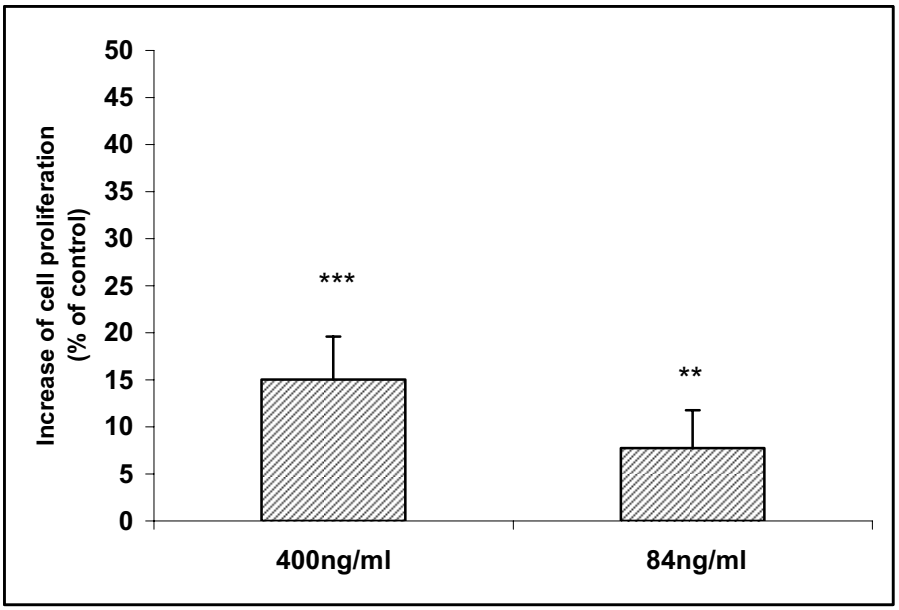


Fig. 3.11.1 Proliferation of human fibroblasts incubated with IGF-I at levels similar to young and elderly men and women by means of MUH fluorescence assay. Breast fibroblasts were seeded at a density of 1,500 cells/well in a 96-well plate and were left 48 h to adhere. On day 0 and 2 they were treated with IGF-I at levels similar to those in 20- and 60-y-old men and women and on day 4 cell proliferation was measured by means of MUH fluorescence assay. Cell proliferation of control cells with no treatment was set at 0% and the cell proliferation of the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (** $p<0.01$, *** $p<0.001$).

3.11.2 IGF-I amplified lipid synthesis in fibroblasts.

After treatment of the breast fibroblasts with IGF-I for 48 h in concentrations similar to the median serum concentration in 20- and 60-y-old women (400 ng/ml and 84 ng/ml, respectively), a significant increase of neutral and polar lipids was observed at both concentrations tested [$p<0.001$] (Fig. 3.11.2). The production of neutral lipids was stronger

stimulated than that of polar lipids. Moreover, the lipids produced by the cells cultured with IGF-I in concentrations similar to those in elderly women showed a slight but not significant decline in comparison to the lipids produced by the cells cultured with IGF-I in concentrations similar to those in young women.

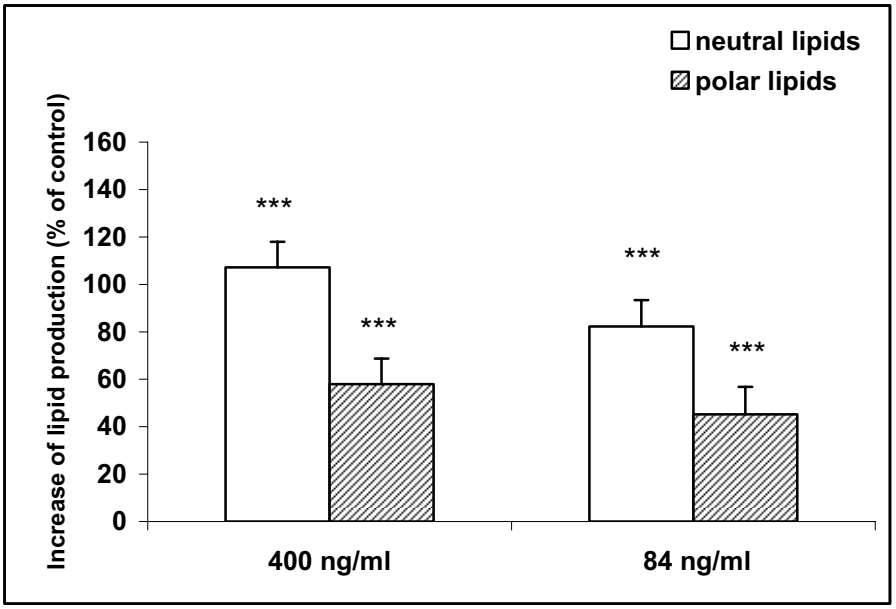


Fig. 3.11.2 Content of lipids in human fibroblasts incubated with IGF-I by means of nile red microassay. Breast fibroblasts were seeded at a density of 1,500 cells/well in a 96-well plate and were left 48 h to adhere. Then, they were treated with IGF-I for 48 h in levels similar to young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (*** $p<0.001$).

3.11.3 17β-estradiol amplified lipid synthesis in fibroblasts.

After treatment of the breast fibroblasts with 17β-estradiol in concentrations similar to the median serum concentration in 20- and 60-y-old women (1000 pmol/l and 74 pmol/l, respectively), a significant increase of neutral and polar lipids was observed ($p<0.001$) in contrast to control cells with no treatment. The lipids produced by the fibroblasts cultured with 17β-estradiol in concentrations similar to those in postmenopausal women showed no significant difference in comparison to the lipids produced by the cells cultured with 17β-estradiol in concentrations similar to those in young women (Fig. 3.11.3).

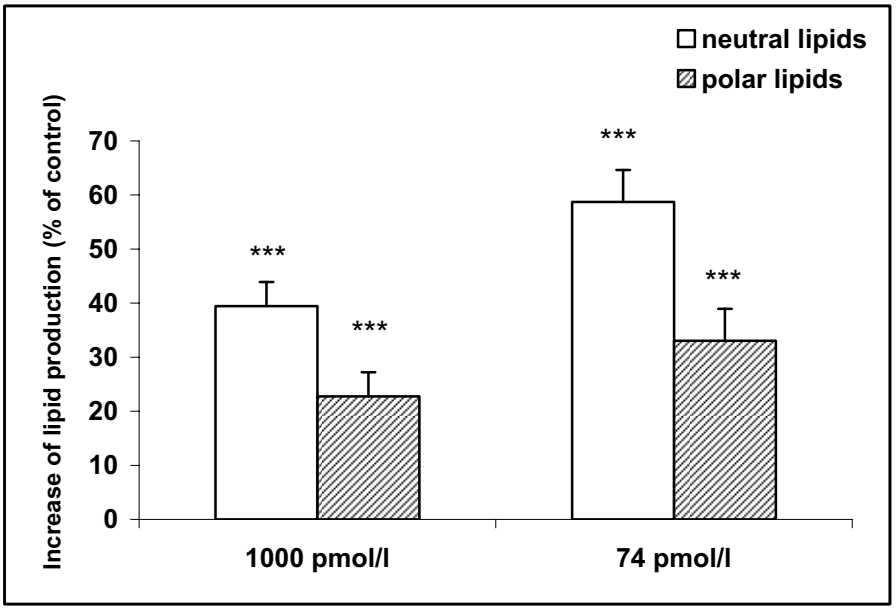


Fig. 3.11.3 Content of lipids in human fibroblasts incubated with 17ß-estradiol by means of nile red microassay. Breast fibroblasts were seeded at a density of 1,500 cells/well in a 96-well plate and were left 48 h to adhere. The following day they were treated with 17ß-estradiol for 48 h in levels similar to those circulating in young and elderly individuals. Neutral lipids (light columns) and polar lipids (dark columns) were measured with the nile red microassay. Lipid production of control cells with no treatment was set at 0% and the lipid production in the treated cells was calculated as percentage of control. Values represent the mean of ten experiments ± SD (*** $p<0.001$).

3.12 17β-estradiol and IGF-I synthesis in fibroblasts after treatment with IGF-I and 17β-estradiol

3.12.1 IGF-I synthesis in fibroblasts was enhanced after treatment with 17β-estradiol.

The IGF-I synthesis in breast fibroblasts treated with 17β-estradiol in a concentration similar to the median serum concentration circulating in young women was enhanced (+29%, $p<0.01$) at 72 h with no effect detected at 48 h of treatment (Fig. 3.12.1A).

3.12.2 17β-estradiol synthesis in fibroblasts was enhanced after treatment with IGF-I.

The 17β-estradiol synthesis also increased but not significantly in breast fibroblasts [+19.6% and +14.3% after 48 and 72 h of treatment, respectively] (Fig. 3.12.1B).

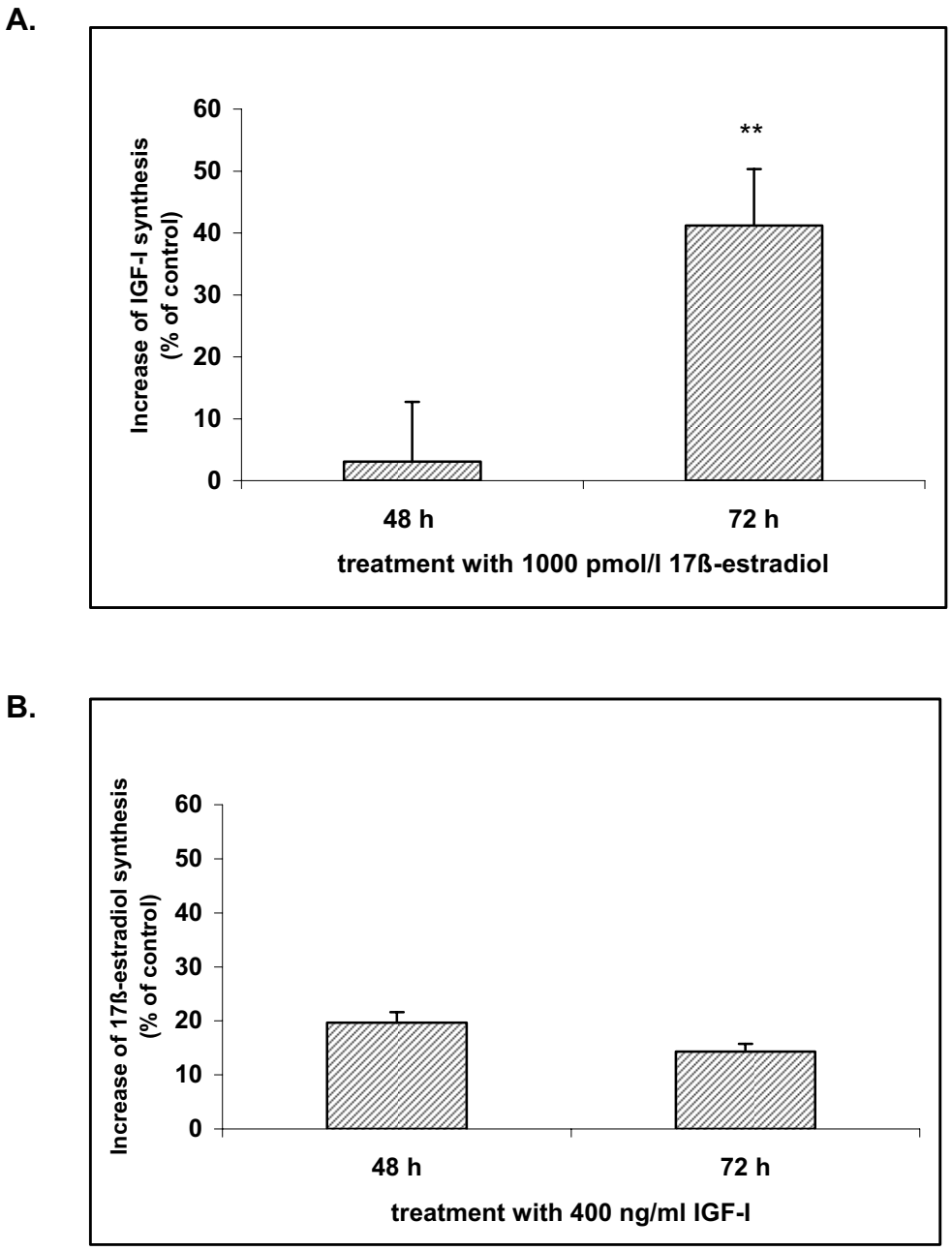


Fig. 3.12.1 IGF-I and 17ß-estradiol synthesis in human fibroblasts incubated with 17ß-estradiol and IGF-I, respectively, by means of ELISA. Breast fibroblasts were seeded at a density of 100,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. The following day they were treated with 17ß-estradiol (A) and IGF-I (B) for 48 h and 72 h in levels similar to those circulating in young female individuals. The production of IGF-I and 17ß-estradiol was measured by means of ELISA. Control cells with no treatment were set at 0% and the production of the hormones in the treated cells was calculated as percentage of control. Values represent the mean of three experiments ± SD (** $p<0.01$).

3.13 Effects of IGF-I and 17ß-estradiol on IGF-IR and ERα in SZ95 sebocytes as single agents

3.13.1 IGF-IR expression was influenced under IGF-I and 17β-estradiol treatment in SZ95 sebocytes.

The regulation of IGF-IR in SZ95 sebocytes was investigated after exposure of the cells to IGF-I and 17β-estradiol in concentrations similar to the median serum concentration in young women (400 ng/ml and 1000 pmol/l, respectively). In contrast to control cells receiving no treatment, IGF-IR expression was first increased in SZ95 sebocytes under IGF-I treatment after 3 and 6 h [+13% and +21.4% ($p<0.05$), respectively], then reduced after 12 and 24 h treatment [-30.7% ($p<0.05$) and -27.6%, respectively] and was followed by an increase after 48 and 72 h [+19.9% ($p<0.01$) and +9% ($p<0.05$), respectively], (Figs. 3.13.1, 3.13.2A). On the other hand, after treatment with 17β-estradiol, a slight decrease of the IGF-IR expression was shown after 3 h (-9.6%), an increase after 6 h (+24%, $p<0.05$), followed by a decrease of IGF-IR after 12 , 24 and 48 h [-35.2% ($p<0.01$), -3.7%, -20% ($p<0.01$), respectively] and finally by an increase of IGF-IR after 72 h [+42.9% ($p<0.05$)] (Figs. 3.13.1, 3.13.2B).

3.13.2 ERα expression under IGF-I and 17β-estradiol treatment in SZ95 sebocytes.

ERα expression was initially increased under IGF-I treatment after 3, 6, 12 and 24 h [+49%, ($p<0.05$), +4.4%, +3.9%, +13% ($p<0.001$), respectively] in contrast to untreated cells, and was followed by normalization after 48 h [-0.5%] (Figs. 3.13.1, 3.13.3A). On the other hand, after treatment with 17β-estradiol, an increase of the ERα expression was detected after 3 and 6 h (+37.3% and +11.8%), followed by a slight decrease after 12 h (-2.5%) and an increase after 24 and 48 h [+19.7% ($p<0.001$) and +6.7 %, respectively]. After 72 h treatment no expression of ERα was detected.

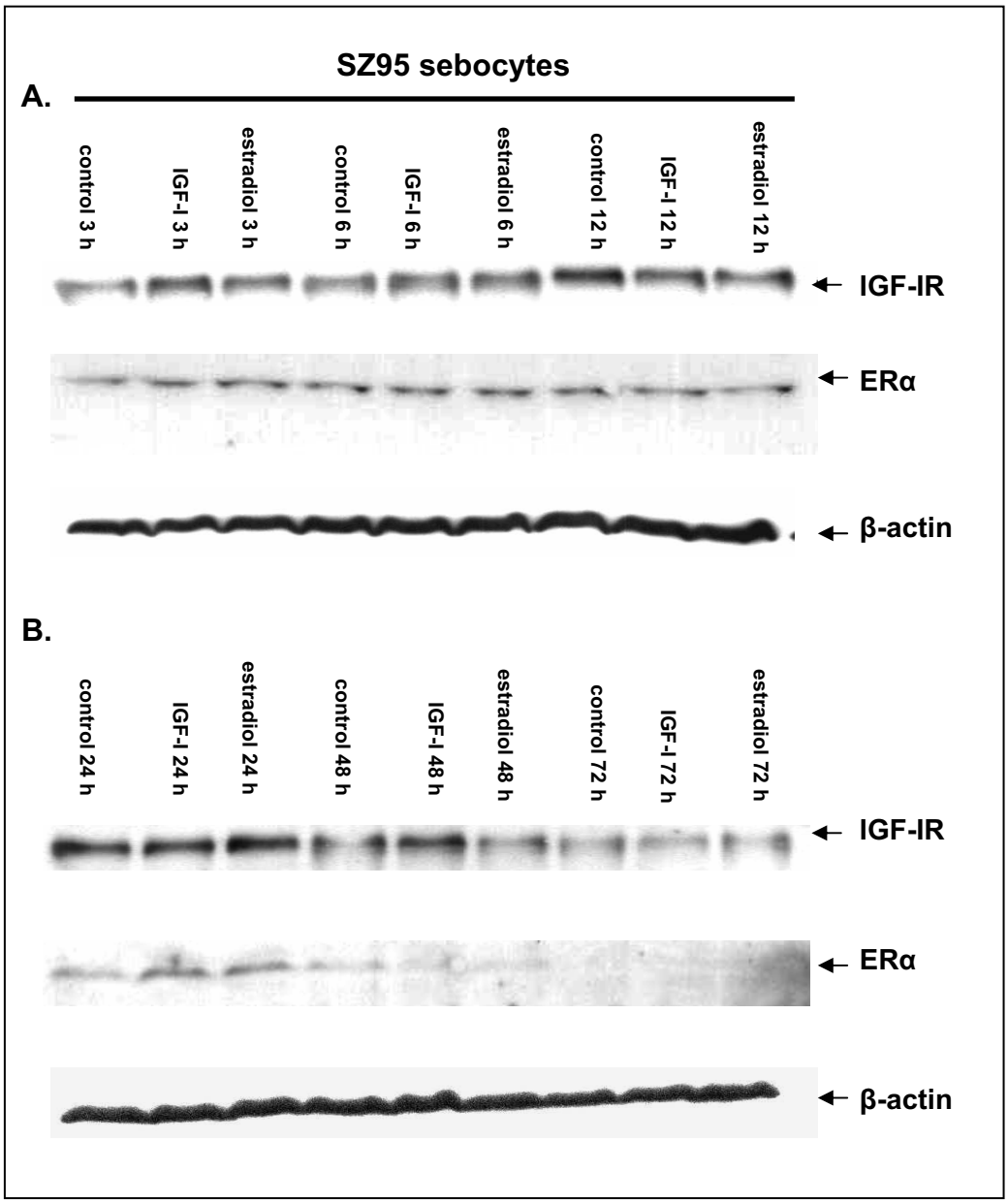
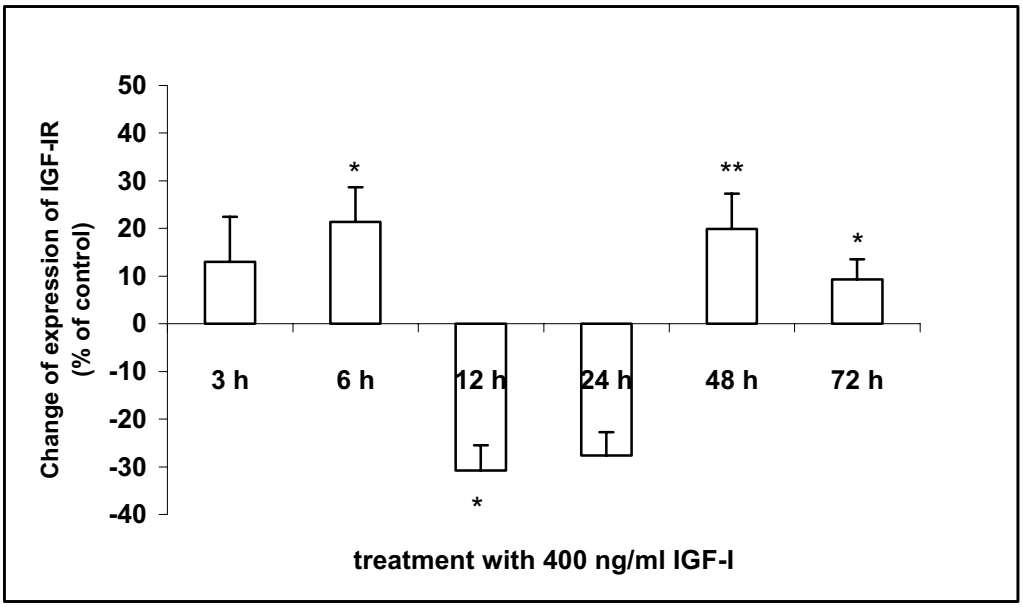


Fig. 3.13.1 Expression of IGF-IR and ERα in SZ95 sebocytes incubated with IGF-I and 17β-estradiol by means of Western blotting. Cells were seeded at a density of 700,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. On day 0 cells were treated with IGF-I and 17β-estradiol, respectively at levels corresponding to 20-y-old females. After 3, 6, 12, 24, 48 and 72 h treatment, cell proteins were collected and 40 µg of protein from each probe was used for the detection of IGF-IR, and ERα via Western blotting. Picture also showing the expression of β-actin of the tested probes, which was used as control of the protein quantity.

A.



B.

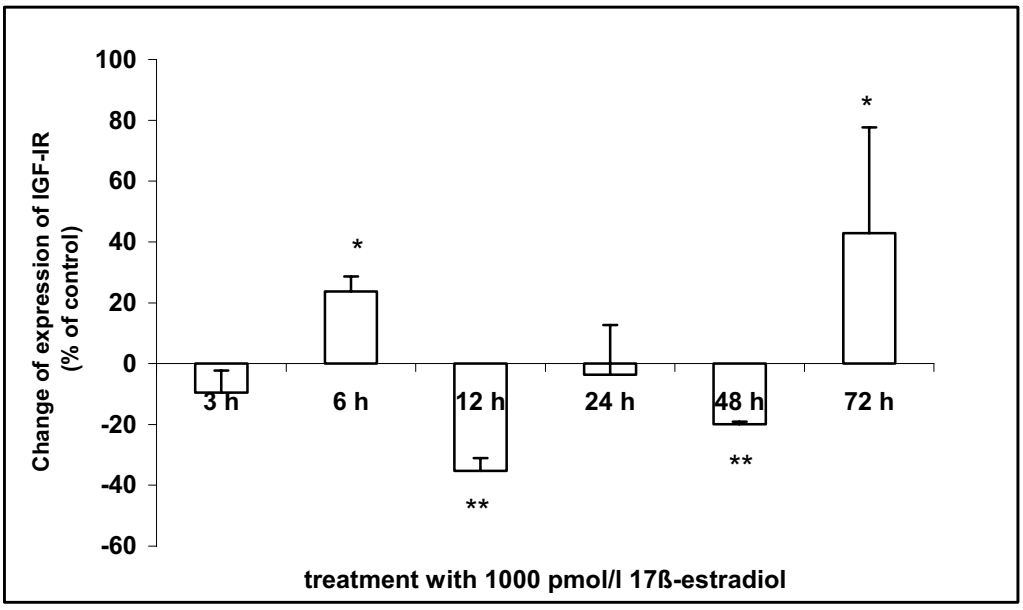
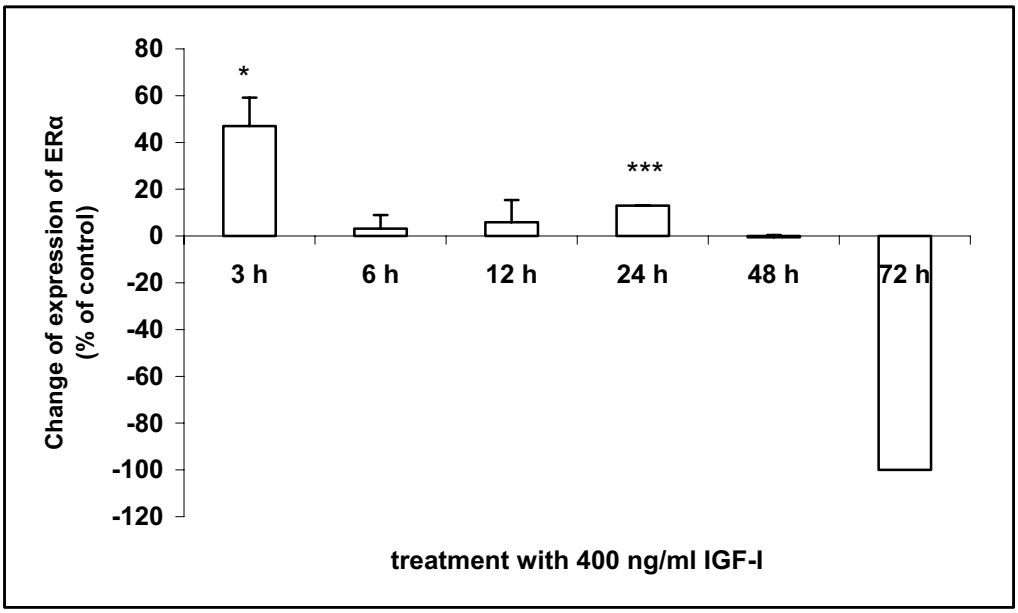


Fig. 3.13.2 Diagrams showing the time-dependent pattern of the expression of IGF-IR in SZ95 sebocytes after treatment with 400 ng/ml IGF-I (A) or 1000 pmol/l 17β-estradiol (B) by means of Western blotting. The receptors expression of control cells with no treatment was set at 0% and expression in the treated cells was calculated as percentage of control. Diagrams showing the expression of IGF-IR relating to the β-actin expression of the probes. Values represent the mean of three experiments ± SD (* $p<0.05$, ** $p<0.01$).

A.



B.

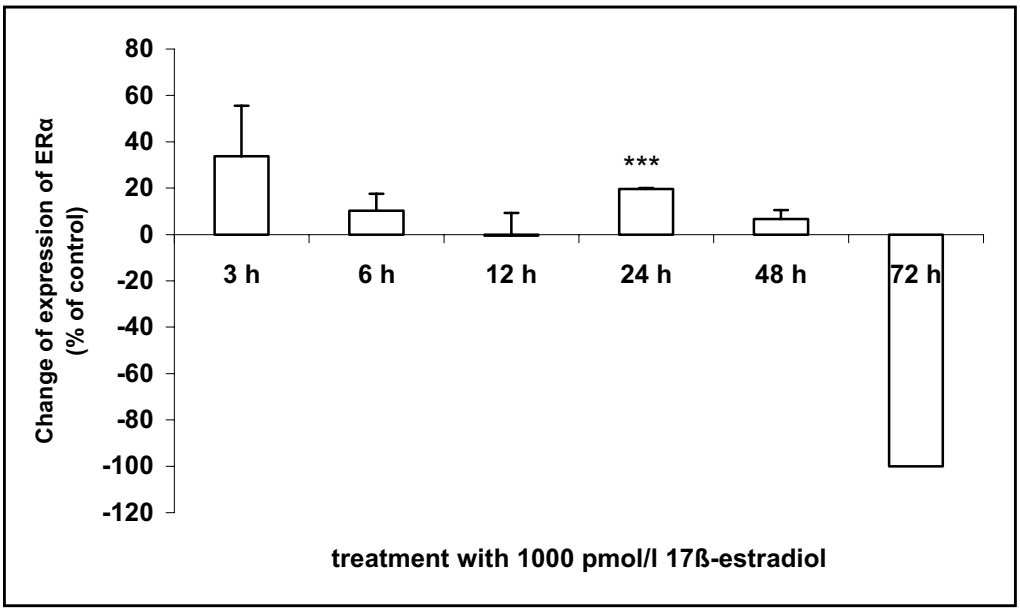


Fig. 3.13.3 Diagrams showing the time-dependent pattern of the expression of ERα in SZ95 sebocytes after treatment with 400 ng/ml IGF-I (A) or 1000 pmol/l 17β-estradiol (B) by means of Western blotting. The receptors expression of control cells with no treatment was set at 0% and expression in the treated cells was calculated as percentage of control. Diagrams showing the expression of ERα relating to the β-actin expression of the probes. Values represent the mean of three experiments ± SD (* $p<0.05$, *** $p<0.001$).

3.14 Effects of IGF-I and 17ß-estradiol on IGF-IR in fibroblasts as single agents

3.14.1 IGF-IR expression was reduced under IGF-I treatment

In breast fibroblasts, IGF-IR expression was significantly reduced under IGF-I treatment at 48 h (-60.3%, $p<0.01$) and this reduction remained detectable after 72 h [-60.6%, $p<0.01$] (Figs. 3.14.1, 3.14.2A). After treatment with 17β-estradiol, no change was initially observed in contrast to the control, however, IGF-IR expression declined significantly after 72 h [-18%, $p<0.01$] (Figs. 3.14.1, 3.14.2B).

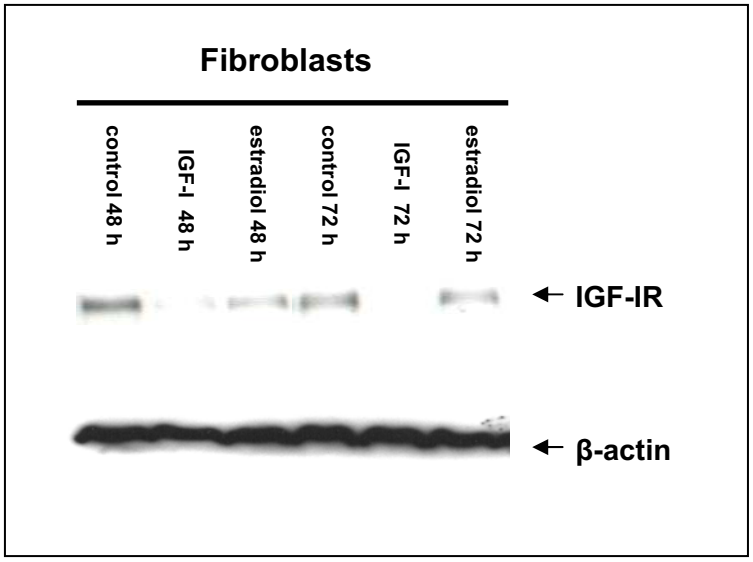
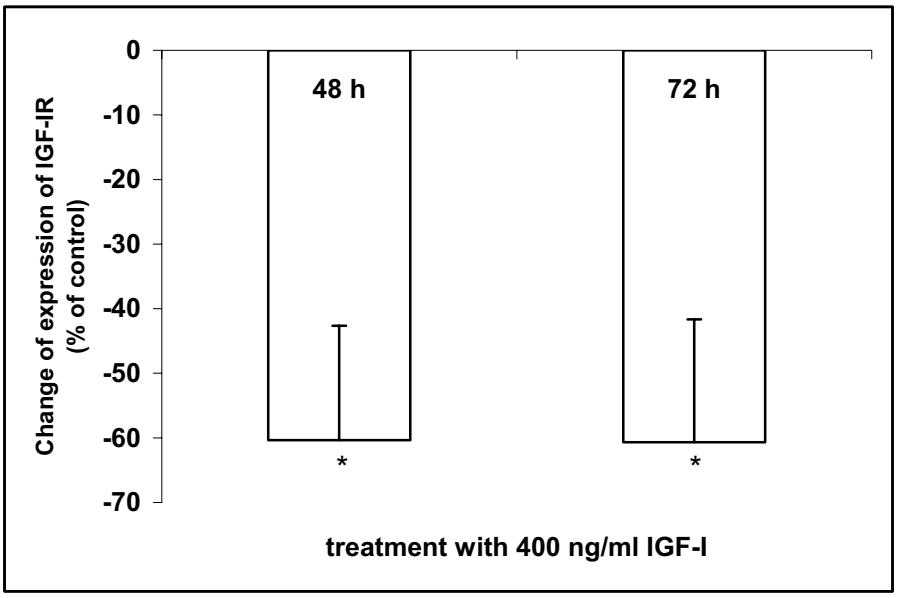


Fig. 3.14.1 Expression of IGF-IR in breast fibroblasts incubated with IGF-I and 17β-estradiol by means of Western blotting. Cells were seeded at a density of 100,000 cells/flask in a 75 cm^2 flask and were left 48 h to adhere. On day 0 cells were treated with IGF-I and 17β-estradiol, respectively at levels corresponding to 20-y-old females. After 48 and 72 h treatment, proteins were collected from each probe and 40 µg of protein was used for the detection of IGF-IR via Western blotting. Picture also showing the expression of β-actin of the tested probes, which was used as control of the protein quantity.

A.



B.

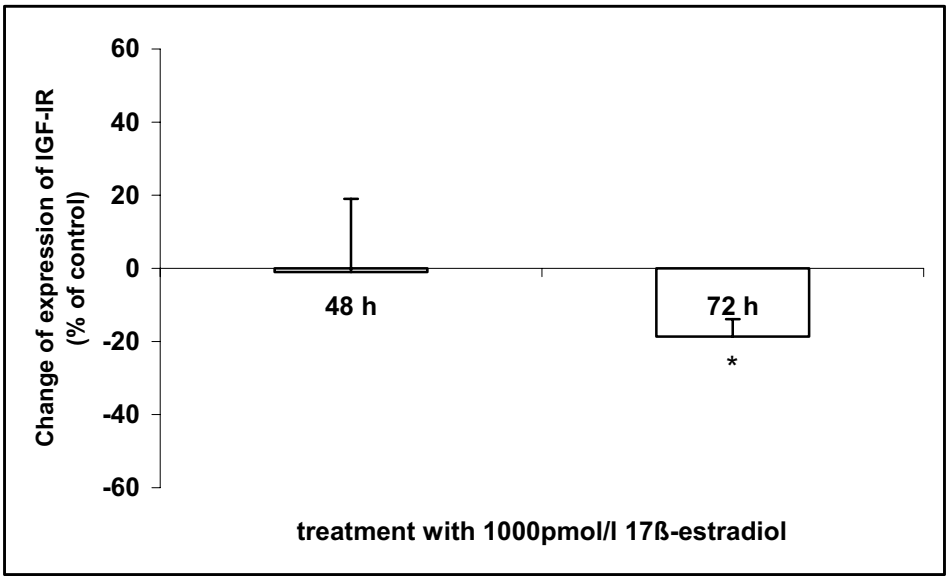


Fig. 3.14.2 Diagrams showing the time-dependent pattern of the expression of IGF-IR in breast fibroblasts after treatment with 400 ng/ml IGF-I (A) or 1000 pmol/l 17β-estradiol (B) by means of Western blotting. The IGF-IR expression of control cells with no treatment was set at 0% and in the treated cells it was calculated as percentage of control. Diagrams showing the expression of IGF-IR relating to the β-actin expression of the probes. Values represent the mean of three experiments ± SD (* $p<0.05$).