

MENOPAUSE

Oral 17 $\beta$ -estradiol and sequential progesterone in menopause: Effects on insulin-like growth factors and their binding proteins

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Abstract

**Objective.** We evaluated the acute effects of low-dose oral estradiol and sequential progesterone on the insulin-like growth factor (IGF)/growth hormone (GH) axis, IGF-binding proteins (IGFBPs) 1 and 3, and plasma levels of sex hormone-binding globulin (SHBG) in postmenopausal subjects.

**Study design.** Thirty healthy normal-weight women (mean age: 54.2  $\pm$  5.7 years) spontaneously postmenopausal for at least 6 months were enrolled. None had used hormone replacement therapy (HRT). Appropriate investigations excluded renal, glucose, lipid and coagulation abnormalities. Breast X-ray and endometrial ultrasound examinations excluded organic pathologies. They received oral cyclical HRT for 1 year, based on the administration of oral estradiol (1 mg/day) for 28 consecutive days plus progesterone (200 mg/day) from day 15 to day 28; out of the whole group, 15 subjects received progesterone orally (group A), while in 15 progesterone was administered transvaginally (group B). On the day before treatment (T0), on day 14 (T14) and on day 28 (T28) of the first cycle, plasma levels of estradiol, progesterone, SHBG, GH, IGF-I and -II, IGFBP-1 and -3, insulin and C-peptide were assayed in all patients. The same parameters were evaluated at T14 and T28 during the 12th month of treatment.

**Results.** At T14, we observed significant increases in the levels of estradiol (from 20  $\pm$  16 to 115  $\pm$  71 pg/ml,  $p < 0.001$ ), SHBG (from 132  $\pm$  42 to 182  $\pm$  55 nmol/l,  $p < 0.001$ ) and IGFBP-1 (from 92  $\pm$  57 to 127  $\pm$  87 ng/ml,  $p < 0.004$ ), while the level of IGF-I decreased (from 197  $\pm$  138 to 129  $\pm$  85 ng/ml,  $p < 0.003$ ). At T28, progesterone levels were significantly higher in the women receiving it orally than transvaginally (8.4  $\pm$  6.1 vs. 3.7  $\pm$  3.2 ng/ml,  $p < 0.025$ ). However, while oral progesterone did not affect the estrogen-induced variations, transvaginal progesterone abrogated the increase in the levels of IGFBP-1. The levels of IGF-II, IGFBP-3, GH, glucose, C-peptide and insulin did not change at any time. At 1 year, the values maintained the same trends. The estrogen-induced variations of SHBG were correlated directly with those of estradiol ( $r = 0.48$ ) and inversely with those of IGF-I ( $r = -0.424$ ).

**Conclusions.** Low-dose oral estradiol reduces plasma levels of IGF-I and increases IGFBP-1 and SHBG concentrations, while GH is unchanged. These effects, significant and immediate, lead us to hypothesize a direct action of estradiol on hepatocytes.

**Keywords:** Hormone replacement therapy, estradiol, progesterone, insulin-like growth factors I and II, insulin-like growth factor binding proteins 1 and 3, growth hormone, sex hormone-binding globulin

Introduction

The insulin-like growth factors (IGF-I and -II) and their binding proteins (IGFBPs) are peptides that affect and mediate hormonal actions at target tissues by endocrine, paracrine and autocrine pathways [1]. Levels of IGFs have been shown to vary during menopause. An age-related decline in the functionality of

the IGF/growth hormone (GH) axis [2] would be expected to lead to an age-dependent decrease [3] in the IGFs; however, some investigators have observed lower IGF-I levels in the premenopause than in the early postmenopause, probably because of the relative hyperestrogenism typical of the late fertile age [4].

IGF-I is a peptide, structurally similar to pro-insulin, that modulates most of the effects of GH,

particularly at bone and muscle sites. In the breast, IGF-I has a mitogenic and anti-apoptotic effect [5,6] and interacts synergistically with estrogens in the induction of cell proliferation, in both normal and neoplastic cell lines [7-9]. In several studies circulating IGF-I levels have been shown to be positively correlated with either an increased mammographic density [10] or an increased risk of breast cancer, particularly in premenopausal patients [11-14]. Quite recently, studies have suggested a positive correlation between circulating levels of IGF-I and the risk of breast cancer in postmenopausal women undergoing hormone replacement therapy (HRT) [15] and in women in the upper tertile of body mass index [16]. Therefore, IGF-I levels could be a risk factor in women who are currently exposed to estrogens, both of endogenous and of exogenous origin. Moreover, higher circulating levels of IGF-I, as well as of its binding protein 3 [17], have been found to be associated with a higher incidence of colorectal cancer [18].

The metabolic functions of IGF-II are not so closely correlated to GH action [16,19]; moreover, the role of IGF-II in regulating cell growth seems to be limited to the early stages of life and appears less important in postnatal life [20].

The effects of IGFs are modulated by their binding proteins, among which IGFBP-1 and IGFBP-3 seem to play a pivotal role. The levels of these proteins remain stable during menopause. The most important circulating reservoir for IGFs is IGFBP-3 in its ternary form comprising the acid-labile subunit; its concentration is much higher than that of the others; it is very stable and is almost completely saturated with IGFs, for which its affinity is the highest observed [21]. On the contrary, the other binding proteins are largely unsaturated and have a lower affinity for IGFs; they might serve to mop up free IGFs as they dissociate from IGFBP-3 and eventually influence their final destination in tissues [22]. The actual bioavailability of IGFs to specific tissues, however, seems dependent on the action of site-specific IGFBP proteases, which lower the levels of IGFBPs, and on the dephosphorylation of IGFBP-1 to phosphoforms which bind IGF-I with a lower affinity; both these mechanisms would modulate the levels of free IGFs, and mainly IGF-I, available for receptor interaction [23].

The claims that HRT leads to an increased incidence of breast tumors, the possibility that IGF-I levels are correlated to risk factors for breast cancer in both premenopausal patients/postmenopausal women undergoing HRT and obese subjects [5-16], and the abundance of papers showing that HRT can modify plasma IGF-I levels [24-32] prompted us to design the present study. Our purpose was to investigate whether the administration of low-dose oral 17 $\beta$ -estradiol sequentially combined with oral or transvaginal progesterone affects the levels of IGFs

and whether this effect appears early, during the first cycle of HRT administration.

## Materials and methods

### Study population

Thirty postmenopausal women were enrolled in this study to be treated with low-dose estradiol and sequential micronized progesterone, a hormone devoid of any androgen effect [31,32]. The main demographic and clinical characteristics of the study population are shown in Table I. All patients had been postmenopausal for at least 6 months, as confirmed by plasma level of follicle-stimulating hormone  $>30$  mU/ml and of estradiol  $<30$  pg/ml. None out of them had previously used HRT. Informed consent was obtained from all patients according to the indications of the Padova University Ethical Committee.

Before each patient entered the study, we investigated her lipid profile, hepatic and renal function, and coagulation parameters, and performed an oral glucose tolerance test, to exclude metabolic abnormalities. Breast X-ray and transvaginal ultrasound (antero-posterior evaluation) examinations excluded any suspected breast lesion and endometrial abnormalities (thickness  $>4$  mm). If some abnormality was found, the patient was not considered suitable for the study.

### Study design

Fifteen patients (group A) started a sequential treatment with 17 $\beta$ -estradiol (Estrofem<sup>®</sup>; Novo Nordisk A/S, Novo Allé, Bagsvaerd, Denmark) given orally at the daily dose of 1 mg for 28 consecutive days plus

Table I. Characteristics of the patients according to progesterone administration route.

	Oral	Transvaginal	p Value
Age (years)	54.6 $\pm$ 5.7	53.8 $\pm$ 5.7	NS
Duration of menopause (years)	6.0 $\pm$ 5.8	5.6 $\pm$ 4.4	NS
BMI (kg/m <sup>2</sup> )	23.7 $\pm$ 3.4	24.9 $\pm$ 4.3	NS
SBP (mmHg)	146 $\pm$ 19	133 $\pm$ 23	NS
DBP (mmHg)	86 $\pm$ 8	86 $\pm$ 9	NS
Glucose (mg/dl)	84.5 $\pm$ 9.7	85.1 $\pm$ 12.4	NS
TC (mg/dl)	221 $\pm$ 41	231 $\pm$ 31	NS
HDL-C (mg/dl)	69 $\pm$ 197	68 $\pm$ 14	NS
LDL-C (mg/dl)	135 $\pm$ 39	143 $\pm$ 31	NS
TG (mg/dl)	83 $\pm$ 38	83 $\pm$ 35	NS

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; NS, not significant; results are expressed as mean  $\pm$  standard deviation and analyzed using the Wilcoxon non-parametric test; no differences were evident between the two groups.

micronized progesterone added in the last 14 days of each cycle at the oral daily dose of 200 mg (Prometrium® cpr 100 mg every 12 h; Rottapharm SpA, Milan, Italy). The remaining 15 (group B) started the same 17 $\beta$ -estradiol treatment, while progesterone was administered transvaginally (Esolut® vaginal suppository 200 mg; Angelini, Roma, Italy) from day 14 to day 28. On the day before beginning HRT (T0), on the 14th day (T14) (i.e. the last day of estradiol only administration) and on the 28th day (T28), a blood sample was drawn from the cubital vein, after an overnight fast, for determination of study parameters. After centrifugation (15 min at 2500 rev/min), plasma was divided into small aliquots and stored at -80°C until the assays, which were performed when all patients had completed the study. The same kits were used for all three samples from each patient in the first treatment cycle.

#### Biochemical determinations

We performed our assays using kits from Diagnostic Systems Laboratories (Webster, TX, USA). A radioimmunoassay was used for 17 $\beta$ -estradiol (Ultra) and immunoradiometric assays for progesterone, insulin and C-peptide; IGF-I and IGF-II (Fast); IGFBP-1, IGFBP-3, SHBG and GH.

The intra- and inter-assay coefficient of variation for each parameter is respectively as follows. 17 $\beta$ -Estradiol (pg/ml): 7.6 and 8.0; progesterone (ng/ml): 4.8 and 9.2; insulin ( $\mu$ IU/ml): 4.6 and 9.6; C-peptide (ng/ml): 3.5 and 10.6; IGF-I (ng/ml): 3.9 and 4.2; IGF-II (ng/ml): 4.7 and 4.5; IGFBP-1 (ng/ml): 2.7 and 3.6; IGFBP-3 (ng/ml): 3.9 and 0.6; GH (ng/ml): 5.4 and 8.8; SHBG (nmol/ml): 3.4 and 8.7. The cross-reactivity of the insulin assay was 6.2% for C-peptide and 32.7% for pro-insulin; the cross-reactivity of the C-peptide assay was <0.2% for insulin and <12.8% for pro-insulin.

#### Statistical analyses

We compared characteristics between our two groups of patients using the Wilcoxon non-parametric test for two independent samples. Statistical analysis of results was carried out using the non-parametric permutation test for paired data [33–36]. For each parameter, we compared – separately for each group of patients – first the change from T0 to T28 with that from T0 to T14; and then the values at T0 and T14. The latter were evaluated also in the whole group of 30 patients, as in the first 14 days they were administered the same estradiol treatment and can be considered a homogeneous group. The calculations were performed using MatLab 7.0 routines (MathWorks Inc., Natick, MA, USA). The correlations between our parameters at each time and between their variations after the treatments were

evaluated using Pearson's exact permutation test for correlation [33–36].

#### Results

The plasma levels of our parameters at T0, T14 and T28 during the first month of treatment are reported in Tables II and III. For each one, we compared – separately for each group of patients – first the change from T0 to T28 with that from T0 to T14; and then the values at T0 and T14. At the end of progesterone administration (T28) its levels were significantly higher in the women who received it orally ( $8.4 \pm 6.1$  vs.  $3.7 \pm 3.2$  ng/ml,  $p < 0.025$ ); the effects of estradiol were maintained in both groups with the exception of IGFBP-1 levels, which returned to their basal values in the patients treated transvaginally.

In the first 14 days all of the patients received oral 17 $\beta$ -estradiol and thus they constitute a truly homogeneous group. A comparison of each parameter between T0 and T14 is given in Table IV. The increases in the levels of estradiol and SHBG were highly significant; as were the increase in IGFBP-1 and the decrease in IGF-I levels.

Insulin was correlated directly with body mass index, both in basal conditions ( $p = 0.0038$ ) and after estradiol administration ( $p = 0.0043$ ). The correlation remained significant after oral progesterone administration ( $p = 0.0003$ ). As expected, the variations in SHBG levels from T0 to T14 were correlated directly with those of estradiol ( $p = 0.015$ ) (Figure 1). In addition, they were correlated inversely with those of IGF-I ( $p = 0.03$ ) (Figure 2). No correlation was found between the levels of insulin and either IGF-I or IGF-II, or between the levels of C-peptide and either IGF-I or IGF-II, at any time of our study.

Twenty-three patients were still continuing the treatment at 1 year: 13 from group A and 10 from group B. We grouped them together and compared parameter values at T0 and T14 in the first month with those from T14 in the 12th month of treatment. The data, which are shown in Table V, confirmed that the estrogen effect observed in the first cycle was still present after 1 year.

#### Discussion

HRT administered during menopause has been reported to modify plasma IGF-I levels, likely through a direct action on hepatocytes. In particular, oral conjugated equine estrogens (CEE) [24–32,37] and selective estrogen receptor modulators [38–40] lower IGF-I levels, while oral androgens and androgen-derived progestins increase its levels [32,37]. Also, hypoestrogenic conditions like those induced in premenopausal patients by the oral administration of anastrozole [41] (an aromatase inhibitor) or by the

Table II. Plasma levels of parameters for group A (oral 17β-estradiol plus sequential oral progesterone) (n = 15) on the day before treatment (T0), on day 14 (T14) and on day 28 (T28) of the first cycle.

	T0	T14	T28	p Value
Insulin (μIU/ml)	5.08 ± 2.43	4.73 ± 1.64	4.95 ± 1.47	NS
C-peptide (ng/ml)	1.45 ± 0.29	1.38 ± 0.32	1.46 ± 0.38	NS
IGF-I (ng/ml)	174 ± 90	125 ± 77	112 ± 55	0.028*
IGF-II (ng/ml)	154 ± 14	147 ± 30	152 ± 20	NS
IGFBP-1 (ng/ml)	105 ± 64	135 ± 96	145 ± 89	NS
IGFBP-3 (ng/ml)	3740 ± 486	3703 ± 750	3515 ± 541	NS
SHBG (nmol/l)	141 ± 40	191 ± 52	200 ± 49	0.001*
GH (ng/ml)	0.63 ± 0.63	0.63 ± 0.78	0.66 ± 0.72	NS
Estradiol (pg/ml)	19 ± 14	114 ± 82	86 ± 43	0.002*
Progesterone (ng/ml)	2.4 ± 1.1	1.9 ± 0.9	8.4 ± 6.1	0.003†

IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; SHBG, sex hormone-binding globulin; GH, growth hormone; NS, not significant; results are expressed as mean ± standard deviation and analyzed using the non-parametric permutation test for paired data; \*significantly different, T14 vs. T0; †significantly different, change from T0 to T28 vs. change from T0 to T14.

Table III. Plasma levels of parameters for group B (oral 17β-estradiol plus sequential transvaginal progesterone) (n = 15) on the day before treatment (T0), on day 14 (T14) and on day 28 (T28) of the first cycle.

	T0	T14	T28	p Value
Insulin (μIU/ml)	6.15 ± 2.57	5.9 ± 2.27	5.80 ± 2.56	NS
C-peptide (ng/ml)	2.04 ± 1.15	1.56 ± 0.40	1.86 ± 0.59	NS
IGF-I (ng/ml)	217 ± 172	132 ± 94	151 ± 114	0.008*, 0.028†
IGF-II (ng/ml)	162 ± 21	156 ± 24	170 ± 18	NS
IGFBP-1 (ng/ml)	80 ± 50	121 ± 80	85 ± 44	0.021*
IGFBP-3 (ng/ml)	3878 ± 913	3586 ± 802	3624 ± 616	0.047*
SHBG (nmol/l)	124 ± 44	181 ± 54	183 ± 57	0.0001*
GH (ng/ml)	0.62 ± 0.81	0.82 ± 0.85	1.22 ± 1.13	NS
Estradiol (pg/ml)	21.05 ± 18	116 ± 62	79 ± 47	0.0001*, 0.026†
Progesterone (ng/ml)	1.5 ± 0.6	1.5 ± 0.9	3.7 ± 3.2	0.05†

IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; SHBG, sex hormone-binding globulin; GH, growth hormone; NS, not significant; results are expressed as mean ± standard deviation and analyzed using the non-parametric permutation test for paired data; \*significantly different, T14 vs. T0; †significantly different, change from T0 to T28 vs. change from T0 to T14.

Table IV. Plasma levels of parameters, on the day before treatment (T0) and on day 14 (T14) of treatment, for the 30 study patients considered together in the first two weeks of treatment with oral 17β-estradiol.

	T0	T14	p Value
Insulin (μIU/ml)	5.51 ± 2.54	5.02 ± 1.91	NS
C-peptide (ng/ml)	1.73 ± 0.59	1.48 ± 0.38	NS
IGF-I (ng/ml)	197 ± 138	129 ± 85	0.001
IGF-II (ng/ml)	158 ± 18	152 ± 27	NS
IGFBP-1 (ng/ml)	92 ± 57	127 ± 87	0.004
IGFBP-3 (ng/ml)	3812 ± 728	3642 ± 764	NS
SHBG (nmol/l)	132 ± 42	182 ± 55	0.0001
GH (ng/ml)	0.6 ± 0.7	0.7 ± 0.8	NS
Estradiol (pg/ml)	20 ± 16	115 ± 71	0.0001

IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; SHBG, sex hormone-binding globulin; GH, growth hormone; NS, not significant; results are expressed as mean ± standard deviation and analyzed using the non-parametric permutation test for paired data.

administration of gonadotropin-releasing hormone analogs [42] lead to an increase in IGF-I levels. Transdermal administration of sex steroids can

modify IGF-I levels significantly only when the administered dose is sufficient to ensure high estrogen levels at the hepatocyte [43].

The progestins more similar to natural progesterone seem to have no effect on IGF-I synthesis even when administered orally [27].

In the present study we administered orally a low estrogen dose, according to the general suggestions in the literature, and evaluated its effects in the first month and after 1 year of treatment. In order to obtain a hormone replacement as close to physiology as possible, we prescribed 17β-estradiol sequentially combined with progesterone. While estradiol was given orally to all 30 of our patients, progesterone was administered orally to only 15 of them, while it was given transvaginally to the others. Oral estradiol has a half-life of about 20 h and gains steady levels in plasma after 4–5 days [44] when administered in a single daily dose. The half-life of oral progesterone is about 9 h, which led us to divide the daily dose into two administrations. Vaginal progesterone was administered in a single dose as suggested.

As reported above, the effects of oral estrogens on the levels of IGF-I, its binding proteins and SHBG are well known [24–32,37]. In a previous study [45] we pointed out that oral CEE decreased IGF-I and increased SHBG plasma levels after 6 months of sequential treatment, while no effect was observed after transdermal estrogens.

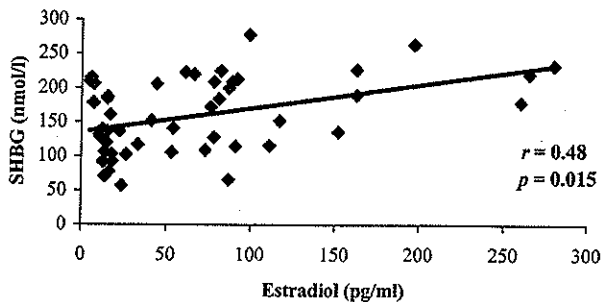


Figure 1. The correlation between sex hormone-binding globulin (SHBG) and estradiol. Among the 30 patients, variations in the levels of SHBG and estradiol from T0 (the day before treatment) to T14 (day 14 of treatment) were determined during the first cycle and the correlation evaluated using Pearson's exact permutation test for correlation.

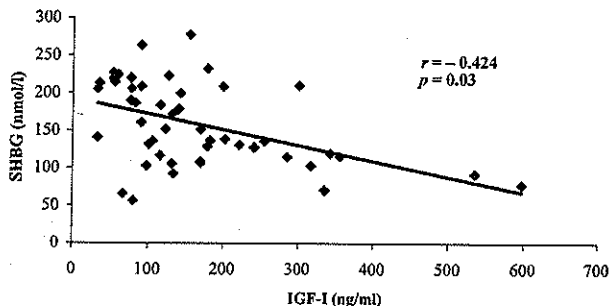


Figure 2. The correlation between sex hormone-binding globulin (SHBG) and insulin-like growth factor-I (IGF-I). Among the 30 patients, variations in the levels of SHBG and IGF-I from T0 (the day before treatment) to T14 (day 14 of treatment) were determined during the first cycle and the correlation evaluated using Pearson's exact permutation test for correlation.

In the present study we confirmed that even low-dose oral estradiol is able to reduce the levels of IGF-I and increase those of SHBG when associated with sequential natural progesterone for 1 year. However, we also demonstrated that these effects are extremely precocious and occur very soon after starting the treatment. Moreover, GH levels were unaffected at any time, which confirms that the variations are independent of it and are likely due to the action of estrogens on the hepatocyte.

In fact, oral  $17\beta$ -estradiol, at the daily dose of 1 mg for 14 days, led to a significant increase in its levels in plasma during the first cycle of treatment, as well as increases in plasma levels of SHBG and IGFBP-1 but a significant reduction in plasma levels of IGF-I. The circulating levels of SHBG were correlated directly with those of estradiol, and inversely with those of IGF-I.

At the end of progesterone administration, its levels were higher in women treated orally. Most of the effects induced by estradiol were maintained in both groups: only the levels of IGFBP-1 returned to their basal values in the group treated transvaginally.

Considered together, our findings suggest that the liver, when over-stimulated by estrogens, in some way downregulates expression of some of the main agents that modulate or mediate estrogen effects at target tissues. Progesterone, on the other hand, seems devoid of any hepatic effect, mainly when administered orally.

Data in the literature seem to indicate that the liver responds in opposite ways to estrogen under- and over-stimulation. In fact, when exposed to the bolus of portal estrogens, the liver could reduce the synthesis of IGFs and increase the level of estrogen-binding proteins. This mechanism has been demonstrated in rats, in which high hepatic doses of estrogens are reported to inhibit the hepatic synthesis of IGF-I mRNA [46], exactly the opposite of what happens in the uterus [47]. This reinforces the hypothesis that estrogens directly suppress the hepatocyte synthesis of IGF-I during their first

Table V. Plasma levels of parameters, on the day before commencing treatment (T0), day 14 of the first treatment cycle (T14, 1st month) and day 14 of the final treatment cycle (T14, 12th month), for the 23 patients continuing treatment at 1 year.

	T0	T14, 1st month	T14, 12th month	p Value
Insulin ( $\mu$ IU/ml)	5.51 $\pm$ 2.54	5.02 $\pm$ 1.91	5.33 $\pm$ 1.98	NS
C-peptide (ng/ml)	1.73 $\pm$ 0.59	1.48 $\pm$ 0.38	1.63 $\pm$ 0.90	NS
IGF-I (ng/ml)	197 $\pm$ 138	129 $\pm$ 85	131 $\pm$ 71	NS
IGF-II (ng/ml)	158 $\pm$ 18	152 $\pm$ 27	162 $\pm$ 11	NS
IGFBP-1 (ng/ml)	92 $\pm$ 57	127 $\pm$ 87	119 $\pm$ 74	NS
IGFBP-3 (ng/ml)	3812 $\pm$ 728	3642 $\pm$ 764	3709 $\pm$ 513	NS
SHBG (nmol/l)	132 $\pm$ 42	182 $\pm$ 55	188 $\pm$ 35	NS
GH (ng/ml)	0.6 $\pm$ 0.7	0.7 $\pm$ 0.8	0.8 $\pm$ 0.9	NS
Estradiol (pg/ml)	20 $\pm$ 16	115 $\pm$ 71	99 $\pm$ 34	NS

IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; SHBG, sex hormone-binding globulin; GH, growth hormone; NS, not significant; results are expressed as mean  $\pm$  standard deviation and analyzed using the non-parametric permutation test for paired data.

passage across the hepatic-portal system. In our study the oral estradiol decreased the levels of IGF-I and increased those of IGFBP-1, while the levels of IGFBP-3 remained unchanged. These results are consistent with the hypothesis of a prevalent estradiol action on the hepatocytes: in fact, both IGF-I and IGFBP-1 are synthesized by the liver parenchymal cells, while the prevalent site of synthesis for IGFBP-3 seems to be the Kupffer cells [48].

The hepatic estrogen effect seems to be dose-dependent and may be linked to an inhibition of GH signaling at the cellular level [49]. However, the biochemical or molecular mechanisms of estradiol action are far from being clarified: in fact, to date, no estrogen response elements have been detected near the IGF-I gene, either in humans or mice [47], and estrogen receptors might function as secondary transcription factors, interacting with other DNA-bound transcriptional activators.

The increase in the levels of SHBG appears inversely correlated to IGF-I variations; in a human hepatoma cell line, insulin – which is structurally and functionally similar to IGF-I – has been shown to directly inhibit the production of SHBG [50,51]. Their inverse correlation seems then quite reliable.

Our results can be of interest in the debate concerning HRT and breast cancer. In fact, the reduction in IGF-I levels and the increase in SHBG levels in our patients might suggest some impact on the risk of this disease.

Breast cancer cells express IGF-I receptors and IGF-I is also synthesized by the stromal tissue supporting the tumor [52]; additionally, it seems that the cells expressing IGF-I receptors are the same ones that express estrogen receptors. On the basis of these findings, a downregulation in circulating bioavailable IGF-I, such as appears after oral estradiol, might hardly be considered a negative event. As well, the increase in SHBG levels should decrease the share of free estrogens and androgens. These changes are the same as observed after tamoxifen, the drug administered to breast cancer patients in order to prevent tumor relapse or the onset of a primitive tumor in the contralateral breast [38]: as does estradiol in our study, the use of tamoxifen [40] increased the levels of IGFBP-1, which proved able to induce apoptosis in human breast cancer cell lines, even independently from IGF-I [53].

Concerning IGFBP-3 and IGF-II, we observed no variations in their levels during the treatment.

The effects we observed immediately after the oral administration of low-dose 17 $\beta$ -estradiol were not modified by sequential progesterone administered orally. Moreover, progesterone administered by the vaginal route did not modify estradiol-induced variations in the levels of SHBG and IGF-I. We did expect this result, as only androgen-derived

progestogens seem able to abrogate estradiol-induced responses [32] and progesterone is devoid of any androgenic activity [31]. In particular, any modulation of progestogens on the synthesis of growth regulating factors and SHBG by the hepatocytes would be mediated by androgen receptors, while progesterone receptors seem to be absent in these cells [54].

On the other hand, these considerations make it difficult to understand why the levels of IGFBP-1 returned to their basal values after progesterone administration by the transvaginal route, which leads to lower circulating levels of the hormone and likely to still higher metabolic neutrality. We have no hypotheses to explain this fact.

The use of low-dose oral estradiol and natural progesterone might be suggested for long-term HRT schedules, with the purpose of minimizing the risk of breast cancer which is supposed to accompany HRT. In fact, at least from a speculative point of view, our data do not support the hypothesis that their administration should increase breast cancer risk.

In our study, the levels of progesterone obtained by the oral route were significantly higher than those gained transvaginally; this can be explained by the possible transport of the hormone from the vagina directly to the uterus [55], partially by-passing the systemic circulation. However, the uterine effects of progesterone were similar in both groups, at least in terms of the occurrence of menstrual-like bleeding. In 12/15 (transvaginal) versus 11/15 (oral) patients a light menstrual flow occurred at the end of the first cycle; in the following months it persisted in most of these patients, and transvaginal ultrasounds performed in the pseudo-luteal phase of the 12th cycle never evidenced an endometrial thickness exceeding 4 mm.

## Conclusions

Our data confirm that oral estradiol significantly reduces the plasma levels of IGF-I and increases IGFBP-1 and SHBG concentrations. These effects appear at once, during the first cycle, even with the low estradiol dose of 1 mg daily. The lack of effects on GH levels leads us to hypothesize a direct action of estradiol on the hepatic synthesis of IGF-I and IGFBP-1.

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