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Bone Mineral Content and Estrogen Receptors in Patients with Breast Cancer*

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Abstract—*A standardized* in vivo *bone mineral concentration was measured at the distal forearm of* 24 *untreated patients presenting with breast cancer. This measurement was compared with the presence of unoccupied high affinity estrogen receptors in the tumour tissue excised. Patients with detectable levels of estrogen binding/*mg *tissue protein had significantly higher bone mineral concentration than patients with undetectable estrogen binding/*mg *protein, although in neither group did the bone measurement differ significantly from age and sex matched normal controls.*

INTRODUCTION

IT HAS been shown that the response to estrogen therapy is correlated to the existence of estrogen receptors in breast tumour tissue [1–3] whereas no relation to estrogen level has been found. These findings indicate that it is the ability of estrogen to act through an estrogen receptor which is responsible for the control of tumour cell proliferation in some breast tumours.

A relation between bone mass and estrogen has been reported in several studies [4–6]. This suggests that bone mass is to some extent a reflection of estrogen action and since both the existence of estrogen receptors and the bone formation ability seems to be closely related to the action of estrogen, it could be of interest to investigate, whether there is a relation between the estrogen receptor content in breast tumour tissue and the bone mass in the same patient.

To elaborate on this problem we have determined bone mineral content (BMC) in the forearm by photon absorptiometry and the amount of estrogen receptors in breast cancer tissues, by a dextran–charcoal estrogen receptor assay.

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Key words: bone mineral cotent, breast cancer, densitometry, estrogen receptors, photon absorptiometry.

MATERIALS AND METHODS

Patients

Twenty four women aged 34–78 yr (mean 56.8 yr) admitted to the Radium Centre over an 8 month period for untreated breast cancer were included in the study. The final diagnosis was based on microscopic examination of tissue removed during operation.

The patient group consisted of 13 cases with local tumour, 6 cases with metastatic spread to regional lymphnodes or to the skin and 5 cases with bone metastases.

All patients had normal kidney function and none of the patients had clinical symptoms of benign bone disease or gastrointestinal disease. Only one patient (No. 8) had been treated with estrogen over a peroid of 18 months within the last 3 yr before the study.

Methods

The bone mineral content (BMC) in the forearm was measured using a GAMMATEC osteodensitometer model GT 30 with a 50 mCi ^{125}I source. This model was a digital version of that described by Christiansen *et al.* [7].

The scan site was selected automatically by the instrument as the most distal position where the separation of radius and ulna exceeds 8 mm. Six scans with a separation of 4 mm between each were carried out and the average BMC displayed. Recording of the absorption was performed with a Servogor two-channel potentiometric recorder. The time constant for the densitometer was 50 msec and the recorder had a rise time of 130 msec/cm. The scanning speed was 2 mm/sec. The beam of the source was collimated to a diameter of 1 mm. BMC expressed as g ash/cm was calculated as the mean values of the two arms.

The bone mineral concentration was calculated as BMC/bone area (g ash/cm^3). Bone area = cross sectional bone area was calculated from the formula: bone area (cm^2) = 1.35 × BW (cm) − 1.53, as found in a study of the interrelationship between densitometric and histomorphometric measurements of radius and ulna in the distal part of the forearm in 12 cadavers [8, 9]. The combined width of radius and ulna (BW) was measured on the densitometric absorption curves, as the width of the part of the curve different from the zero-line minus 1 mm (thickness of the ^{125}I beam [8, 9].

BMC and BMC′ were expressed in absolute values and in % of normal mean for same age and sex. The reproducibility of BMC was about 1% and of BMC′ about 3% for normal individuals.

Preparation of the cytosol from breast tumour biopsy specimens

The tissue was minced with a pair of scissors, cooled in liquid nitrogen and homogenized in a Schwingmühle (Retch, West Germany).

The homogenate was weighed and suspended in a three-fold volume of TE-buffer (Tris 10 mM, EDTA 1.5 mM, NaN_3 1.0 mM, pH 7.4). The suspension was centrifuged at 100,000 ***g*** and 4°C for 1 hr (Beckman, Spinco Ultracentrifuge L 50). The supernatant, the cytosol, was assayed for high affinity estrogen receptors as described below.

The estrogen receptor assay

The method used was originally described by Mester *et al.* [10], Fehretry *et al.* [11] and later modified by Daehnfeldt [12]. The assay is a dextrancoated charcoal assay, which allows the calculation both of the total binding capacity of unoccupied high affinity receptors as well as the dissociation constant [13, 14].

Briefly, 50 µl cytosol was incubated at 0°C with 3-H-17-beta-estradiol (90 ci/mmol, The Radiochemical Centre, Amersham, U.K.) and at least five different concentrations of 17-beta-estradiol (5 µl solution in 99% ethanol + 25 µl TKE buffer) ranging from 2.9 × 10^{-10} M to 7.2 10^{-9} M for 2 hr in duplicates. The TKE buffer consited of: Tris 10 mM, KCl 50 mM EDTA 1.0 mM, NaN_3 1.0 mM, pH = 7.4.

The incubation was terminated by addition of 250 µl dextran-coated suspension (5.0 mg dextran T-70, 500 mg charcoal, 200 ml TKE buffer). After absorption for 30 min at 0°C the charcoal was spun down (800 ***g***, 10 min, 4°C) and the radioactivity in an aliquot of the supernatant was determined by liquid scintillation (Packard Tricarb 3003 spectrophotometer, efficiency for tritium approximately 25%). Quench correction was carried out by the channel ratio method. To correct for deficient absorption controls were used containing 3-H-17-beta-estradiol, TKE buffer and bovine albumin and the radioactivity was subtracted from the experimental values.

The binding capacities were read from a Scatchard plot (Fig. 1) which allows a correction for low-affinity binding as well as a determination of K_d (dissociation constant) values.

When the results were scattered along a sloping straight line the biopsies were defined as "*receptor-positive*". When the binding data

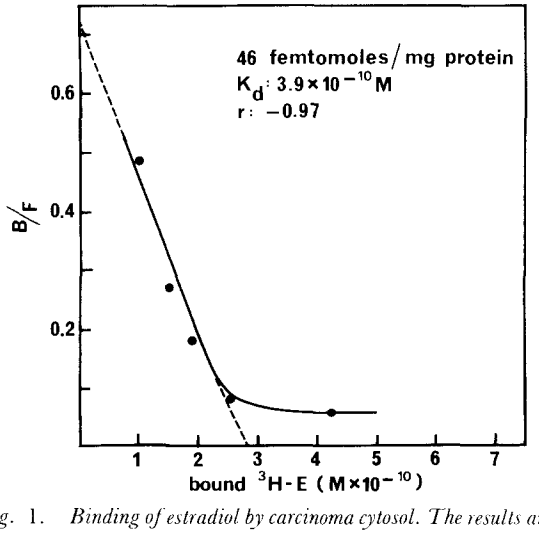

Fig. 1. Binding of estradiol by carcinoma cytosol. The results are presented in a Scatchard plot with molar concentration on the abscissa and ratio bound to free estradiol on the ordinate. Regression line is calculated by the square fit from the results in the steepest part of the curve, and the interception of this line with the abscissa gives the binding capacity, whereas the slope of this line gives an approximate value of the association constant of the estradiol-receptor complex.

were scattered along a straight line with no slope, when no straight line could be drawn with any confidence, or when no significant binding of estradiol was recorded the biopsies were defined as "*receptor-negative*".

Protein measurements were carried out by the method described by Lowry *et al.* [15] and the binding capacities are presented as fmole estrogen bound/mg cytosol protein.

Statistical evaluations were carried out using the Mann–Whitney U-test and Spearman's rank correlation test.

RESULTS

Clinical data and results of measurements of bone mineral content and estrogen receptors in women with breast cancer are given in Table 1. BMC and BMC′ expressed as per cent of normal values did not differ from normals, neither in receptor-positive nor in receptor-negative patients compared with age and sex matched normal controls. Both BMC ($R=-0.71$, $P<0.001$) and BMC′ ($R=-0.82$, $P<0.001$) decreased significantly with age, whereas no significant relation was seen between bone width (BW) and age ($R=0.06$).

The amount of estrogen receptors increased significantly with age ($R=0.49$, $P<0.02$), whereas the K_d values were not significantly related to age ($R=0.38$).

In receptor-positive patients the BMC′% was significantly elevated compared with receptor-negative patients ($P<0.01$, Fig. 2) and a significant positive correlation was found between BMC′% and the amount of estrogen receptors in breast cancer tissue ($R=0.58$, $P<0.01$), whereas no significant cor-

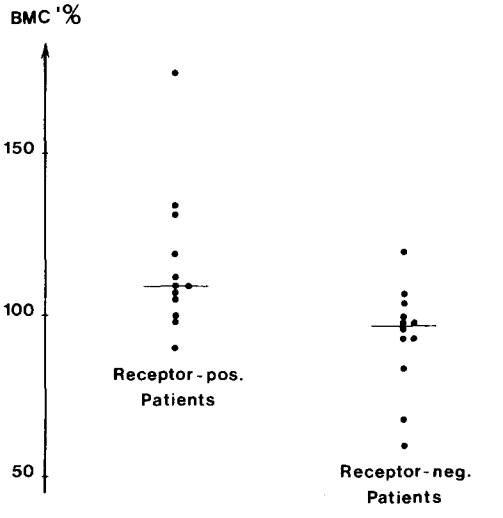

Fig. 2. Bone mineral concentration expressed in per cent of age and sex matched normal controls (BMC′%) in receptor-positive and receptor-negative patients with breast cancer. Medians are indicated.

Table 1. Clinical data, bone mineral content and estrogen receptors in patients with breast cancer

Pat. No.	Age (yr)	Clinical state	BMC g ash/cm	BMC %	BMC′ g ash/cm³	BMC′ %	BW cm	Estrogen receptors fmole/mg protein	Estrogen receptors $K_d \times 10^{-10}$ M
1	60	N	0.74	88	0.40	119	2.5	69	5.3
2	45	T	1.20	97	0.52	98	2.9	—	—
3	45	T	1.50	121	0.56	104	3.1	—	—
4	61	T	0.93	110	0.36	107	3.1	29	3.9
5	77	T	0.73	106	0.30	109	3.0	57	5.0
6	49	N	1.33	107	0.57	109	2.9	46	3.9
7	58	T	1.40	122	0.44	90	3.5	69	3.1
8	61	N	0.95	113	0.37	112	3.0	31	2.9
9	69	N	0.65	78	0.33	98	2.6	83	6.8
10	34	M	1.03	84	0.51	96	2.6	—	—
11	55	T	0.86	75	0.46	93	2.5	—	—
12	59	M	0.76	66	0.30	60	3.0	—	—
13	78	M	0.58	85	0.29	105	2.6	55	2.4
14	74	N	1.03	150	0.48	175	2.7	246	14.0
15	37	M	1.10	90	0.54	100	2.7	13	9.0
16	51	T	1.15	100	0.41	84	3.2	—	—
17	54	N	1.59	138	0.53	107	3.4	—	—
18	65	T	1.01	120	0.44	131	2.8	48	0.8
19	34	T	1.10	90	0.65	120	2.4	—	—
20	47	T	0.98	79	0.53	100	2.5	—	—
21	59	T	0.96	83	0.34	68	3.3	—	—
22	76	M	0.69	100	0.27	98	3.0	—	—
23	67	T	0.79	94	0.31	93	3.0	—	—
24	48	T	1.86	150	0.70	133	3.2	70	4.2

T = localised tumour; N = breast cancer with involvement of lymphnodes or the skin; M = bone metastases.

relation was found between the BMC% and the amount of estrogen receptors ($R=0.40$, N.S.).

Similar to this finding a significant relationship was found between BMC'% and the dissociation constant K_d ($R=0.56$, $P<0.01$), whereas no significant relation was seen between the BMC% and K_d ($R=0.34$). No relation was found between bone width (BW) and the amount of estrogen receptors ($R=-0.03$). The BMC% and the BMC'% were unrelated to the clinical state of the patients according to the TMN classification (Table 1).

Patient No. 8, who received estrogen therapy during 18 months prior to the study did not show BMC or BMC' values different from the other patients.

DISCUSSION

As found in several studies of healthy women [16, 17] the present study shows decreasing bone mineral content and bone mineral concentration with age in patients with breast cancer. After correcting the BMC' for age-variation, i.e., expressing the values as BMC' in per cent of the values for age and sex matched normal individuals, a markedly and significant increase in bone mineral concentration has been found with increasing amount of estrogen receptors. This finding is somewhat surprising since the amount of unoccupied estrogen receptors in this study and in most earlier studies [1] increased after the menopause, when the production of estrogen hormones is reduced.

As a relation between bone mass and estrogen has previously been reported [4–6] one could have expected an inverse relationship between bone mass and the amount of estrogen receptors in breast tissue. The positive relationship found between BMC'% and the amount of estrogen receptors may reflect a strong estrogen effect in receptor-positive patients as compared with the receptor-negative patients. This relation is, however, only found after correction of the BMC' for age-variation indicating that the negative influence of high age on BMC' is more pronounced than the positive influence of the amount of estrogen receptors.

Several investigators have been looking for estrogen receptors in bone [18] and have not found them, and consequently it has been suggested that bone does not contain estrogen receptors. The methods used for determination of estrogen receptors need, however, a relatively high amount of protein which was also found in this investigation. The lack of finding estrogen receptors in bone might be due to contamination with matrix protein resulting in an unknown, but probably great, dilution of protein from bone formation cells (osteoblasts).

The present study may suggest that bone mass in patients with breast cancer is not only related to age and estrogen hormones, but also to receptor-positivity or receptor-negativity. The findings could indicate that a high estrogen effect in the organism might be mediated both by high levels of estrogen hormones and by a high amount of estrogen receptors.

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