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# Plasma Insulin-like Growth Factor-1 (IGF-1) Concentrations in Human Breast Cancer

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**Insulin-like growth factor-1 (IGF-1) is capable of stimulating breast cancer cell growth *in vitro* and the presence of IGF-1 receptors has been demonstrated in primary breast cancers. We determined plasma IGF-1 in a primary breast cancer population and in a control population. Radioimmunoassays were performed either directly on plasma, IGF-1 (NE), or after an acid–ethanol extraction of the plasma, IGF-1 (E). We demonstrated an inverse correlation between age and IGF-1: for this reason, only results obtained in females of the same age range (> 35 years) were compared. Median concentrations of IGF-1 were significantly higher in primary breast cancers [IGF-1 (E) = 152 ng/ml, IGF-1 (NE) = 26 ng/ml, $n$ = 44] than in controls [IGF-1 (E) = 115 ng/ml, IGF-1 (NE) = 20 ng/ml, $n$ = 92]. To our knowledge such a growth factor increase has never been described in breast cancer. We conclude that IGF-1 could be an important factor involved in the development of breast cancer and that treatment reducing IGF-1 levels could be beneficial for patients.**
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## INTRODUCTION

INSULIN-LIKE growth factor-1 (IGF-1), which is also termed somatomedin-C, is a polypeptide (76 amino acids) involved in the growth of many cell types [1]. The plasma concentration of IGF-1 primarily reflects growth hormone (GH) activity [3] and a highly significant correlation between IGF-1 levels and integrated daily GH secretion has been found [4]. Other factors are known to affect the IGF-1 plasma concentration: nutritional status, sex, oestrogen and age [3, 5]. IGF-1 decreases in acute starvation, is low in chronic malnutrition and is restored by nutritional repletion [6, 7]. It is slightly higher in females than in males, but the effect of oestradiol is variable with plasma IGF-1, decreased in some conditions and increased in others [3]. Finally, a dramatic plasma IGF-1 decline is observed with age [3, 4].

IGF-1 stimulates the growth of human breast cancer cell lines [8–12]. The first step of IGF-1 action is its binding to membrane receptors. IGF-1 receptors have been characterised by saturation or competitive binding and cross-linking techniques in cultured breast cancer cell lines [8, 9, 13–15] and in breast cancer biopsies [16]. Moreover, we and others have demonstrated the presence of IGF-1 receptors in most operable breast cancers [16–19]; histo-autoradiographic studies have allowed the localisation of IGF-1 receptors on ductal epithelia of human primary breast cancers [20]. In our experience, IGF-1 receptor concentrations were much higher in breast cancer than in benign breast disease [21] and the presence of IGF-1 receptors in breast cancers was associated with a better prognosis [22]. These results suggest that IGF-1 could be an important factor involved in the growth of breast cancer. The *in vivo* pathway of IGF-1 action is not precisely known. It appears that IGF-1 would not act via the autocrine pathway: immunoreactive IGF-1 had been found in the medium conditioned by breast cancer cell lines [10, 23, 24], but the demonstration of the absence of IGF-1 mRNA in these cells [25] evidenced that this immunoreactive IGF-1 either represents an IGF-1-related protein or an IGF-1 binding protein [26, 27]. However, IGF-1-like activity and IGF-1 mRNA has been found in human breast cancer biopsies, suggesting a possible paracrine role in these tumours [17, 25]. IGF-1 from endocrine sources may also act on breast cancers: we have shown that, in explant culture, IGF-1 can diffuse freely from the external medium to the breast cancer tissue [28].

It is thus of interest to determine IGF-1 circulating levels in breast cancer patients. In the present work we detected IGF-1 plasma concentrations in such patients and, as age is an important parameter of IGF-1 variation, compared it to IGF-1 in a control population of the same age.

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## PATIENTS AND METHODS

### *Subjects*

Included in this study was an unselected sequence of female breast cancer patients undergoing surgery for locoregional disease in the Centre Oscar Lambret. None of them underwent cancer treatment before surgery. Blood samples were collected at 9 a.m. the day before surgery in tubes containing ethylenediaminetetraacetic acid (EDTA). The plasma was separated by centrifugation, frozen, and stored at −20°C until assayed. For control samples, plasma was collected by the CRTS (Centre Regional de Transfusion Sanguine, Lille, France) from the Centre Oscar Lambret employees, and, for older controls (> 65 years), on healthy women at an old people's home (Barbieux Medical Center, Roubaix, France). All the control plasma was obtained from 9 a.m. to 11 a.m.

### *IGF-1 assay*

The antiserum (UBK487) used for the radioimmunoassay (RIA) was a gift from Drs Underwood and Van Wyk [29]; it was distributed by the Hormone Distribution Program of the

National Institute for Diabetes, Digestive and Kidney Diseases (NIDDK-USA) through the National Hormone and Pituitary Program. As specified by the NIDDK [29], the antiserum was used in a final dilution of 1/18 000 and has 0.5% crossreactivity with IGF-2, it crossreacts minimally with insulin at $10^{-6}$ mol/l. The IGF-1 for standards was purchased from Amersham (ref. ARN 4010), 1 ng of this recombinant DNA-derived ThR-59 analogue of IGF-1 is equivalent to 0.0067 unit. This product was labelled with $^{125}I$ using a low chloramin T concentration method (to 7.4 MBq/μg) [15].

For IGF-1 plasma determination we used the two RIA procedures suggested by the NIDDK. In both procedures, the final incubation volume was 0.5 ml and the buffer used was phosphate/Tween pH 7.5 containing protamine (grade I) $SO_4$ 200 mg/l, sodium phosphate (monobasic) 40 mmol/l, Na azide 0.02%, EDTA 0.01 and Tween 20 0.05%. The first assay of IGF-1 (NE), was the non-equilibrium RIA of furnaletto [29] allowing non-extracted plasma and standard to incubate for 72 h at 4°C with the antibody before [$^{125}I$]IGF-1 addition. The second, IGF-1 (E), was that proposed by Daughaday *et al.* [30] which, thanks to an acid–ethanol-extracted serum, decreases artifacts induced by quantitative and qualitative variations in the IGF-1-binding protein complex. For the plasma extraction we added 0.8 ml of a mixture of 87.5% ethanol and 12.5% 2N hydrochloric acid (vol/vol) into 0.2 ml plasma with thorough mixing in glass tubes. After 30 min (at room temperature) the tubes were centrifuged at 2000 *g* for 30 min at 4°C. Supernatant (0.5 ml) was removed and transferred to a fresh glass tube, and 0.2 ml 0.855 mmol/l Tris base was added and mixed to neutralise the acid–ethanol extract of plasma. Standards and unknowns were incubated for 1 h at 4°C with antibody before the addition of labelled IGF-1. In the two procedures, an overnight incubation with [$^{125}I$]IGF-1 was achieved, and the antibody-bound [$^{125}I$]IGF-1 was precipitated using goat anti-rabbit gammaglobulin as carrier. The assay was performed in duplicate in polypropylene tubes [31]. Each IGF-1 concentration was checked by a second determination. The sensitivity of the assay was 10 ng/ml (B/Bo = 90%). The intra-assay coefficient of variation was 6%; the interassay coefficient of variation was 12%.

*Statistical methods*

As the distribution of IGF-1 plasma concentration values could not be established as normal, non-parametric tests were used. The population localisation was indicated by median value, the population dispersion by lowest and highest values. Correlations between parameters were evaluated using the Spearman-rank correlation, and putative differences between populations were tested using the Mann–Whitney analysis. Nevertheless the corresponding parametric tests, that are generally used in such studies, were also noted as indication. Graphic representations of the studied populations were performed using the box-plot method [32]. In this representation the box represents the middle 50% of the data. The line inside the box is the median. The upper and lower bars estimate the upper and lower quartiles. Single points are considered as outliers.

## RESULTS

*IGF-1 in the control population*

Table 1 shows the statistical characteristics of the control population. IGF-1 (E) and IGF-1 (NE) are quite different. The ratio of the median concentrations of IGF-1 (NE) on IGF-1 (E) was 0.15. Moreover, a high correlation was found between IGF-1 (E) and IGF-1 (NE) by both Spearman coefficient ($r_s = 0.67$; $P < 10^{-4}$) and regression analysis ($r = 0.748$; $P < 10^{-4}$).

*Table 1. Characteristics of the control population* (n: *number of values*; S.D.: *standard deviation*)

| | *n* | Median | Range | Average | S.D. |
|---|---|---|---|---|---|
| Age | 134 | 45.5 | 20–80 | 47.8 | 17.2 |
| IGF-1 (E) | 134 | 142 | 9.5–356 | 147 | 70.4 |
| IGF-1 (NE) | 134 | 23 | 2–64.8 | 23.2 | 12.3 |
| IGF-1 (NE)/IGF-1 (E) | 134 | 0.15 | 0.03–0.92 | 0.17 | 0.095 |

A significant negative correlation could be observed between IGF-1 (E) and age ($r_s = -0.66$; $r = -0.66$; $P < 10^{-4}$) (Fig. 1a) and between IGF-1(NE) and age ($r_s = -0.6$; $r = 0.6$; $P < 10^{-4}$) (Fig. 1b). Conversely no correlation could be observed between age and IGF-1 (NE)/IGF-1 (E) ratio.

*IGF-1 in the breast cancer patient population*

Table 2 shows the statistical characteristics of the breast cancer population. The ratio of the median concentration of IGF-1 (NE) on IGF-1 (E) was 0.18. A correlation was found between IGF-1 (E) and IGF-1 (NE) ($r_s = 0.53$; $P = 0.0003$). A significant inverse correlation between age and IGF-1 (E) was observed ($r_s = -0.41$; $P = 0.0054$) (Fig. 2a), but there was

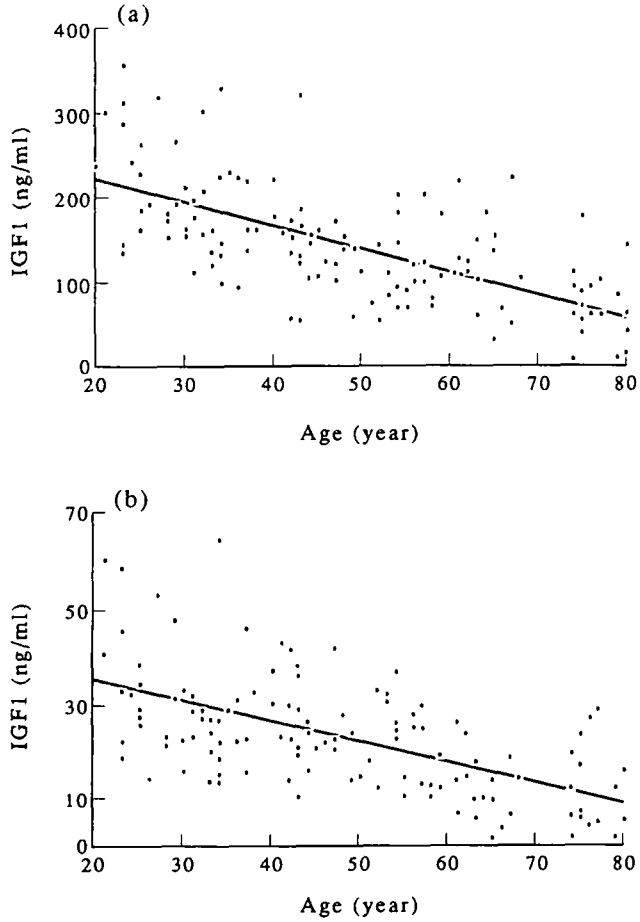


**Fig. 1. Linear regression between plasma IGF-1 concentration and age in the adult control population. The dependent variable is IGF-1 and the independent variable is the age. (a) IGF-1 (E) = −2.69 age+ 275; $r = -0.66$; $n = 133$. (b) IGF-1 (NE) = 0.43 age + 43.7; $r = -0.60$; $n = 133$.**

*Table 2. Characteristics of the breast cancer population*

| | *n* | Median | Range | Average | S.D. |
|---|---|---|---|---|---|
| Age | 47 | 61 | 31–86 | 57.7 | 12.6 |
| IGF-1 (E) | 46 | 152 | 52–300 | 155.6 | 61.1 |
| IGF-1 (NE) | 47 | 26 | 6.2–57 | 28.6 | 11.3 |
| IGF-1 (NE)/IGF-1 (E) | 46 | 0.18 | 0.07–0.5 | 0.2 | 0.08 |

no correlation between age and IGF-1 (NE) (Fig. 2b). No correlation was found between age and IGF-1 (NE)/IGF-1 (E) ratio.

Obesity of the patients was appreciated using two parameters: the difference ($d$) between measured and ideal {height $-100$ $-$ [(height $-$ 150)/4]} patient weight, the Quarterlet index [$i$ = weight/(height)$^2$]. In the population the $d$ median value was 9.6 kg (corresponding to a weight excess), $d$ ranged from $-12$ to 65 kg. There was no correlation between $d$ and IGF-1 concentrations or between $i$ and IGF-1 concentrations.

*Comparison between control and breast cancer population*

The comparison between IGF-1 concentrations in the two populations demonstrates that IGF-1 was higher in breast cancer than in controls ($P < 10^{-2}$), but Tables 1 and 2 indicate also that the median age in the control population was significantly lower than the median age in the breast cancer population (Mann–Whitney analysis and $t$-test: $P < 0.0005$). This difference was due to a large number of controls (42 values) under 35 years. When only women older than 35 years were considered in both populations, Mann–Whitney analysis and $t$-test show that no difference in age distribution could be observed. Thus comparison of IGF-1 levels could be performed in these subpopulations. Results are detailed Table 3. The box-plots of the results are shown on Fig. 3. After the age corrections IGF-1 was still higher in breast cancer patients than in controls, considering either IGF-1 (E) or IGF-1 (NE). There was no difference in IGF-1 (NE)/IGF-1 (E) ratios between the two populations.

Above 50 years of age the difference between IGF-1 in controls ($n$ = 58) and breast cancer patients was very high; the ratio IGF-1 (NE)/IGF-1 (E) was higher in the breast cancer population than in controls. The difference in the population aged from 35 to 50 years was of borderline significance for IGF-I (NE) as well as for IGF-1 (E) and there was no difference in the IGF-1 (NE)/IGF-1 (E) ratio (Table 4).

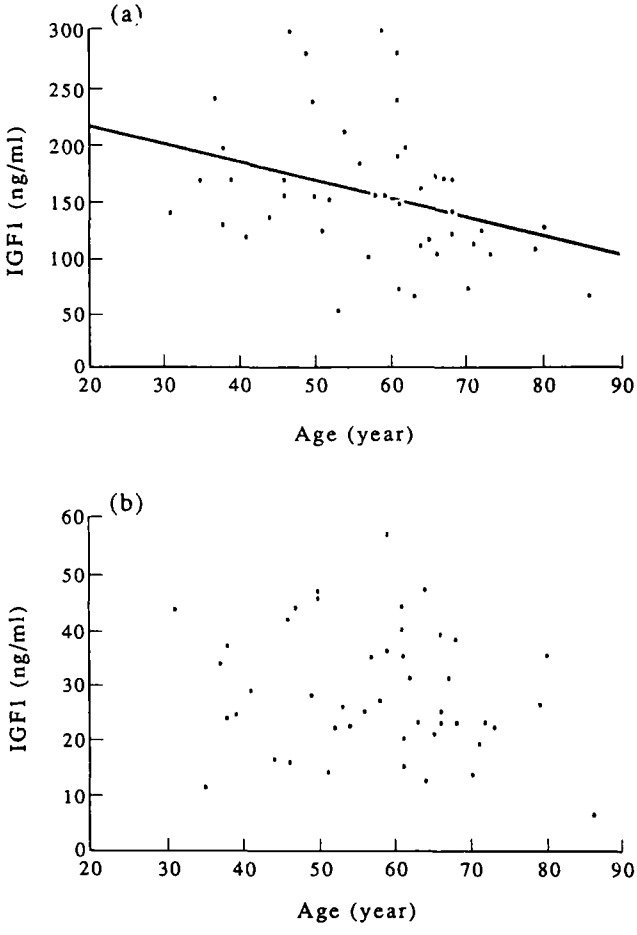


**Fig. 2. Linear regression between plasma IGF-1 concentration and age in the breast cancer population. (a) IGF-1 (E) = −1.66 age + 251; $r$ = −0.35; $n$ = 45; $P$ = 0.018. (b) IGF-1 (NE) = −0.17 age + 38; $r$ = −0.19; $n$ = 45; $P$ = 0.20.**

## DISCUSSION

We demonstrated that IGF-1 plasma concentration was increased in a population of human breast cancers when compared with IGF-1 plasma concentration in a control population.

Two assays for plasma IGF-1 measurement were used and both led to significant increases, higher difference being observed with IGF-1 (NE) ($P < 10^{-4}$) than with IGF-1 (E) ($P$ = 0.003). As determined by RIA for IGF-1-binding protein the acid–ethanol extraction removes 90% of binding proteins from serum and allows the detection of almost all the total serum IGF-1 [30] (conversely, in ovine fetal and adult plasma, the acid–ethanol extraction cannot be used reliably [33], suggesting species differences in the nature of IGF-binding proteins and in their ability to survive to acid–ethanol treatment). We confirmed that assays on unextracted serum detected only a part of the serum IGF-1 (E) on account of binding protein interferences. The two assays of IGF-1 were justified by the fact that, in absence of RIA available for each binding protein, the IGF-1 (NE)/IGF-1 (E) ratio is an estimator of binding proteins: the ratio is, for example, higher in patients with hypopituitarism, by reason of lower concentrations of binding proteins in sera [30]. Then, owing to this ratio, we would expect to detect variation in heterogenous binding proteins present in the plasma, which could be produced by breast cancer cells [26, 27]. We found ratios of 1/5 to 1/6, these results were in agreement with those found by Daughaday *et al.* [30] in controls. In our study the IGF-1 (NE)/IGF-1 (E) ratio in breast cancer was not different from the ratio in controls, suggesting that in plasma breast cancer there was no, or low if any, variation in IGF-1 binding proteins, compared to control.

We noted an IGF-1 decline with age in the control population and this is consistent with other studies [3, 4, 34]. In the breast cancer population, a significant inverse correlation was demonstrated only between age and IGF-1 (E); the absence of correlation between IGF-1 (NE) and age could be due to a more marked perturbation of IGF-1 (NE) than IGF-1 (E) in breast cancer. Elevated IGF-1 was noted when comparing the breast cancer population and the whole control population. In order to avoid any bias due to the negative correlation between IGF-1 and age, we compared populations where age distributions were similar (> 35 years). As the controls were younger than the

*Table 3. Characteristics of the control and the breast cancer populations older than 35 years*

| | *n* | Median | Range | Average | S.D. | MW | *t*-test |
|---|---|---|---|---|---|---|---|
| Age | | | | | | | |
| Control | 92 | 55.5 | 36–80 | 56.6 | 13.1 | NS | NS |
| Cancer | 45 | 61 | 37–86 | 58.8 | 11.7 | | |
| IGF-1 (E) | | | | | | | |
| Control | 92 | 115 | 10–322 | 120.5 | 55 | $P = 0.003$ | $P = 10^{-3}$ |
| Cancer | 44 | 152 | 52–300 | 155.6 | 62.4 | | |
| IGF-1 (NE) | | | | | | | |
| Control | 92 | 20.2 | 2–46 | 20 | 10.3 | $P < 10^{-4}$ | $P = 10^{-5}$ |
| Cancer | 45 | 26 | 6.2–57 | 28.6 | 11 | | |
| IGF-1 (NE)/IGF-1 (E) | | | | | | | |
| Control | 92 | 0.17 | 0.03–0.91 | 0.18 | 0.11 | NS | NS |
| Cancer | 44 | 0.18 | 0.09–0.5 | 0.20 | 0.08 | | |

MW = Mann–Whitney analysis; NS = not significant.

breast cancer patients, the age selection led to a decrease in the average IGF-1 value in the control population. Hence it was logical that the plasma IGF-1 concentration difference between breast cancer and control populations was higher in populations older than 35 years than before age selection.

The IGF-1 decrease with age was continuous without any break at the time of menopause (50 years). This finding is in agreement with results demonstrating the absence of a dramatic effect of oestradiol on IGF-1 [3]. Menopausal women with breast cancer more frequently have oestrogen-(IGF-1) receptor-positive tumours [16] however, and for this reason we compared IGF-1 plasma levels in breast cancer and control groups after 50 years of age (in our patient population, mean age at menopause was 50 years), and between 35 and 50 years of age. We found that the IGF-1 increase was greater in patients over 50 than in those between 35 and 50. Unfortunately, we could not study the direct relation between plasma IGF-1 concentrations and tumour IGF-1 receptors, as we did not assay tumour IGF-1 receptors in this population. But we can hypothesise that the patients with elevated plasma IGF-1 concentrations may have the lowest IGF-1 receptor levels because of increased IGF-1 receptor down regulation and would also have the worse prognosis [22].

A reduced circulating IGF-1 concentration has previously been described in primary human lung cancer [35] that could be explained on the basis of impaired nutritional status [6, 7]. In our breast cancer population, obesity was more common than

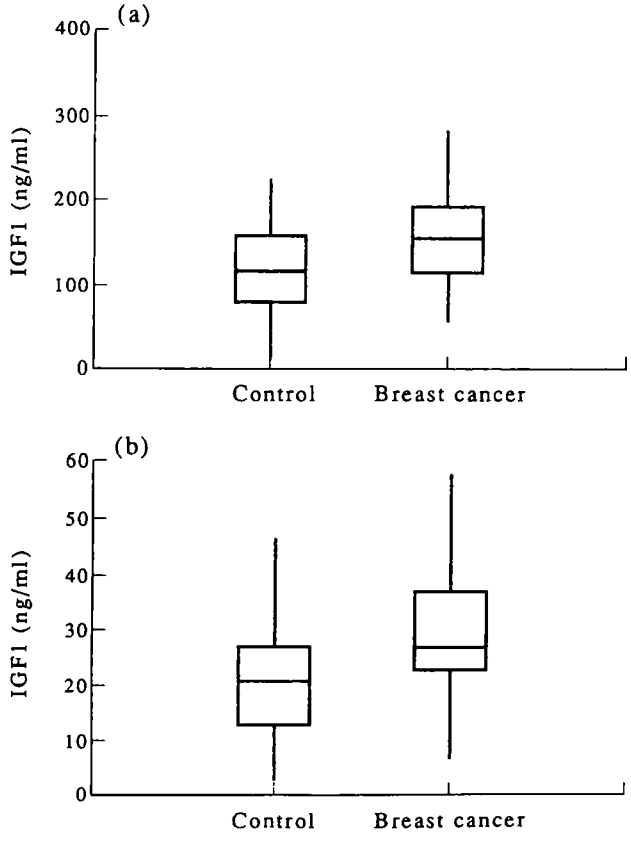


**Fig. 3. Comparison of IGF-1 plasma concentrations in breast cancer and in control after 35 years. (a) IGF-1 (E), (b) IGF-1 (NE). Differences are highly significant whatever the statistical test used (cf. Table 3).**

*Table 4. Characteristics of the control population and the breast cancer population between 35 and 50 years and older*

| | 35 years < age < 50 years | | | Age > 50 years | | |
|---|---|---|---|---|---|---|
| | *n* | Median | MW | *n* | Median | MW |
| Age | | | | | | |
| Control | 34 | 43 | NS | 58 | 64.6 | NS |
| Cancer | 12 | 43.75 | | 33 | 65 | |
| IGF-1 (E) | | | | | | |
| Control | 34 | 148 | $P = 0.05$ | 58 | 104 | $P = 0.002$ |
| Cancer | 12 | 168 | | 32 | 142 | |
| IGF-I (NE) | | | | | | |
| Control | 34 | 26 | $P = 0.06$ | 58 | 16 | $P < 10^{-4}$ |
| Cancer | 12 | 31 | | 33 | 27 | |
| IGF-1 (NE)/ IGF-1 (E) | | | | | | |
| Control | 34 | 0.18 | NS | 58 | 0.13 | $P = 0.045$ |
| Cancer | 12 | 0.16 | | 33 | 0.18 | |

For symbols see footnote to Table 3.

malnutrition, this observation is in agreement with the results of Colleti *et al.* [36] and is consistent with studies suggesting that excessive intake of dietary fat or calories may predispose to development of breast cancer [37]. In the present study we did not find any correlation between IGF-1 and obesity, moreover, a negative correlation between these two parameters has previously been described [35–38]. It is reasonable to conclude that obesity is not the factor responsible for the IGF-1 increase in breast cancer.

The major hormone implicated in the control of IGF-1 secretion is growth hormone (GH) [3], and the observed IGF-1 increase could be the consequence of GH increase. This hypothesis is supported by the results of Emerman *et al.* [39] who have shown that serum GH concentration, detected by RIA, was elevated in 40% ($n$ = 42) of breast cancer patients compared to controls. Elevated plasma lactogenic hormones (human GH plus prolactin) have also been described in women at risk for familial breast cancer [40].

Considering the known *in vitro* effect of IGF-1 on breast cancer cells and the presence of IGF-1 receptors in most breast cancers, these results suggest than an increase in plasma IGF-1 could be involved in breast cancer. These results emphasise the interest of research into IGF-1 lowering drugs that could potentially be used in the treatment of breast cancer [41]: it is interesting to note that tamoxifen has recently been shown [36, 42] to induce a reduction in circulating IGF-1 levels.


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# Oestrogenic Effects of Adjuvant Tamoxifen in Postmenopausal Breast Cancer

**Tommy Fornander, Lars E. Rutqvist, Nils Wilking, Kjell Carlström and Bo von Schoultz**

**Oestrogenic influence of the non-steroidal anti-oestrogen tamoxifen may have consequences for the morbidity pattern among women on long-term adjuvant treatment. Subclinical oestrogenic effects of adjuvant tamoxifen on the tissue level was studied among 16 postmenopausal women in three different organ systems: the pituitary, the liver and bone. After 3 months of adjuvant tamoxifen prolactin levels decreased 66% ($P<0.001$) in comparison with pretreatment levels. There was an 80% increase in basal growth hormone after 3 months of treatment in comparison with pretreatment levels, which did not reach statistical significance ($P = 0.07$). Sex hormone binding globulin levels increased 39% ($P<0.01$) and IGF-1 (somatomedin C) levels decreased 20% ($P<0.05$). The levels of bone GLA protein (BGP; osteocalcin), a marker of bone osteoblastic activity, decreased 28% ($P<0.01$). In 13 of the patients bone mineral density (BMD) was measured before treatment and after 1 year. No significant change in BMD was observed. The results thus suggest a clear oestrogenic effect of tamoxifen on the pituitary, liver and bone in postmenopausal women.**
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## INTRODUCTION

TAMOXIFEN is widely used as an adjunct to surgery in the treatment of primary breast cancer. There are few acute side-effects and the substance is generally well tolerated [1–4]. The effect in human breast cancer is thought to be predominantly anti-oestrogenic through a blockade of oestrogen receptors (ER) in the cancer cells [5, 6]. Other mechanisms have been suggested to explain effects observed in tumours with a low ER content [7]. Although the anti-oestrogenic action of tamoxifen is well established in breast cancer, effects in other human tissues are not fully known. Tamoxifen is one of several triphenylethylene substances all of which are partial oestrogenic agonists/antagonists. The oestrogenic and anti-oestrogen effects of tamoxifen have been shown to be both species- and organ-specific. In the uterus of immature and ovariectomized mice, tamoxifen acts as a pure oestrogen agonist during short-term treatment, but in the immature rat uterus the substance is a partial oestrogenic agonist/antagonist [8–11]. In the chick, tamoxifen is a pure antagonist of oestrogen-stimulated growth of the oviduct [12]. This task seems to be even more complicated considering also the duration of treatment, e.g. long-term tamoxifen therapy to mice has been reported to produce anti-oestrogenic actions [13]. It is obviously not possible to extrapolate results from one system to another and firm conclusions about tamoxifen effects on different tissues in man cannot be drawn from animal results. Although data on tamoxifen effects on several biochemical markers have been published, the information is incomplete and

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