

High Glucose Modulates Responsiveness to Estrogens of Human Derived Female Cultured Osteoblasts and Osteoblastic Cell Lines

D Somjen^{*}, S Katzburg^{1,2}, E Knoll^{1,2}, O Sharon^{1,2} and N Stern^{1,2}

¹Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center, Tel-Aviv - 64239, Israel

²Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Received September 02th, 2019; Revised November 04th, 2019; Accepted November 06th, 2019

ABSTRACT

Human female-derived cultured osteoblasts (hObs) and human female osteoblastic cell lines (SaOS2 and hfoB) express mRNAs involved in bone biology and physiology, such as estrogen receptors α (ER α) and β (ER β), vitamin D receptor (VDR), 1α , 25 (OH) vitamin D₃ hydroxylase (1OHase) and 12 and 15 lipoxygenases (12LO and 15LO). These mRNAs are modulated by estrogenic compounds. Since the skeletal protective effects of estrogens are not discernible in diabetic women, we tested the estrogenic modulations of these parameters in cells grown in growth medium containing high glucose (HG; 9.0 g/L; 44 mM) compared to normal glucose (NG; 4.5 g/L; 22 mM). HG significantly increased DNA synthesis (DNA) and creatine kinase specific activity (CK). Stimulations of DNA but not of CK by E₂, by 4, 4', 4''-[4-propyl-(1H)-pyrazol-1, 3, 5-triyl] tris-phenol (PPT; ER α specific agonist) or by 2, 3-bis (4-hydroxyphenyl)-propio-nitrile (DPN; ER β specific agonist), were modulated by HG.

HG itself up-regulated the expression of mRNA of 12LO and 15LO and to less extent ER β and VDR, but no effect on ER α and 1OHase mRNA expression. The different hormonal treatments modulated the expression of VDR, 1OHase, 12LO and 15LO mRNAs which were reduced in HG, whereas the induction of their products 1α , 25 dihydroxy- vitamin D₃ (1,25D) and 12- and 15-hydroxyeicosatetraenoic acid (12 and 15 HETE) were only slightly affected by HG. The exact mechanism of HG effects on bone cell responses and its relationship to human bone physiology is not yet clear.

Keywords: Human osteoblasts, Hyperglycemia, ERs, VDR, 1OHase, LOs, Estrogens

INTRODUCTION

We have previously studied the effects of estrogens on rat bone physiology using the increase of the specific activity of creatine kinase (CK) as a response marker [1,2]. The brain type (BB) iso-enzyme of CK which is part of the “energy buffer” system, regulates the cellular concentration of ATP and ADP and is a response marker to different hormones including estrogenic compounds, in bone cells *in vivo* and *in vitro* [3,4] which contain detectable concentrations of E₂ receptors [5,6]. Estrogens are known for their beneficial effect in osteoporosis [5], characterized by reduction in bone mineral density, leading to fractures after minimal trauma. The biological effects of estrogens in cells and tissues, is initiated by binding to estrogen receptors (ERs). Two ERs have been identified, ER α and ER β , which differ in their structure and tissue distribution [6]. Although estrogen treatment is efficient in preventing bone loss, it can also stimulate the growth of estrogen-dependent tumors. Hence, new compounds, which can replace the current used

hormonal therapy treatments with no such deleterious effects, are highly desirable [7].

In human-derived cultured osteoblasts (hObs), we found that E₂ increased cell proliferation and CK specific activity in a gender specific manner [8].

Diabetes is associated with a net loss of bone [9,10], with reduction of new bone formation and decreased bone

Corresponding author: Dalia Somjen, PhD, Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center, 6 Weizman St., Tel-Aviv - 64239, Israel, Tel: 972 3 6973306; Fax: 972 3 6973421; E-mail: dsomjen10@gmail.com

Citation: Somjen D, Katzburg S, Knoll E, Sharon O & Stern N. (2020) High Glucose Modulates Responsiveness to Estrogens of Human Derived Female Cultured Osteoblasts and Osteoblastic Cell Lines. *Adv Res Endocrinol Metab*, 2(1): 46-59.

Copyright: ©2020 Somjen D, Katzburg S, Knoll E, Sharon O & Stern N. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

mineral density. In diabetic mice the up-regulation of specific transcription factors is attenuated, resulting in deficiency in conversion of mesenchymal cells to osteoblasts [9,10].

We have found previously that E₂ and other estrogenic compounds stimulate also vitamin D (VDR) and 1OHase mRNA expression and activity, measured by 1 α , 25 hydroxy vitamin D₃ (1,25D) formation [11-13], as well as lipoxigenase mRNA (12 and 15LO) expression and activity, measured by HETE formation (12 and 15HETE) [13,14].

We now analyzed the effects of high glucose (HG) in the growth medium on the response to estrogenic compounds of human-derived cultured bone cells, as well as the human female bone cell lines the SaSO2 and hfoB, which is relevant to the important factors existing in diabetes. The compounds analyzed were E2 and the ER α and ER β specific analogs, i.e., DPN and PPT.

In the present study we analyzed:

1. Modulation by NG and HG of DNA and CK basal activities and their responses to treatment with E2, DPN and PPT.
2. Modulation by NG and HG of VDR and 1OHase basal expression as well as 1,25D formation and their responses to treatment with E2, DPN and PPT.
3. Modulation by NG and HG of 12 and 15 LO basal expression as well as 12 and 15HETE formation and their responses to treatment with E2, DPN and PPT.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade. E₂, DPN (ER β specific agonist, 4, 4', 4''-[4-propyl-(1H)-pyrazol-1, 3, 5-triyl] tris-phenol), PPT (ER α specific agonist; 2, 3-bis 4-hydroxyphenyl)-propionitrile) and creatine kinase (CK) assay kit were purchased from Sigma Chemicals Co. (St. Louis, MO).

Cell cultures

1. Human bones were obtained from biopsies of patients undergoing corrective surgery following accidental injury, hip or knee replacement. All patients (women) were healthy, non-osteoporotic and not receiving hormonal replacement treatment. Two groups were defined: pre-menopausal women, ranging between 37-55 years old (n=5) and post-menopausal women, ranging between 60-84 years old (n=5). The non-enzymic method for isolation and culture of human bone cells and their characterization as osteoblasts was described previously [8]. Cell outgrowth from bone explants was apparent after 6-10 days. First passage cells were seeded at a density of 3×10^5 cells per 35 mm tissue culture dish in phenol red free DMEM with

10% charcoal stripped FCS and incubated at 37°C in 5% CO₂. To obtain "high glucose" (HG) conditions, the medium including the FCS, was supplemented with glucose up to a final concentration of 44 nM (9.0 g/L), whereas glucose concentration in the regular medium (NG) was 22 nM (4.5 g/L).

2. SaSO2 and hfoB human female derived bone cell lines were obtained from ATCC and were grown as instructed.

Hormonal treatment

At sub-confluence cells were treated with 30 nM E₂, 300 nM DPN or 3 μ M PPT for 24 h, followed by harvesting for the different assays.

Creatine kinase (CK) extraction and assay

Cells were scraped off the culture dishes and homogenized by freezing and thawing three times in cold isotonic extraction buffer. Supernatant extracts were obtained by centrifugation at 14000x g for 5 min at 4°C in an Eppendorf micro-centrifuge. CK specific activity was measured in a Kontron Model 922 Uvicon Spectrophotometer at 340 nm using a Sigma coupled assay kit (procedure 47-UV). Protein was assayed by Coomassie brilliant blue dye binding, using BSA as the standard [8].

Assessment of DNA synthesis

Cells were grown until sub-confluence and then treated with various hormones as indicated. 22 h later, ³[H] thymidine was added for 2 h. Cells were then treated with 10% ice-cold trichloroacetic acid (TCA) for 5 min and washed twice with 5% TCA and then with cold ethanol. The cellular layer was dissolved in 0.3 ml of 0.3 N NaOH, samples were aspirated and ³[H] thymidine incorporation into DNA was assayed [1].

Determination of mRNA for ER α , ER β , VDR, 25 hydroxy vitamin D₃ 1- α hydroxylase (1OHase), 12LO and 15LO by real time PCR

RNA was extracted from cultured human bone cells and subjected to reverse transcription as previously described for the different parameters [12,14-16].

Assessment of 1OHase activity

1OHase activity was assessed by measuring 1,25 (OH)₂D₃ (1,25D) generated in hObs within 60 min after the addition of 25(OH)D₃ (200 ng/ml) to cultures, using 1,25D ¹²⁵I RIA kit from Dia Sorin, Mn, USA. Protein was assayed by Coomassie brilliant blue dye binding, using BSA as standard.

Assessment of 12 and 15LO activity

12 lipoxigenase (12LO) and 15 lipoxigenase (15LO) activities were assessed by measuring 12HETE and 15HETE formation. Cells and medium were extracted for HETE

formation and analyzed by HPLC as previously described [13].

Statistical significance

The significance of differences between experimental and control values P, was evaluated using a non-paired, two-tailed Student's t-test in which n=number of donors.

RESULTS

Modulation of DNA synthesis and CK specific activity by E₂ and ERs specific agonists in human female-derived osteoblasts by HG

Basal activities of the different cells show that hObs from pre-menopausal females show the highest activity of both parameters (Figure 1a). Growing the cells in HG increased constitutive level of the specific activity of CK in pre-menopausal osteoblasts by 146 ± 5% and in post-

menopausal osteoblasts by 134 ± 8%, in SaOS2 by 135 ± 15% and no effect in hfoB 110 ± 8% (Figure 1b). Growing cells in HG increased basal level of DNA synthesis (DNA) in pre-menopausal osteoblasts by 153 ± 20% and in post-menopausal osteoblasts by 165 ± 13% (Figure 1b), in SaOS2 by 200 ± 12% but not in hfOB by 108 ± 8% (Figure 1b). Female derived hObs treated with E₂, DPN or PPT for 24 h, showed a significant increase in CK (Figure 2b), in both age groups (Figure 2b) and both cell lines. The response of pre-menopausal cells was higher than post- and more than in SaOS2 and the same as in hfoB with E₂ (Figure 2b). Growth of the cells in HG led to reduction of the response of CK to treatment with E₂, DPN and PPT in cells from both age groups and in both cell lines (Figure 2a). Growth of the cells in HG led to abolishment of the response of DNA to treatment with E₂ or DPN but not PPT in cells from both age groups and in both cell lines (Figure 2a).

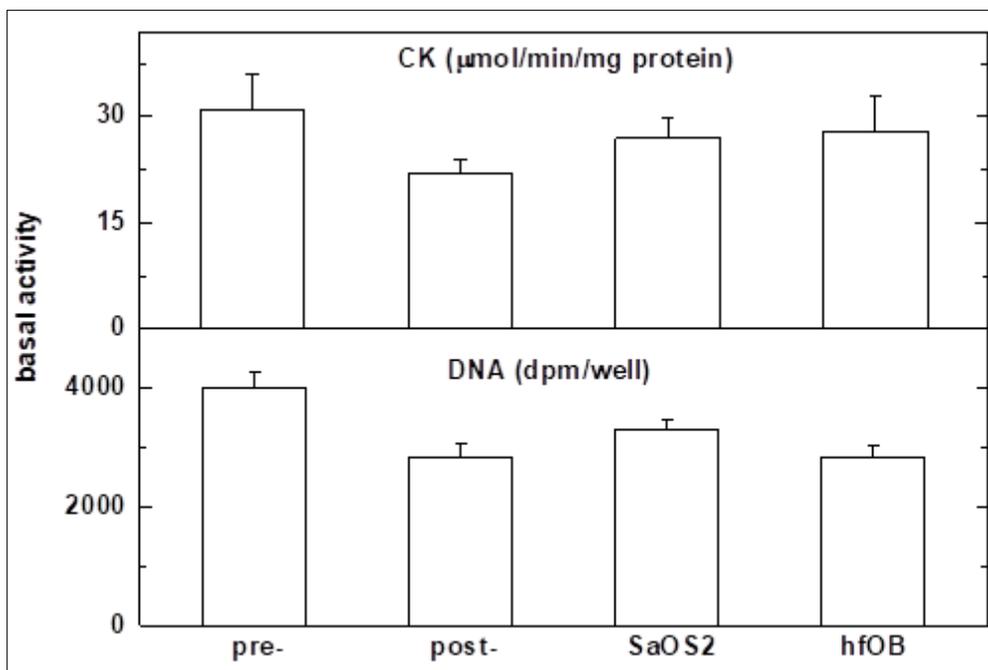


Figure 1a. Basal levels of DNA synthesis and CK specific activities in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

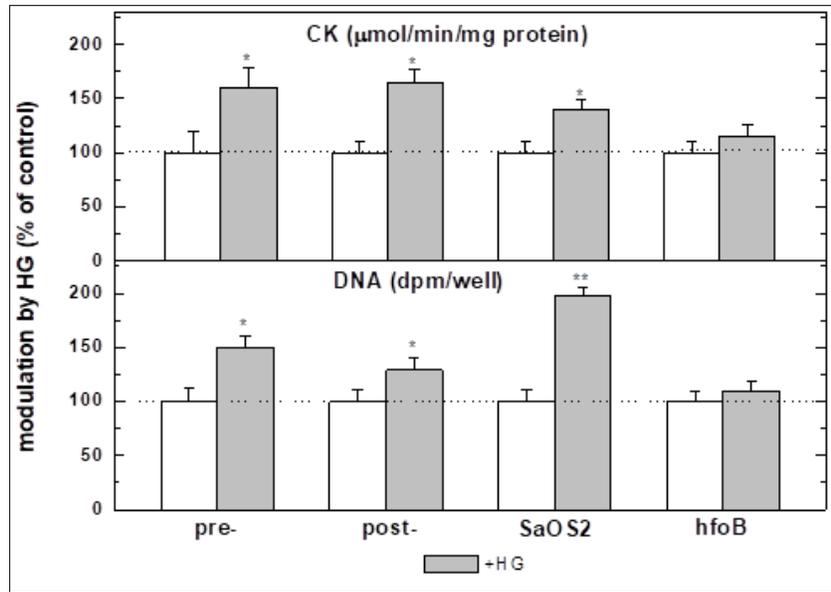


Figure 1b. Modulation by HG (44 nM compared to 22 nM) of basal levels of DNA synthesis and CK specific activities in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

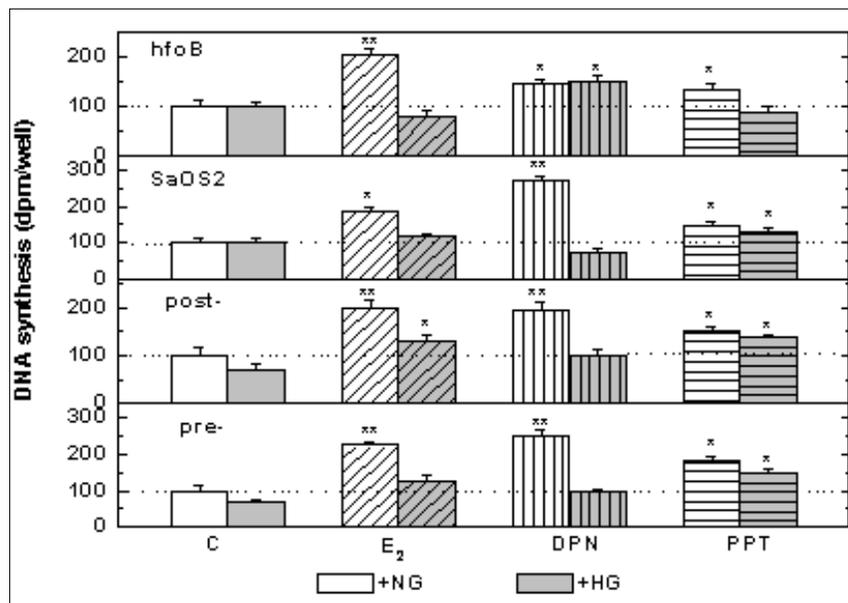


Figure 2a. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of DNA synthesis in primary human female-derived osteoblasts as well as SaOS2 as well as hfoB human female bone cell lines. Bone cells were obtained, cultured, treated and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P < 0.05; **, P < 0.01.

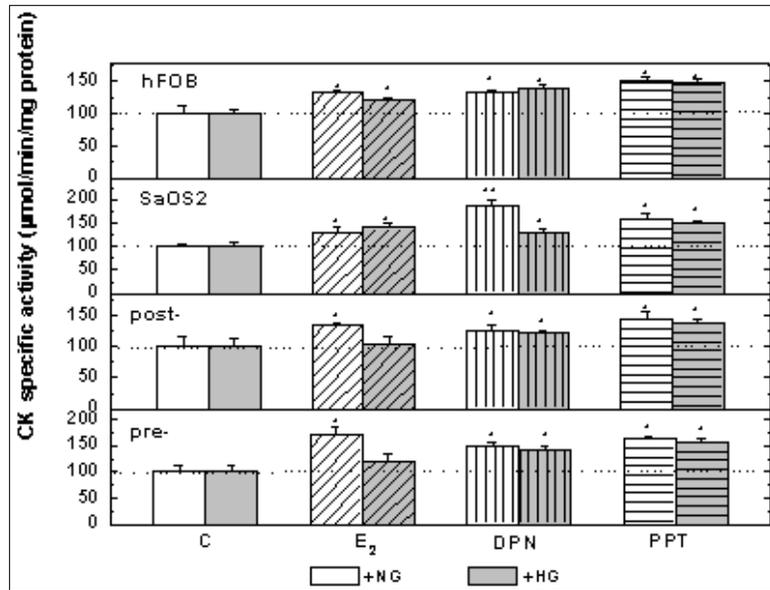


Figure 2b. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of CK specific activity in primary human female-derived osteoblasts and SaOS2 as well as hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

Expression and modulation of ERα and ERβ in human female-derived osteoblasts by HG

Female-derived osteoblasts from both female age groups and both cell lines expressed mRNA for both ERα and ERβ as measured by real time PCR (Figure 3a). High glucose modulated the expression of both ERα and ERβ, in these cells to different extents (Figure 3b). Female derived hObs

treated with E₂, DPN or PPT, showed a significant modulations in ERα (Figure 4a), in all cells with different patterns of modulations of these effect by HG (Figure 4a). Similar results were obtained also when ERβ was assayed (Figure 4b).

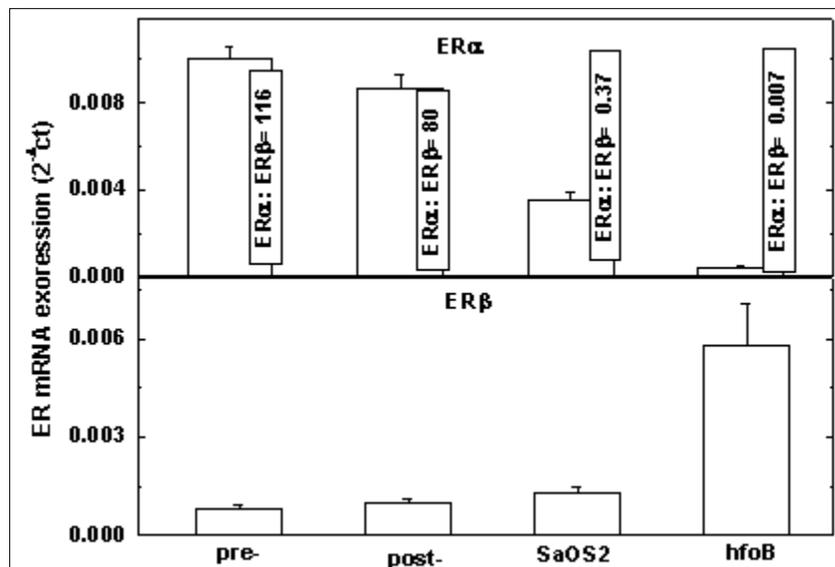


Figure 3a. Basal expression of ERα and ERβ mRNAs in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

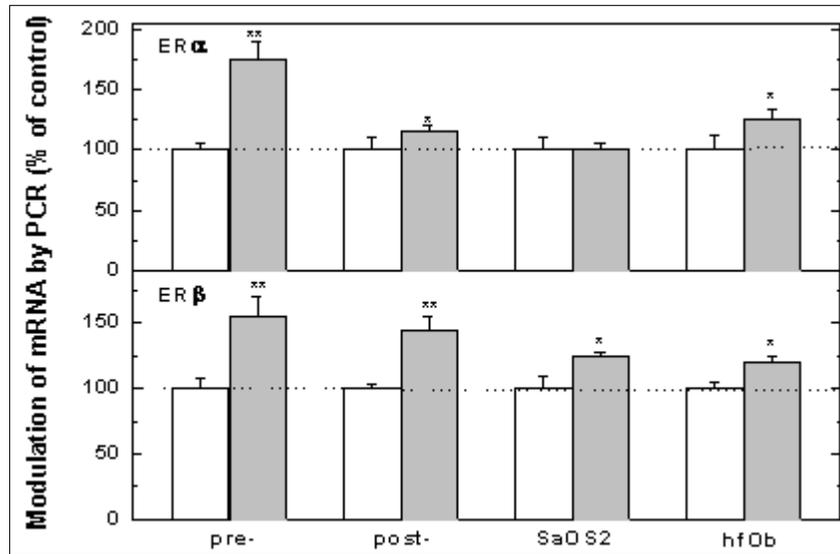


Figure 3b. Modulation by HG (44 nM compared to 22 nM) on basal expression of ER α and ER β mRNAs in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means \pm SEM for triplicate cultures from 5 donors for each group.

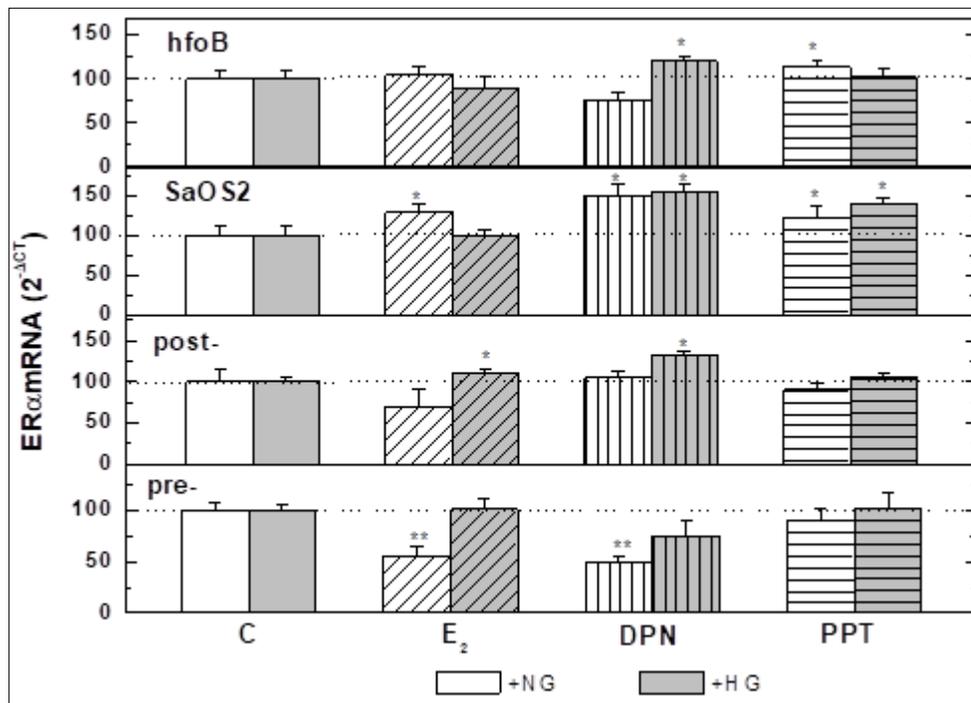


Figure 4a. Modulation by HG (44 nM compared to 22 nM) of the modulation by E₂, DPN and PPT of the expression of mRNA for ER α in primary human female-derived osteoblasts and SaOS2 as well as hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means \pm SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, $P < 0.05$; **, $P < 0.01$.

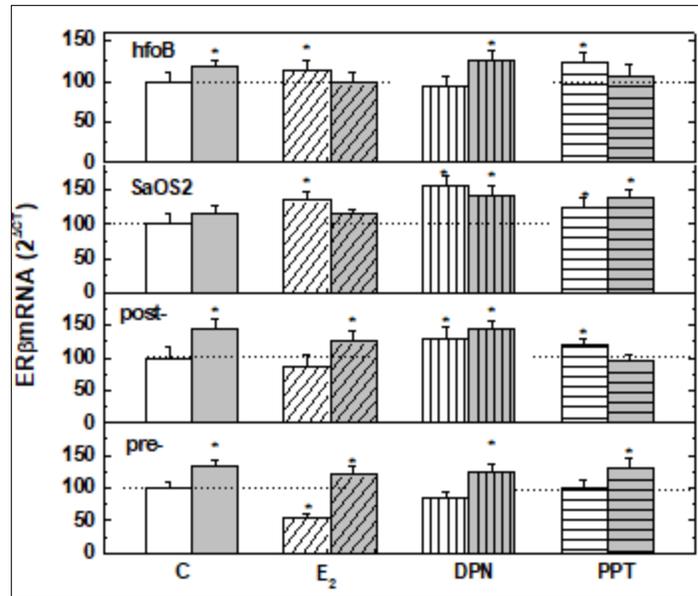


Figure 4b. Modulation by HG (44nM compared to 22nM) of the modulation by E₂, DPN and PPT of the expression of mRNA for ERβ in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

Expression and modulation of VDR and 1OHase in human female-derived osteoblasts by HG

Female-derived bone cells from both female age groups and cell lines expressed mRNA for VDR and 25 hydroxy vitamin D₃ 1-α hydroxylase (1OHase) as measured by real time PCR, corrected for RNase P mRNA and also produced 1,25(OH)₂D₃ (1,25D) as measured by radio-immunoassay

(Figure 5a). Growing the cells in HG decreased the expression of 1OHase as well as 1,25D production respectively, in both age groups and SaSO2 and hfoB (Figure 5b). While growing the cells in HG increased VDR in all cells tested (Figure 5b).

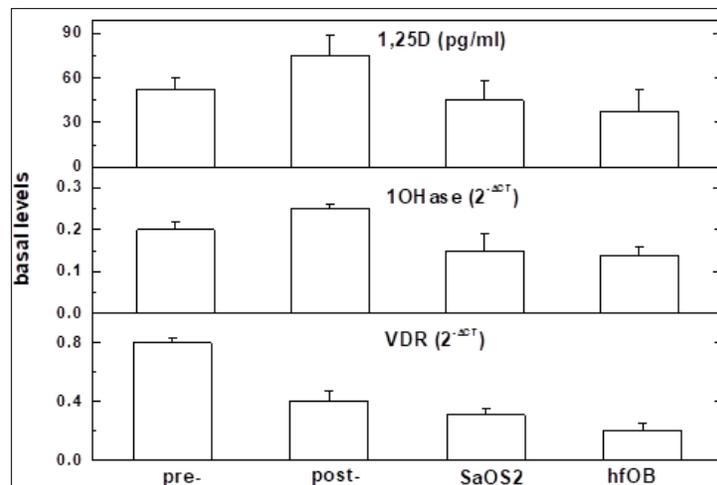


Figure 5a. Basal expression of VDR and 1OHase mRNAs and 1,25D formation in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

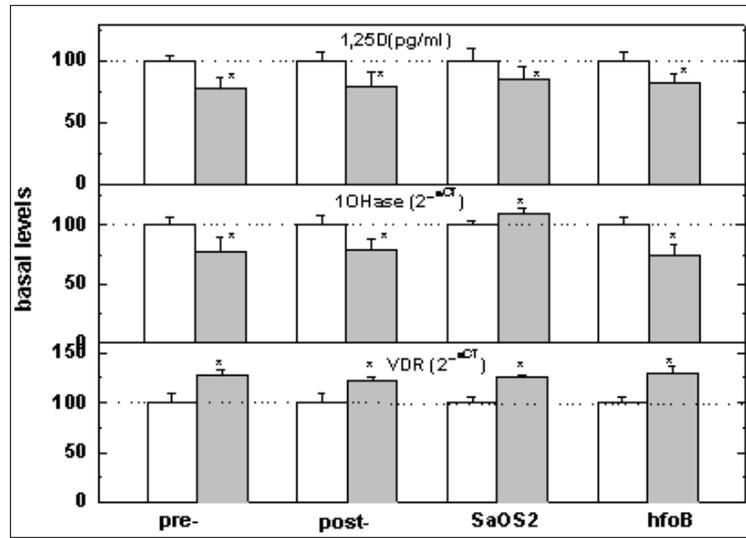


Figure 5b. Modulation by HG (44 nM compared to 22 nM) of VDR and 1OHase mRNAs and 1,25D formation in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

Female derived hObs treated with E₂, DPN or PPT, showed a significant modulation in VDR (Figure 6a). In all cells HG decreased its expression (Figure 6a). Similar results were

obtained when 1OHase was assayed for both mRNA expression and its activity as measured by 1,25D production (Figures 6b and 6c).

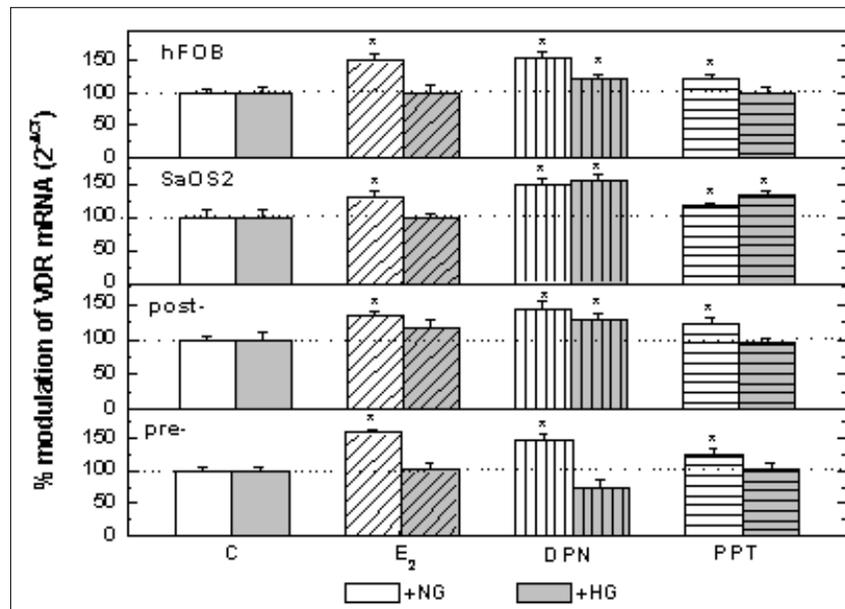


Figure 6a. Modulation by HG (44 nM compared to 22 nM) of the modulation by E₂, DPN and PPT of the expression of mRNA for VDR in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

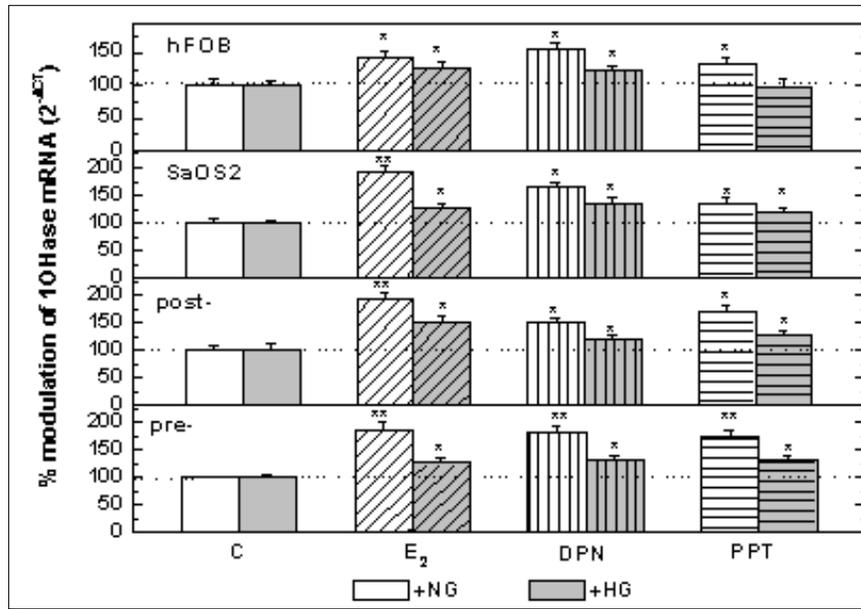


Figure 6b. Modulation by HG (44 nM compared to 22 nM) of the modulation by E₂, DPN and PPT of the expression of mRNA for 10Hase in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

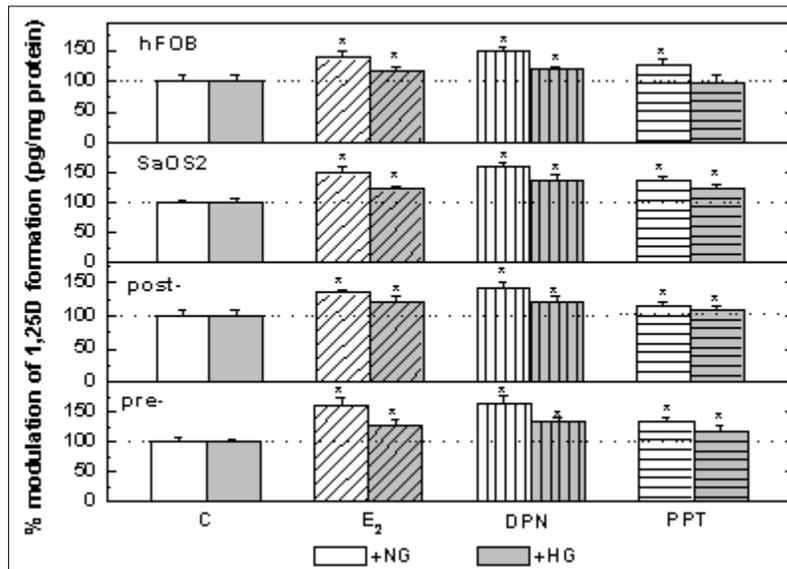


Figure 6c. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of the formation of 1,25D in primary human female-derived osteoblasts and SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

Expression and modulation of 12LO and 15LO in human female-derived osteoblasts by HG

Female-derived bone cells from both female age groups or SaOS2 and hfoB cell lines expressed mRNA for 12LO and

15LO as measured by real-time PCR (Figure 7a). Growing the cells in HG increased the expression of 12LO and 15LO in all cells to different extent (Figure 7b). Female derived hObs treated with E₂, DPN or PPT increased 12LO mRNA expression, in all cells (Figure 7b). Growing the cells in HG

lowered 12LO mRNA, increased expression by estrogenic compounds tested (Figure 8a). Treatment with E₂ or DPN as well as PPT in NG increased the 15LO mRNA expression

(Figure 8b). In HG, the increase in 15LO mRNA expression by the different estrogens was slightly reduced by HG compared to NG (Figure 8b).

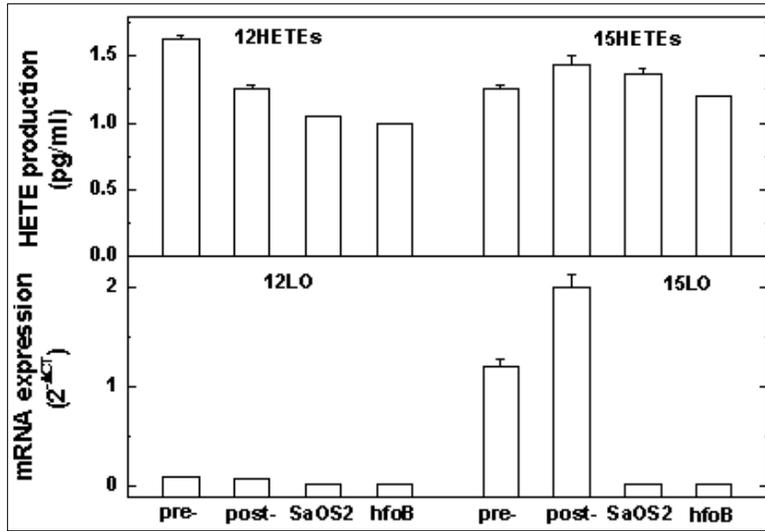


Figure 7a. Basal expression of 12 and 15LO mRNAs and 12 and 15HETE formation in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained, cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

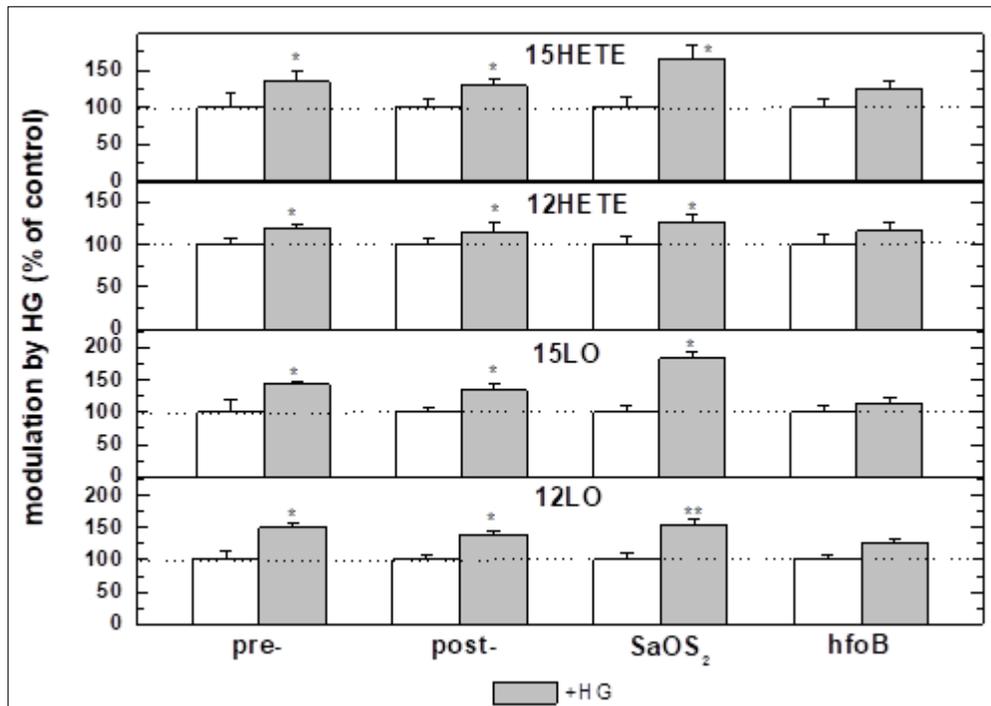


Figure 7b. Modulation by HG (44 nM compared to 22 nM) of 12 and 15LO mRNAs and 12 and 15HETE formation in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group.

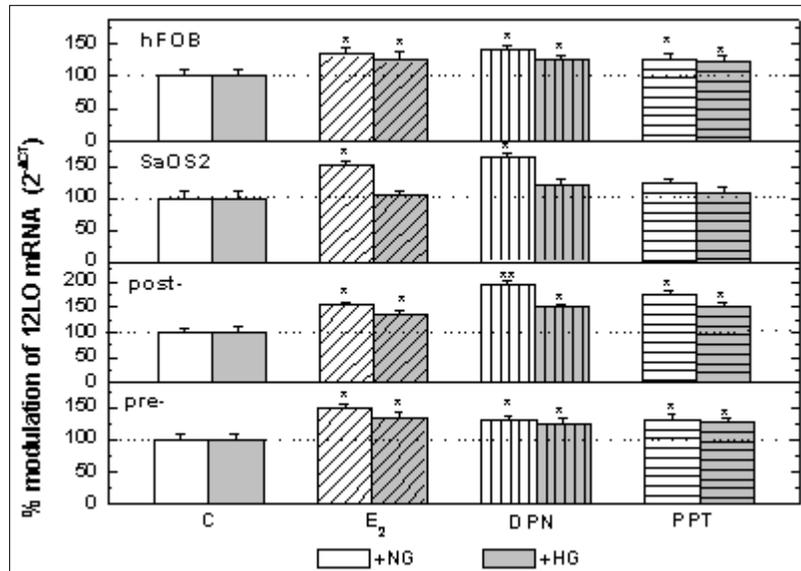


Figure 8a. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of the expression of mRNA for 12LO in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

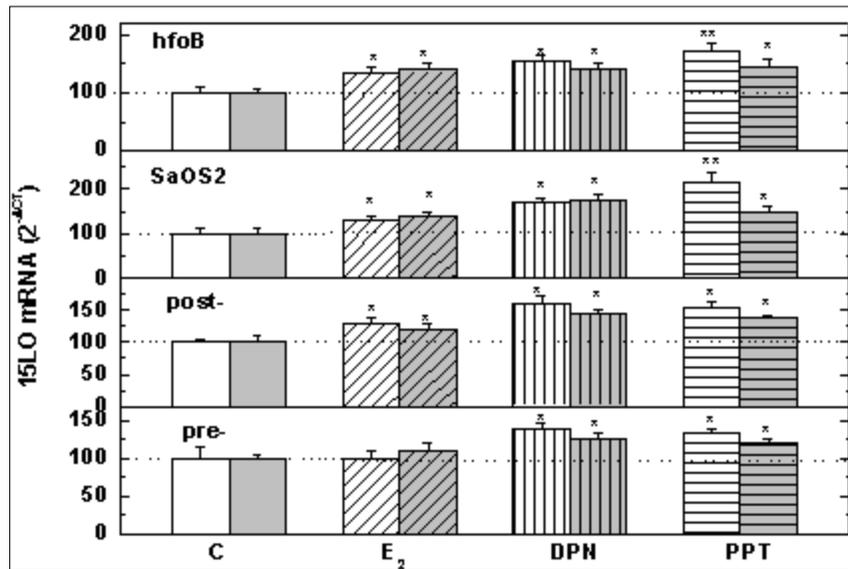


Figure 8b. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of the expression of mRNA for 15LO in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for real time PCR analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

Modulation of the production of 12HETE and 15HETE in human female-derived osteoblasts by HG

Female-derived bone cells from both female age groups and SaOS2 or hfoB cell lines produced 12HETE and 15HETE (Figure 7a). Growing the cells in medium containing HG

increased the production of 12HETE and 15HETE in all cells to different extent (Figure 7b). Female derived hObs treated with E₂, DPN or PPT increased 12HETE and 15HETE production, in all cells tested (Figures 8c and 8d). In HG, the increase in 12HETE production by E₂ or DPN was reduced, whereas the increase by PPT was up-regulated

(Figure 8c). In HG, the increase in 15HETE production by DPN was reduced, while the increase by PPT was up-regulated, but the increase by E₂ was not affected by HG (Figure 8d).

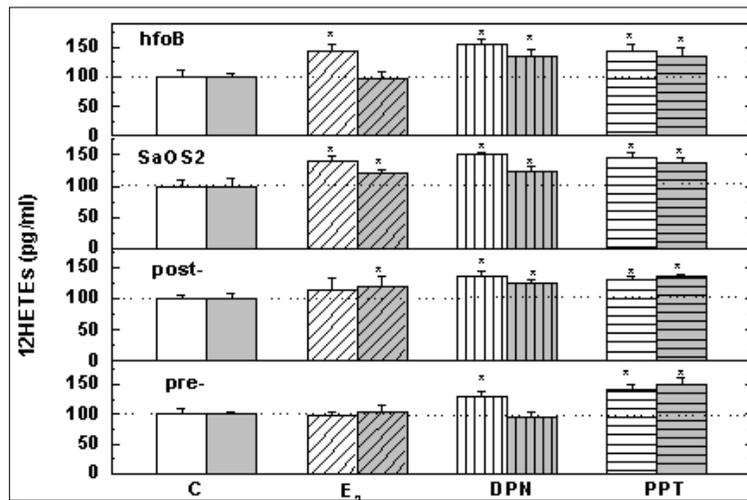


Figure 8c. Modulation by HG (44nM compared to 22nM) of the stimulation by E₂, DPN and PPT of the formation of 12HETEs in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01

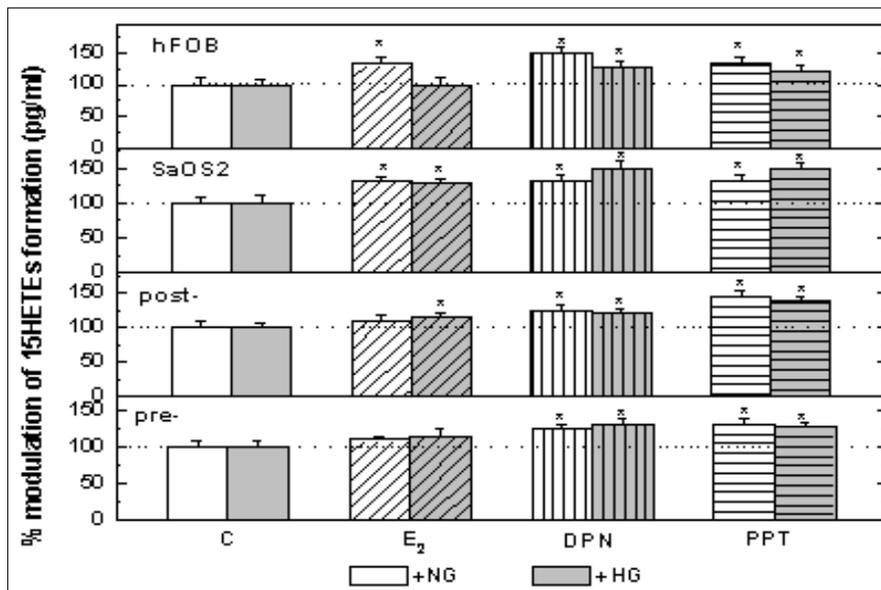


Figure 8d. Modulation by HG (44 nM compared to 22 nM) of the stimulation by E₂, DPN and PPT of the formation of 15HETEs in primary human female-derived osteoblasts as well as SaOS2 and hfoB human female bone cell lines. Bone cells were obtained and cultured and extracts prepared for analysis as described in Materials and Methods. Results are means ± SEM for triplicate cultures from 5 donors for each group. Means of cells grown in high glucose (grey bars) were compared to cells grown in normal glucose (open bars): *, P<0.05; **, P<0.01.

DISCUSSION AND CONCLUSION

The estrogenic compounds tested in our studies can be divided into classes based on their ER specificity, in human female bone cells. Similarly to E₂, DPN and PPT showed higher stimulation in pre-menopausal than in post-

menopausal cells and similar results in SaOS2 and hfoB human female osteoblastic cell lines.

Growing the cells in high glucose concentration (HG; 44 mM instead of NG; 22 mM) sharpens the ability to distinguish between the groups. First of all, the

hyperglycemia increased the constitutive levels of DNA and of CK in all cells except hfoB cell line (**Figure 1b**). Moreover, the stimulation of DNA and CK by E₂ was abolished by hyperglycemia in both age groups but not in the cell lines, the stimulation of DNA and CK by DPN and PPT was slightly decreased by hyperglycemia in both age groups (**Figures 2a and 2b**). It is important to note that the constitutive levels of DNA synthesis and CK specific activity were increased by HG in age group bone cells and SaSO2 and hfoB cell lines (**Figures 4a and 4b**). In order to understand the mechanism of the changes induced by hyperglycemia, we show that the abolition of estrogenic stimulation by hyperglycemia was accompanied in contrast, by increases in mRNA levels of ER α and to less extent in ER β in all female cells tested (**Figures 4a and 4b**). This parallels our previous findings [17,18], using human vascular smooth muscle cells. Attempt to correlate estrogen receptors mRNAs with the changes in nuclear and/or membrane binding failed also in the human vascular smooth muscle cells [13,17,18].

The modulation of ERs by hyperglycemia is a recent addition to the spectrum of changes induced by hyperglycemia, which stimulates the differentiation of osteoblasts and osteoclasts and stimulates osteoblasts to produce osteocalcin and alkaline phosphatase.

Bone growth which is disturbed in diabetes [9,14] is also not enhanced to the same extent by hormone replacement therapy; Consensus opinion of The North American Menopause Society (2000) and might be the result of lower hip BMD in young women due to their type 1 diabetes [14]; therefore the use of the specific phytoestrogens and their synthetic derivatives that we are currently using, might provide an alternative solution. Bone cells also express VDR and 1OHase which is the enzyme synthesizing the active of vitamin D metabolite of the 1, 25 (OH)₂D₃ (1,25D) (**Figure 5a**). Hyperglycemia increased only slightly the VDR expression without affecting 1OHase expression and activity as measured by 1,25D formation (**Figure 5b**). Whether these changes are leading to bone physiology changes due to possible changes in 1,25D is not yet clear. The modulation of VDR and 1OHase as well as 1,25D, is an addition to the spectrum of changes induced by hyperglycemia.

Recent publications linked 15LO and 12LO (platelet type) to bone density. In the present study we show that the expression of the LOs in these cells (**Figure 7a**) is modulated by growing the cells in HG (**Figure 7b**). 12LO mRNA is increased by HG by 55% and 15LO mRNA by 84% (**Figure 7b**). Growing the cells in HG modulated the induction of 12LO mRNA by E₂ and DPN (**Figure 8a**) and slightly affect the stimulation of 15LO mRNA by E₂, DPN and PPT (**Figure 8b**). The expression of these enzymes results in the ability of bone cells to produce and secrete 12HETE and 15HETE, the products of LOs. 12HETE production is increased by growing the cells in high glucose

(**Figure 8c**). Growing the cells in HG decreased the induction of 15HETE by E₂ and DPN but increased its induction by PPT (**Figure 8d**). Growing the cells in HG did not affect the stimulation of 15HETE by E₂, but slightly affected the response to DPN and PPT (**Figure 8d**).

The exact mechanism of the effects of growing the cells in HG in the growth medium on bone cell responses to estrogenic compounds is yet to be investigated and its relationship to human physiology is not yet clear. We believe that we should explore agents that are more effective in HG conditions alone and/or a combination of different drugs which might be less affected by hyperglycemia. If these experiments show promising results, we will analyze animal models that might lead to human studies.

ACKNOWLEDGEMENT

I want to thank my colleagues Katzburg S, Knoll E, Sharon O and Stern N for their help in this research study and Posner G for providing the vitamin D analogs.

REFERENCES

- Somjen D, Weisman Y, Harell A, Berger E, Kaye AM (1989) Direct and sex specific stimulation by sex steroids of creatine kinase activity and DNA synthesis in rat bone. Proc Natl Acad Sci U S A 86: 3361-3363.
- Kaye AM, Weisman Y, Harell A, Somjen D (1990) Hormonal stimulation of bone cell proliferation. J Steroid Biochem Mol Biol 37: 431-435.
- Reiss N, Kaye AM (1981) Identification of the major component of the estrogen induced protein of rat uterus as the BB isozyme of creatine kinase. J Biol Chem 256: 5741-5749.
- Fournier B, Haring S, Kaye AM, Somjen D (1996) Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and anti-estrogens and its modulation by calciotropic hormones. J Endocrinol 150: 275-285.
- Komm BS, Terpening CM, Benz DJ, Graeme KA, Kox M, et al. (1988) Estrogen binding, receptor mRNA and biological response in osteoblast-like osteosarcoma cells. Science 241: 81-84.
- Enmark E, Gustafsson JA (1999) Oestrogen receptors - An overview. J Intern Med 246: 133-138.
- Delmas PD (2000) Treatment of post-menopausal osteoporosis. Lancet 359: 2018-2026.
- Katzburg S, Ornoy A, Hendel D, Lieberherr M, Kaye AM, et al. (2001) Age and gender specific stimulation of creatine kinase specific activity by gonadal steroids in human bone-derived cells in culture. J Endocrinol Invest 24: 166-172.

9. He H, Liu R, Desta T, Leon C, Gerstenfeld LC, et al. (2004) Diabetes causes decreased osteoclastogenesis, reduced bone formation and enhanced apoptosis of osteoblastic cells in bacteria stimulated bone loss. *Endocrinology* 145: 447-452.
10. (2000) Effects of menopause and estrogen replacement therapy or hormone replacement therapy in women with diabetes mellitus: Consensus opinion of The North American Menopause Society. *Menopause* 7: 87-95.
11. Somjen D, Tordjman K, Katzburg S, Knoll E, Sharon O, et al. (2008) Lipoxygenase metabolites are mediators of PTH-dependent human osteoblast growth. *Bone* 42: 491-497.
12. Somjen D, Katzburg S, Knoll E, Sharon O, Posner GH, et al. (2010a) Vitamin D analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cells. *J Steroid Biochem Mol Biol* 121: 265-267.
13. Somjen D, Katzburg S, Grafi-Cohen M, Knoll E, Sharon O, et al. (2011a) Vitamin D metabolites and analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cell line. *J Steroid Biochem Mol Biol* 123: 85-89.
14. Somjen D, Katzburg S, Stern N, Kohen F, Sharon O, et al. (2007) 25 hydroxy-vitamin D₃-1 α hydroxylase expression and activity in cultured human osteoblasts and their modulation by parathyroid hormone, estrogenic compounds and dihydrotestosterone. *J Steroid Biochem Mol Biol* 107: 238-244.
15. Somjen D, Kohen F, Lieberherr M, Gayer B, Schejter E, et al. (2005) Membranal effects of phytoestrogens and carboxy derivatives of phytoestrogens on human vascular and bone cells: New insights based on studies with carboxy-biochanin A. *J Steroid Biochem Mol Biol* 93: 293-303.
16. Somjen D, Katzburg S, Sharon O, Posner GH, Jaccard N, et al. (2011b) Mutual interaction of special phytoestrogenic compounds, their synthetic carboxy-derivatives and the less-calcemic vitamin D analog activities in human derived female cultured osteoblasts. *J Steroid Biochem Mol Biol* 127: 351-357.
17. Somjen D, Kohen F, Gayer B, Sharon O, Limor R, et al. (2004a) Role of putative membrane receptors in estradiol's effects on human vascular cell growth. *Am J Hypertens* 17: 462-469.
18. Somjen D, Paller CJ, Gayer B, Kohen F, Knoll E, et al. (2004b) High glucose blocks estradiol's effects on human vascular cell growth: differential interaction with estradiol and raloxifene. *J Steroid Biochem Mol Biol* 88: 101-110.
19. Manolagas SC, Kousteni S, Jilka RL (2002) Sex steroids and bone. *Recent Progr Horm Res* 57: 385-409.
20. Somjen D, Katzburg S, Sharon O, Grafi-Cohen M, Knoll E, et al. (2010b) The effect of ER α and ER β specific agonists on cell proliferation and energy metabolism in human cultured bone cells. *Abs Am Endocrinol*.