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The luteinising hormone–releasing hormone analogue triptorelin with or without the aromatase inhibitor formestane in premenopausal breast cancer: effects on bone metabolism markers

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Abstract

Background: the combination of a luteinising hormone–releasing hormone (LH–RH) analogue and an aromatase inhibitor (AI) induces greater oestrogen suppression than the analogue alone in premenopausal breast cancer. However, very few data on the biological effects of such a combination are currently available. *Aim of the study:* the short-term effects of treatment with the LH–RH analogue triptorelin alone or in association with the AI formestane on bone metabolism were investigated in premenopausal breast cancer. Circulating levels of the bone formation markers carboxy-terminal and amino-terminal propeptides of type I procollagen (PICP and PINP) and the bone resorption marker cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) were assessed. In addition, serum levels of insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3 and interleukin 6 (IL-6) were evaluated. *Patients and methods:* twenty-one patients with advanced breast cancer were randomly given triptorelin monthly alone ($n = 10$, arm A) or in combination with formestane fortnightly ($n = 11$, arm B). Blood samples were collected over a 3-month period. *Results:* serum PICP and PINP levels increased significantly over time ($P = 0.0065$ and 0.0197 in arm A and B, respectively); no change in ICTP levels was observed. A rise in IGF-I and IGFBP-3 levels was seen in each treatment group, but only the increase in IGF-I was significant ($P = 0.0138$, always). The on-treatment levels of the bone turnover markers and IGF-system components were inversely correlated with serum oestrogens. Neither treatment modalities significantly affected serum IL-6 levels over time. No difference in the behaviour of any of the assessed biomarkers was observed between patients with or without skeletal metastases. *Conclusion:* it is worth noting that complete oestrogen depletion, at least in our case series, seems to increase only osteoblastic activity markers. The observed modifications appear to be related to oestrogen depletion per se rather than the degree of oestrogen suppression or the different therapeutic regimen administered.

Keywords: Breast cancer; Bone metabolism markers; Growth factors; Cytokines; Oestrogens; Gonadotrophin releasing-hormone; Aromatase inhibitors

1. Introduction

The goal of endocrine therapy in breast cancer is to inhibit the oestrogen-stimulated growth of tumour cells. This can be accomplished in two major ways — by blocking the oestrogen receptor (ER) at the target cell or by inhibiting the oestrogen supply to tumour tissue [1]. The latter approach relies on the suppression of oestrogen biosynthesis by means of inhibitors of the aromatase enzyme complex [2,3]. Although the role of aromatase inhibitors (AIs) in the management of post-menopausal breast cancer is well established, a high degree of ovarian aromatase activity together with the

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compensatory endocrine loops induced by oestrogen blockade have as yet prevented any meaningful sex steroid suppression by AIs in premenopausal patients [4,5]. Accordingly, in menstruating patients oestrogen deprivation has been induced by surgical oophorectomy or medical castration using luteinising hormone–releasing hormone (LH–RH) analogues [6,7]. However, oestrogen levels approximate those observed in postmenopausal women, probably because neither surgical, nor medical castration suppresses peripheral aromatisation [8]. It has been reported that breast cancer patients who relapsed while on LH–RH analogue treatment alone, experienced a further tumour remission when serum oestrogen levels were additionally decreased by aromatase inhibition [8,9]. However, very few data on the therapeutic and biological effects of such an association are currently available.

We have recently reported on the endocrine effects of the combination of the LH–RH analogue triptorelin with or without the AI formestane (4-hydroxyandrostenedione, 4-OHA) in advanced premenopausal breast cancer [10]. Since the combination induced a much greater suppression of circulating 17β-oestradiol (E_2), oestrone (E_1) and oestrone sulphate (E_1-S) levels than did the analogue alone, we investigated whether there was any differential influence on some relevant biomarkers of bone metabolism in the two treatment groups. Here we report on the short-term effects of two therapeutic modalities on circulating levels of the bone formation markers, carboxy-terminal and amino-terminal propeptides of type I procollagen (PICP and PINP, respectively), and the bone resorption marker, cross-linked carboxy-terminal telopeptide of type I collagen (ICTP). Since the insulin-like growth factor (IGF) system and the cytokine, interleukin 6 (IL-6), have been shown to be involved in bone remodelling, serum levels of IGF-I, IGF binding protein-3 (IGFBP-3) and IL-6 were also assessed [11,12].

Table 1
Main patient characteristics[a]

	Treatment group	
	Triptorelin ($n=10$)	Triptorelin + 4-OHA ($n=11$)
Median age (years; range)	45 (30–49)	45 (39–52)
Median weight (kg; range)	61 (53–68)	61 (48–81)
Disease-free interval		
<2 years	2	3
≥2 years	8	8
Receptor status		
ER positive	10	6
ER negative	–	4
ER unknown	–	1
PgR positive	6	9
PgR negative	3	–
PgR unknown	1	2
Dominant disease status[b]		
Soft tissue	4	4
Viscera	7	7
Bone	1	7
Number of disease sites		
1	10	7
≥2	–	4
Previous adjuvant therapy[b]		
None	2	1
Cytotoxics	4	2
Radiotherapy	6	9

[a] 4-OHA, 4-hydroxyandrostenedione; ER, oestrogen receptor; PgR, progesterone receptor.
[b] Some patients appear in more than one category.

2. Patients and methods

2.1. Patients

Twenty-one consecutive unselected premenopausal patients with previously untreated advanced breast cancer were randomly allocated to receive an i.m. depot formulation of triptorelin (Decapeptyl®) 3.75 mg once monthly administered alone (ten patients) or in combination with formestane (Lentaron®) 500 mg i.m. fortnightly (11 patients). Both drugs were injected by nurses in an outpatient setting during the first 3 months of treatment, after which the patients were trained to inject themselves. The treatment was continued until disease progression or the occurrence of any severe adverse event. Patients entered the study if they had a positive ER and/or progesterone receptor (PgR) tumour status, and a performance status ≤ 2 (ECOG scale), provided they did not suffer from any endocrine disorders. All eligible patients were actively menstruating and were allowed to have received adjuvant cytotoxic chemotherapy but no previous endocrine adjuvant therapy. A minimum 3-week washout period was required prior to entry into the study. None of the patients received any other form of endocrine treatment, anticancer treatment or drugs known to influence drug or hormone disposition during the study period. In order to avoid any influence on the assessment of circulating IL-6 levels due to the use of nonsteroidal anti-inflammatory agents inhibiting prostaglandins, the patients were given alternative drugs, if indicated. Written informed consent was obtained from all patients, and the study protocol was approved by the local Bioethical Committee.

The main characteristics of the study population are shown in Table 1. The treatment groups were comparable in terms of age, body weight, disease-free interval and previous adjuvant therapy. No patient was obese

Table 2
Geometric mean levels (and corresponding 95% confidence intervals) of bone metabolism markers at each time point and in each treatment group[a]

	Treatment week	Treatment group	
		Triptorelin ($n = 10$)	Triptorelin + 4-OHA ($n = 11$)
PICP (ng/ml) (95% CIs)	Baseline	93.88 (84.16–104.62)	106.06 (84.15–133.63)
	Week 1	100.18 (86.96–115.41)	119.58 (97.88–146.23)
	Week 2	94.92 (76.10–118.30)	119.58 (93.88–152.30)
	Week 4	105.32 (93.76–118.18)	103.34 (81.96–130.27)
	Week 8	107.99 (93.78–124.26)	116.05 (96.31–139.80)
	Week 12	135.91 (101.60–181.96)	134.42 (107.35–168.31)
PINP (ng/ml) (95% CIs)	Baseline	42.56 (32.99–54.91)	50.96 (32.18–80.69)
	Week 1	45.51 (33.41–62.00)	55.20 (35.33–86.27)
	Week 2	49.65 (32.80–75.11)	51.62 (32.51–81.99)
	Week 4	50.80 (31.17–82.82)	55.71 (31.67–97.98)
	Week 8	48.38 (33.18–70.53)	62.99 (39.48–100.58)
	Week 12	63.75 (38.94–104.42)	102.10 (47.46–106.05)
ICTP (ng/ml) (95% CIs)	Baseline	4.04 (3.18–5.13)	4.72 (4.00–6.05)
	Week 1	3.87 (3.02–4.96)	4.12 (3.80–5.86)
	Week 2	3.89 (2.83–5.33)	4.75 (3.06–6.01)
	Week 4	4.46 (3.39–5.86)	5.05 (3.58–6.31)
	Week 8	4.68 (3.44–6.36)	4.74 (3.69–6.91)
	Week 12	4.93 (4.00–6.05)	4.73 (3.92–5.47)

[a] 4-OHA, 4-hydroxyandrostenedione; CIs, confidence intervals; PICP, carboxy-terminal of type I procollagen; PINP, amino-terminal of type I procollagen; ICTP, carboxy-terminal of type I collagen.

or had severely impaired hepatic and/or renal function. No episodes of hypercalcaemia occurred during the study period.

2.2. Endocrine investigations

Blood samples were taken at baseline and 1, 2, 4, 8 and 12 weeks after the start of the therapy. Throughout the study, the blood samples were collected at the same time of day from each patient (between 9:00 and 10:00 h) after an overnight fast and before drug administration. The serum for the assessment of biomarkers was separated by centrifugation immediately after clotting and stored at -70°C until endocrine measurement. All endocrine measurements were performed at the laboratory of the Unit of Nuclear Medicine Division.

The PICP, PINP, and ICTP serum levels were measured through the study by means of radioimmunoassays (RIAs) supplied by Orion Diagnostic (Espoo, Finland). The lowest detectable doses were 1.2, 2 and 0.5 ng/ml for PICP, PINP, and ICTP, respectively. The normal value ranges were 50–170 ng/ml for PICP, 19–84 ng/ml for PINP and 1.8–5.0 ng/ml for ICTP. The intra- and interassay coefficients of variation (CVs) were $< 6\%$ in all the cases.

Alteration in the IGF-system were evaluated at baseline and 4, 8, and 12 weeks after the start of the therapy. The IGF-1 and IGFBP-3 serum levels were estimated by means of RIAs purchased from Italiana Laboratori Bouty (Milan, Italy) and CIS Diagnostici (Tronzano Vercellese, Italy), respectively. The methodology of these assays has been previously described [13]. Serum IL-6 levels were determined by a solid-phase enzyme amplified sensitivity immunoassay (EASIA) purchased from Medgenix Diagnostics (Fleurus, Belgium). The IL-6 EASIA minimum detectable concentration was 2 pg/ml and the intra- and interassay CVs were $< 7.5\%$.

The accuracy of the assays was tested against serum samples with known concentrations of the tested analytes. The assays were performed in duplicate and all samples from the same patients were analysed in the same assay batch.

2.3. Statistical methods

The endocrine data were log-transformed in order to approximate a Gaussian distribution: geometric mean values and 95% confidence intervals were therefore used rather than arithmetic means.

The variation over time of each analyte was analysed by adopting a mixed effect linear modelling approach, in such a way as to account for possible correlations among longitudinal measurements within the same subject [14]. To compare the two treatment groups, time, treatment and the time–treatment interaction were entered into the models using 0–1 indicator variables; pre-treatment measurements were also included as covariates in order to adjust for possible baseline imbalances between the two groups. The possible influence of

bone metastases on marker levels was also investigated by including this factor (and its interaction terms) in the models.

A number of correlation structures between the longitudinal measurements were tried. The reported statistical results were obtained using a first-order autoregressive correlation structure with heterogeneous variances, which generally provided the best fit. As currently suggested, the estimation algorithm adopted was the restricted maximum likelihood.

To investigate the possible association between bone metabolism markers and the remaining analytes, Spearman's correlation coefficients (and corresponding P values) were computed. Accordingly, a graphical exploration of associations was obtained by means of scatterplots of standardised values (z-scores). The conventional 5% significance level was adopted in all the analyses. All computations were made using SAS software (Cary, USA) [15].

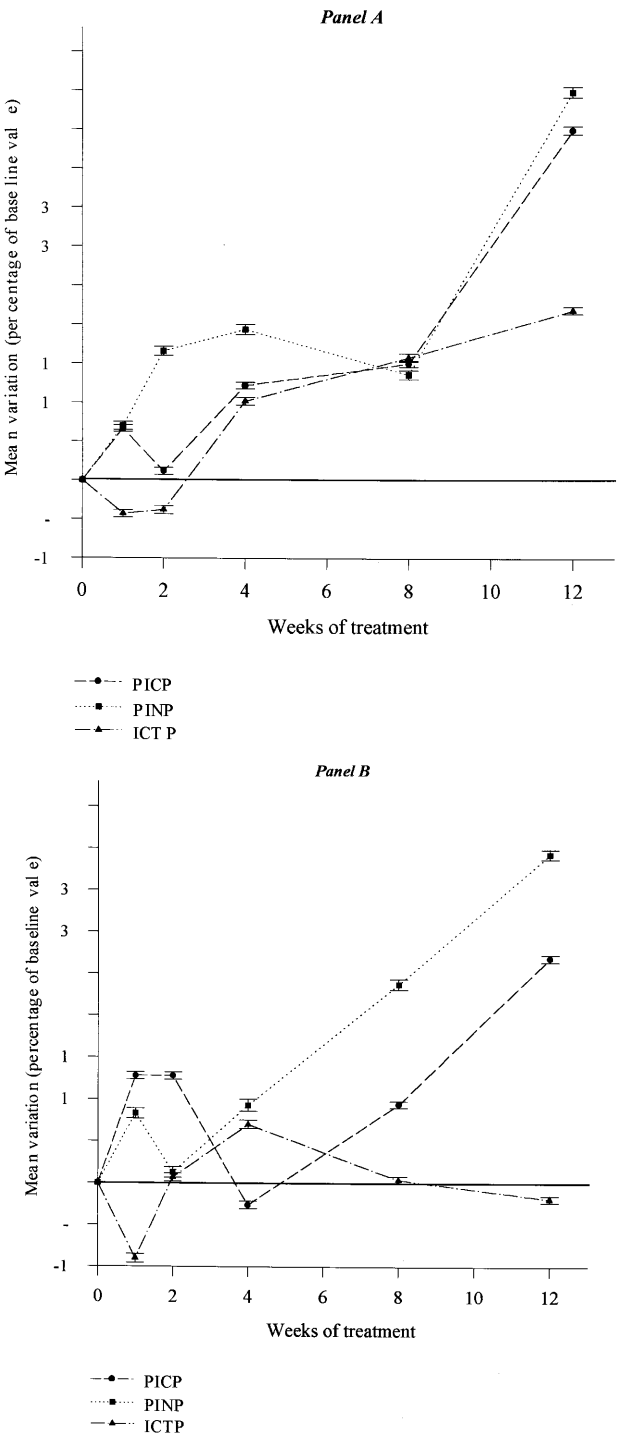

Fig. 1. On-treatment modifications of circulating bone metabolism markers (calculated as percentage with respect to mean baseline values). Mean geometric percentage variations of carboxy-terminal of type I procollagen (PICP), amino-terminal of type I procollagen (PINP) and carboxy-terminal of type I collagen (ICTP) in patients treated with triptorelin alone (panel A) or in combination with formestane (panel B) over a 3-month period.

3. Results

3.1. Circulating oestrogen levels

The effects of each treatment modality on circulating oestrogens have been reported in another paper [10]. Briefly, after 4 weeks of treatment the baseline serum E_2 levels in the analogue alone and combination groups decreased by an average of 86.9% (95% CI, 70.5–94.2%) and 97.3% (95% CI, 98.8–94.1%); E_1 by an average of 48.5% (95% CI, 27.5–63.5%) and 70.4% (95% CI, 52.3–81.6%); and E_1-S by an average of 56.7% (95% CI, 40–68.8%) and 80.5% (95% CI, 69.4–87.6%). All between-group differences were statistically significant and remained unchanged thereafter.

3.2. Circulating bone metabolism marker levels

There was no clear evidence of a possible effect of bone metastases on marker serum levels. Therefore, the results described henceforth disregard this factor.

The mean serum concentrations of PICP, PINP and ICTP over 3 months are reported in Table 2. A graphic representation of bone metabolism marker levels, in terms of mean percentage changes versus baseline is given in Fig. 1. The bone formation markers (PICP and PINP) increased in both the treatment groups. The increase over time was statistically significant for both biomarkers ($P = 0.0065$ for PICP and 0.0197 for PINP), regardless the treatment options neither treatment, nor the time–treatment interaction, were statistically significant. In contrast the circulating bone resorption marker (ICTP) levels remained unchanged in both the treatment groups.

3.3. Circulating growth factor and cytokine levels

The mean serum concentrations of IGF-1, IGFBP-3 and IL-6 are reported in Table 3. A graphic representation of the serum levels in terms of mean percentage

Table 3
Geometric mean levels (and corresponding 95% confidence intervals) of growth factors and interleukin-6 at each time point and in each treatment group[a]

	Treatment week	Treatment group	
		Triptorelin ($n = 10$)	Triptorelin + 4-OHA ($n = 11$)
IGF-1 (ng/ml)	Baseline	171.23 (149.63–196.12)	215.51 (177.89–261.32)
(95% CIs)	Week 4	185.49 (157.44–218.38)	224.92 (183.17–276.51)
	Week 8	205.82 (168.51–251.61)	250.38 (205.76–304.39)
	Week 12	195.00 (143.63–264.75)	257.24 (210.88–313.86)
IGFBP-3 (μg/ml)	Baseline	2.77 (2.41–3.16)	3.06 (2.73–3.43)
(95% CIs)	Week 4	2.87 (2.52–3.27)	3.23 (2.93–3.56)
	Week 8	3.06 (2.51–3.74)	3.36 (3.06–3.68)
	Week 12	2.96 (2.45–3.57)	3.52 (3.18–3.88)
IL-6 (pg/ml)	Baseline	4.73 (2.70–8.26)	3.24 (2.17–4.84)
(95% CIs)	Week 1	4.26 (2.21–8.22)	2.99 (1.75–5.09)
	Week 12	4.45 (2.02–9.78)	2.30 (1.92–2.74)

[a] 4-OHA, 4-hydroxyandrostenedione; CIs, confidence intervals; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein-3; IL-6, interleukin-6.

Table 4
Spearman's correlation coefficients (corresponding P values) among on-treatment circulating levels of bone metabolism markers, growth factors and interleukin-6[a]

	E_1	E_1-S	PICP	PINP	ICTP	IGF-1	IGFBP-3	IL-6
E_2	0.80[b] (0.0001)	0.82[b] (0.0001)	−0.22[b] (0.0135)	−0.20[b] (0.0254)	−0.26[b] (0.0034)	−0.26[b] (0.0187)	−0.32[b] (0.0034)	0.36[b] (0.0039)
E_1	–	0.86[b] (0.0001)	−0.21[b] (0.0190)	−0.22[b] (0.0172)	−0.23[b] (0.0116)	−0.11 (0.3246)	−0.14 (0.1875)	0.28[b] (0.0266)
E_1-S	–	–	−0.22[b] (0.0144)	−0.22[b] (0.0176)	−0.23[b] (0.0119)	−0.16 (0.1403)	−0.27[b] (0.0142)	0.26[b] (0.0401)
PICP	–	–	–	0.74[b] (0.0001)	= 0.54[b] (0.0001)	0.06 (0.5772)	−0.05 (0.6329)	−0.03 (0.7998)
PINP	–	–	–	–	0.74[b] (0.0001)	−0.01 (0.9493)	−0.16 (0.1508)	0.06 (0.66329
ICTP	–	–	–	–	–	0.02 (0.8747)	−0.05 (0.6734)	−0.04 (0.7643)
IGF-1	–	–	–	–	–	–	0.75[b] (0.0001)	−0.27 (0.0773)
IGFBP-3	–	–	–	–	–	–	–	−0.46[b] (0.0021)

[a] E_2, 17β-oestradiol; E_1, oestrone; E_1-S, oestrone-sulphate; PICP, carboxy-terminal of type I procollagen; PINP, amino-terminal of type I procollagen; ICTP, carboxy-terminal of type I collagen; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein-3; IL-6, interleukin-6.
[b] Statistically significant correlations.

changes versus baseline is given in Fig. 2. Although both IGF-1 and IGFBP-3 concentrations increased over time, the rise in IGF-1 proved to be statistically significant ($P = 0.0138$). No change in IL-6 levels was observed in either treatment group.

3.4. Correlation analysis

Table 4 reports the estimated correlation coefficients (and corresponding P values) among on-treatment circulating levels of bone metabolism markers, growth factors and IL-6. Fig. 3 shows the scatterplots of E_2 levels versus PICP (panel A), PINP (panel B) and ICTP (panel C).

Weak, negative significant correlations were observed between all evaluated oestrogens and bone metabolism markers. In our case series, E_2 correlated significantly also with circulating IGF system components and IL-6. Negative correlations were found between E_2 and IGF-1 and between E_2 and IGFBP-3, whilst E_2 showed a positive correlation with IL-6. Circulating E_1 showed a positive significant association only with IL-6, and E_1-S levels significantly correlated with IGFBP-3 and IL-6, albeit in a very weak manner. Weak, positive and

statistically significant correlations were demonstrated between the two procollagen derivatives (PICP and PINP), between PICP and ICTP and between PINP and ICTP. No statistically significant association was found between the IGF system and bone metabolism markers, but circulating IGF-1 was shown to be positively correlated to IGFBP-3 and IL-6 displayed a weak but significant negative correlation with IGFBP-3.

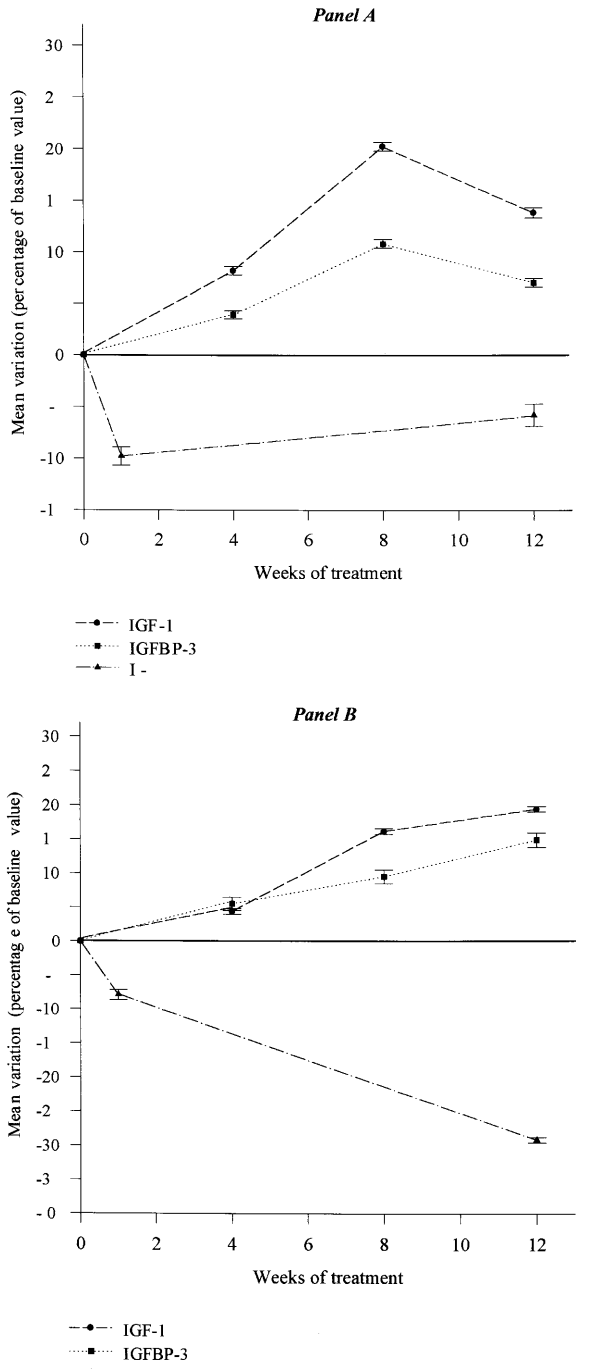

Fig. 2. On-treatment modifications of circulating insulin-like growth factor system markers and interleukin-6 (calculated as percentage with respect to mean baseline values). Mean geometric percentage variations of insulin-like growth factor (IGF)-1 and IGF binding protein-3 (IGFBP-3) in patients treated with triptorelin alone (panel A) or in combination with formestane (panel B) over a 3-month period.

4. Discussion

Bone tissue is continuously remodelled by resorption and formation. This process is referred to as 'coupling' and is responsible for the maintenance of a firmly fixed bone mass [16]. In women steroid hormones play a key role in regulating the equilibrium between osteoblastic and osteoclastic activity, as demonstrated by the observation that a reduction in circulating oestrogen induces bone loss [17,18]. For this reason it is interesting to study the changes in bone formation (PICP and PINP) and resorption (ICTP) markers induced by oestrogen depletion. PICP and PINP are released into the circulation in stoichiometric relation to total collagen synthesis and deposition. Consequently, PICP and PINP measurement is a sensitive tool to evaluate the deposition of type I collagen, the major structural protein of the bone. ICTP is released into the circulation during the resorptive process, and thus its measurement could be clinically employed in detecting changes in bone resorption [19]. The insulin-like growth factor-system and the cytokine IL-6 have been reported to be involved in bone remodelling. IGFs are known to have anabolic effects on bone, but only IGF-1 has been demonstrated to have the ability to promote osteoblast differentiation, so only this component of the IGF system can be properly used as an index of bone formation [20]. Otherwise, it has been demonstrated in humans that IL-6 indirectly promotes osteoclast development, probably acting through other cytokines (e.g. IL-1β and tumour necrosis factor α). At the same time, IL-6 seems to inhibit osteoblast differentiation [21].

Since the main biological effect of hormonal treatments for premenopausal breast cancer is to reduce circulating steroids to postmenopausal levels, it is interesting to investigate if they may induce biological effects similar to those observed in postmenopause on bone metabolism. The postmenopausal oestrogen decline induces increases in both formation and resorption markers; hence, the net bone loss typical of this status is due to an imbalance of the 'coupling' process in favour of the osteoclastic activity. However, the results of correlation study between bone resorption markers and bone status are contradictory. In this regard Cosman et al. in a study on 81 pre- and post-menopausal women conclude that measuring individual markers of bone turnover cannot replace serial bone densitometry for accurate determination of change in bone mass [22,23]. On the contrary, Krall et al. suggest that age-related decrease in bone mineral density (BMD) my vary by gender and skeletal sites, and determinations of os-

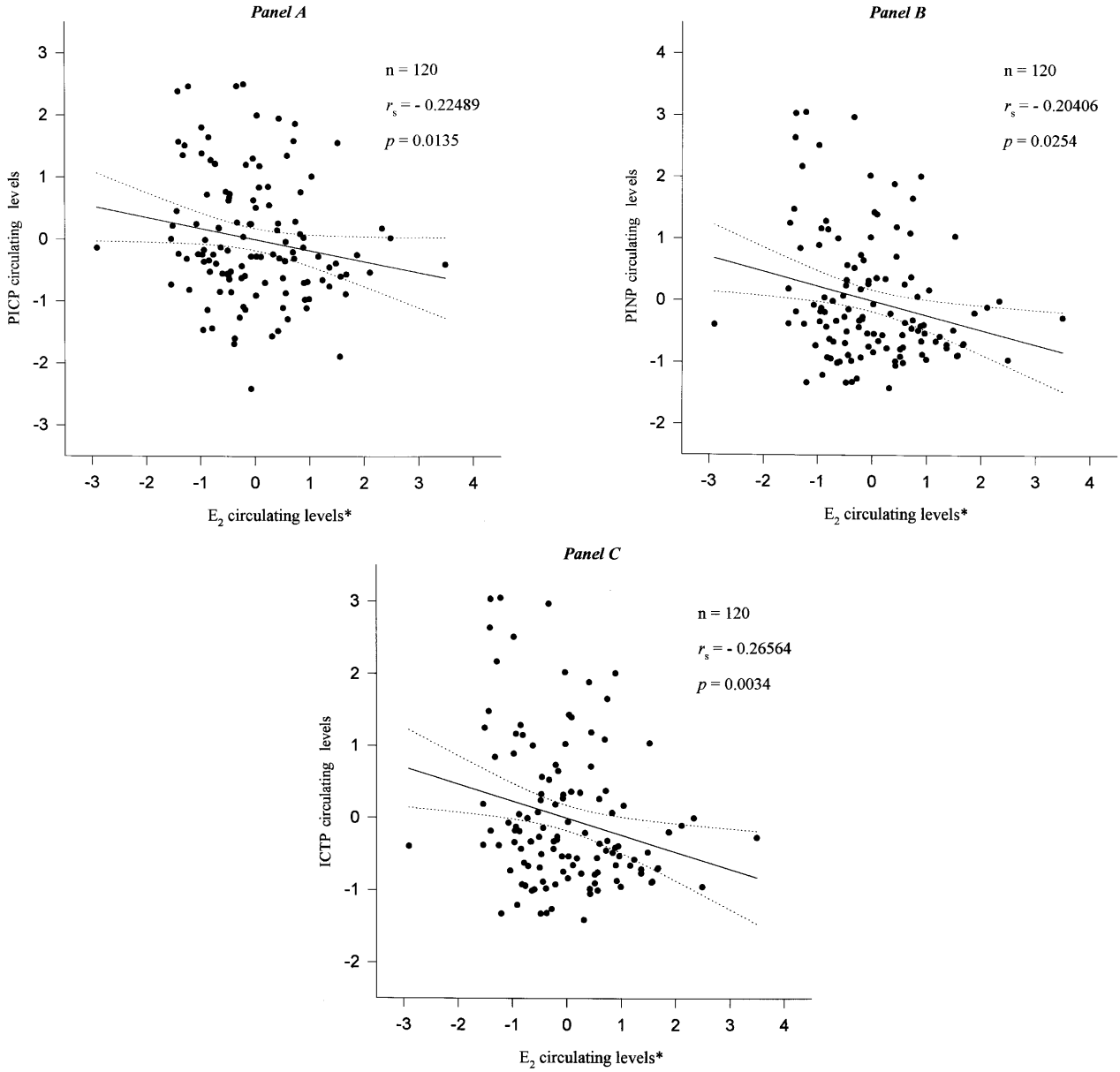

Fig. 3. Scatterplot of 17β-oestradiol levels versus circulating bone metabolism markers PICP (panel A), PINP (panel B), ICTP (panel C).

teocalcin and *N*-telopeptide crosslinks at single point in time may potentially be used as an indicator of current bone status [24,25]. Previous studies reported that the rate of bone turnover in premenopausal women is not related to that observed after menopause; different biological mechanisms are likely to be involved in the two conditions [16,26].

In our study the observed changes in the tested analytes are due to the decrease in circulating oestrogen rather than other factors, as demonstrated by the correlation analysis. Among the oestrogens, E_2 was shown to correlate well with both bone formation (PICP, PINP and IGF-1) and bone resorption (ICTP and IL-6) markers. Our data also confirm that oestrogen suppression influences bone metabolism in pre-menopausal women. The increase in osteoblastic activity markers (PINP, PICP, IGF-1 and IGFBP-3) observed in our patients series had already been reported by other authors during oestrogen depletion [27,28], but such rises in markers of bone formation did not correspond to similar changes in bone resorption marker (ICTP and IL-6). Likely the behaviour of serum levels of bone resorption markers found in our study are related to the short-term examination of our patients [29]. These experimental data may lead to the conclusion that premenopausal steroid depletion might not be accompanied by bone loss as occurs during menopause. However, this hypothesis should be proved by more sound

biological evidences, e.g. the absence of bone resorption processes in bone biopsy specimens from premenopausal women submitted to pharmacological oestrogen suppression.

Statistical analysis show that the alterations in the bone turnover markers were similar in both the treatment groups; the observed alterations may therefore be attributed to circulating oestrogen suppression per se, irrespective of the drug employed to obtain the steroid depletion or the degree of oestrogen reduction.

The addition of an AI to an LH–RH superagonist does not seem to significantly influence biological markers related to bone loss, although a more conspicuous
oestrogen suppression is achieved than with the single agents alone. In our study, patients' blood samples were available only for 3 months, hence our conclusions only apply to the short term. Further studies should be undertaken to verify the long-term effects of complete oestrogen deprivation on premenopausal bone metabolism. The measurement of circulating molecules derived from bone proteins does not allow complete knowledge of the bone status, but it does offer definite advantages over other means of investigations, as it is non-invasive, simple and reproducible.

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