needed to estimate the functional consequences of treatment with confidence, and better matching would thereby be ensured.

Thus a two stage pragmatic trial in patients with stroke such as ours can provide useful information about the success of treatment. Unsatisfactory treatments may be discontinued without exposing large numbers of patients to unnecessary risk, whereas those that give significant functional benefit may be recommended with confidence for conscious patients with stroke admitted to general hospitals with limited facilities for investigation.

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Appendix

The formulas for logistic regression models show how the predicted odds of surviving or going home may be calculated from the model parameters given the age of the patient, the initial conscious level, and so on, individual coefficients (odds ratios) being estimates of the relative risk associated with each factor.

Coefficients excluding treatment are:

$$\frac{P_H}{1-P_H} = 5.8^c \times 2.0^B \times 0.968^{Age}$$

$$\frac{P_s}{1-P_s} = 1.9^c \times 2.9^B \times 1.012^{Age}$$

Coefficients including treatment effect are:

$$\frac{P_H}{1-P_H} = 5.9^c \times 2.0^B \times 0.968^{Age} \times 1.1^T$$

$$\frac{P_s}{1-P_s} = 1.9^c \times 3.0^B \times 1.013^{Age} \times 1.4^T$$

where P_H = probability of returning home in first month; P_s = probability

of surviving the first month; $C=+1$ if patient alert on day 1, -1 if patient drowsy on day 1; $B=+1$ if patient continent on day 1, -1 if patient incontinent on day 1; and $T=+1$ if patient taking placebo, -1 if patient taking a β blocker.

Thus, for instance, the odds of returning home within the first month for a 60 year old who is alert and continent initially are $5.8 \times 2.0 \times 0.968^0 = 1.6:1$ and for an 80 year old $0.9:1$ ($1.0:1$ if taking placebo, $0.8:1$ if taking a β blocker). Note that the model predicts a slight improvement in survival with age provided that the degree of consciousness and continence remain unchanged.

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Aromatase activity in adipose tissue from breast quadrants: a link with tumour site

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Abstract

To determine the importance of local oestrogen biosynthesis within the breast, aromatase activity was measured in adipose tissue from the breast quadrants of 12 consecutive mastectomies from patients with breast cancer. Activity was detected in all samples (range 3.6-35.0 fmol oestrogen/mg protein/h) but varied considerably not only among different patients but also among the quadrants of individual breasts. The highest activity in a breast was always found in a quadrant that contained tumour,

whereas quadrants with the lowest activity were never associated with the presence of tumour.

These results provide evidence of a significant relation between breast adipose tissue and breast cancer. Whether such an association occurs because breast tumours are more likely to develop in areas with enhanced oestrogen biosynthesis or because they secrete into their local environment factors capable of stimulating oestrogen biosynthesis remains to be determined.

Introduction

Unlike other glands in the body the human adult breast is invested with an abundance of adipose tissue.¹ Although the precise role of this adipose tissue remains to be elucidated, there is evidence of an association between mammary fat and the development of breast cancer.^{2,3} Breast adipose tissue is not metabolically inert, being able, for example, to synthesise oestrogens by aromatising androgen precursors.^{4,7} Although aromatase activity in adipose tissue varies among subjects and different sites in the body,^{6,9} little is known about its activity within the breast. Our aims were to investigate the

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variation in aromatase activity among different quadrants of the same breast by measuring activity in adipose tissue in specimens obtained at mastectomy in patients with breast cancer and to relate the activity to the quadrants of the breast in which malignant tumour was identified.

Materials and methods

Tritiated 1β - Δ 4 androstenedione was synthesised from 1β , 2β - Δ 4 androstenedione (specific activity 2.07 TBq/mmol; New England Nuclear) by the method of Berkovitz *et al.*¹⁰ Cofactors were obtained from Sigma, Poole, Dorset; other chemicals and solvents were obtained from BDH Chemicals, Poole, Dorset.

Adipose tissue was obtained from the four quadrants of the breast during 12 consecutive mastectomies performed for breast cancer that had been confirmed histologically; table I gives details of the patients. The adipose tissue was carefully separated from breast parenchyma and fibrous tissue and 2 g homogenised in phosphate buffer (1:1 w/v) pH 7.4 and centrifuged at 800 g for five minutes. The soluble lower layer was then centrifuged at 100 000 g for one hour and the resulting pellet (particulate fraction) resuspended in 600 μ l phosphate buffer.

TABLE I—Details of patients from whom breast adipose tissue was obtained

Case No	Age (years)	Menopausal state	Height (cm)	Weight (kg)	Histological type of cancer	Previous treatment
1	31	Premenopausal	155	57.2	Poorly differentiated	None
2	34	Premenopausal	179	73.5	Poorly differentiated	None
3	75	Postmenopausal	150	50.8	Infiltrating (no special type)	Tamoxifen
4	66	Postmenopausal	160	82.5	Lobular	None
5	76	Postmenopausal	163	74.4	Lobular	Tamoxifen
6	50	Premenopausal	160	70.0	Spindle cell	None
7	61	Postmenopausal	155	66.7	Adenocarcinoma	Aminoglutethimide
8	62	Postmenopausal	155	59.9	Ductal (no special type)	None
9	61	Postmenopausal	163	68.2	Lobular	None
10	63	Postmenopausal	153	69.9	Ductal	None
11	66	Postmenopausal	173	63.5	Intraductal	None
12	50	Postmenopausal	174	57.6	Intraductal with invasion	None

The aromatase assay was based on the release of tritiated water during the aromatisation of 1β - 3 H- Δ 4-androstenedione to oestrone.¹¹ The particulate fraction (500 μ l) was added to 1β - 3 H- Δ 4-androstenedione (100 nmol/l, 37 Bq) and cofactors for generating nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) in a final volume of 1.1 ml. In control incubations the particulate fraction was replaced with bovine serum albumin (1.5 mg/ml, Armour Pharmaceuticals, Eastbourne). Each sample was incubated in air at 37°C for three hours with continuous shaking. To stop the reaction aliquots were dispensed into 3 ml ice cold chloroform, thoroughly shaken, and centrifuged at 2000 g for three minutes. Aliquots from the aqueous supernatant were then transferred into a 5% charcoal solution. After thorough mixing the charcoal was precipitated by centrifugation at 2000 g for 15 minutes and the supernatant decanted into a glass scintillation phial with 10 ml NE260 Scintillant (Nuclear Enterprises, Edinburgh). Liquid scintillation counting was performed with a Packard Tri carb spectrometer. Counts from the control incubations were subtracted from all other counts before correction for recovery losses and counting efficiency. Results were expressed as fmol oestrogen/mg protein/h.

Aromatase activity in the four breast quadrants was compared by the Friedman test, and mean activities in the quadrants containing tumours were compared with those of the quadrants without tumours by the Wilcoxon signed rank test. Multiple regression analysis on the logarithms of all 48 measurements was used to test the combined association of tumour and anatomical site with aromatase activity. The analysis treated patients, site, and presence or absence of tumour as fixed effects with 12, four, and two levels respectively, and tests were performed to assess the significance of site while controlling for patient and tumour and of tumour while controlling for patient and site.

Results

Aromatase activity was detected in all samples studied (range 3.6–35.0 fmol oestrogen/mg protein/h). Considerable variation was observed among patients, average values ranging from 6.3 to 26.9 fmol oestrogen/mg protein/h, and among different quadrants in the same breast, the ratio of activity in

the most to the least active quadrants ranging from 1.4 to 4.9 (table II). There was a significant association between the anatomical site of the quadrant and the aromatase activity ($p < 0.05$). The highest activity was present in the upper outer quadrant in seven cases, the lower outer quadrant in three, and the upper inner quadrant in two. The lowest activity occurred in the lower inner quadrant in five cases, the upper inner quadrant in four, and the lower outer quadrant in the remaining three.

TABLE II—Aromatase activity in adipose tissue in breast quadrants. (Figures in parentheses are ratios of activity to lowest activity in that breast)

Case No	Aromatase activity (fmol oestrogen/mg protein/h) in breast quadrants				Mean
	Lower inner	Lower outer	Upper inner	Upper outer	
1	22.9 (1)	27.4 (1.2)	24.9 (1.1)	32.2* (1.4)	26.9
2	5.6 (1)	7.1 (1.3)	12.2 (2.2)	14.0* (2.5)	9.7
3	4.9 (1)	10.3 (2.1)	10.5 (2.1)	23.7* (4.8)	12.4
4	9.7 (1)	12.3 (1.3)	12.5 (1.3)	15.4* (1.6)	12.5
5	7.7 (1.1)	7.1 (1)	34.9* (4.9)	12.3* (1.7)	15.5
6	11.7 (1.9)	14.0† (2.3)	6.2 (1)	17.3* (2.8)	12.3
7	26.0 (1.1)	23.9 (1)	23.6 (1)	32.0* (1.4)	26.4
8	10.9 (2.1)	5.2 (1)	8.2 (1.6)	15.0* (3.1)	10.1
9	3.6 (1)	3.6 (1)	10.8* (3.0)	7.2* (2.0)	6.3
10	10.5* (1.7)	15.0* (2.4)	6.2 (1)	9.5 (1.5)	10.3
11	15.0 (1.4)	35.0* (3.3)	10.5 (1)	17.8* (1.7)	19.6
12	9.9 (1)	19.0† (1.9)	13.9*† (1.4)	13.1*† (1.3)	14.0

*Quadrant containing clinically detected tumour.

†Additional tumour foci discovered on histological examination.

The figure shows the relation between aromatase activity and tumour site determined clinically and histologically. In eight cases (1–8) the main tumour mass was located entirely in one quadrant, and in all eight this quadrant showed the highest activity. In case 6 a second focus of tumour was detected in the lower outer quadrant, which had the second highest activity. In three patients (cases 9–11) the tumour mass straddled two quadrants, both of which showed higher aromatase activity than the two without the tumour. In the remaining patient (case 12) an invasive tumour was present in three quadrants but not in the quadrant with the lowest activity.

Activity was therefore strongly correlated with the presence of tumour in a quadrant: in all 12 cases the average activity in the quadrants bearing a tumour was significantly higher than that in those not bearing tumours ($p < 0.001$). Multiple regression analysis showed that whereas there was no significant difference in activity among the various quadrants after correction for the presence or absence of tumour, the activity was significantly correlated with the presence of tumour in a quadrant, irrespective of its anatomical site ($t = 5.45$, 32 df, $p < 0.001$). The mean ratio of aromatase activity in quadrants with tumours compared with those without was 1.89 (95% confidence interval 1.56 to 2.31). The average aromatase activity for each patient did not seem to be related to height, weight, or obesity or to the type, size, or stage of the tumour.

Aromatase activity in adipose tissue from breast quadrants in relation to site of tumour detected clinically and pathologically in 12 patients. Activity was ranked as shown on scale and quadrants shaded accordingly.

Discussion

Although obesity has been linked to increased risk of breast cancer,¹² little attention has been given to the role of adipose tissue within the breast. Yet obesity increases the amount of adipose tissue within the breast,¹³ and the relative proportion of adipose to glandular tissue increases with age¹⁴ in parallel with the incidence of breast cancer.¹² A comparison of Japanese immigrants to Hawaii with native Japanese women showed a similar positive correlation between the proportion of mammary adipose tissue and the incidence of hyperplastic breast lesions and breast cancer.³ Animal studies also support a role for mammary adipose tissue in the development of cancer. Species with little mammary adipose tissue seem to be resistant to mammary neoplasia, whereas rodents with a high incidence of mammary cancer have a well developed mammary fat pad.¹⁵ Furthermore, in mice this fat is essential for the development of hyperplastic lesions and cancers.²

Though the precise role of breast adipose tissue in the development of cancer in humans is still uncertain, the ability of adipose tissue to synthesise and metabolise steroids might be important, these hormones being known to influence the growth and function of the adult breast.¹⁶ We and others previously showed that most, if not all, samples of breast adipose tissue have the potential to synthesise oestrogens from androgen precursors.^{4-7 17} We believe that this is the first report of such activity in the different quadrants of individual breasts. We found that all samples of mammary adipose tissue had aromatase activity but that this varied appreciably among different patients and within individual breasts. Though the variation within the breast was to some extent related to anatomical site, the activity was much more significantly related to the presence of tumour in a quadrant: activity in quadrants bearing tumours was 1.5-2.5 that in the other quadrants. The observed trend of high activity in the upper outer quadrant probably reflects the greater incidence of tumours in this site, as recorded here and elsewhere.¹⁸

There are several possible explanations for enhanced aromatase activity in breast quadrants containing tumours. Breast cancers in general show higher aromatase activity than adipose tissue,¹⁹ and samples of adipose tissue from quadrants bearing tumours might contain micrometastatic deposits of tumour. This possibility cannot be totally excluded, but samples of adipose tissue examined histologically showed no evidence of tumour deposits. Furthermore, the adipose tissue was taken from the periphery of breast quadrants in areas remote from the palpable tumour to minimise the risk of including tumour foci within the samples.

Assuming that increased aromatase activity in quadrants containing tumours is not due to contamination of samples by tumour cells, two further explanations may be advanced: (a) that malignant tumours may stimulate aromatase activity in surrounding tissues and (b) that increased aromatase activity in a particular region of adipose tissue might produce a local environment that promotes malignant growth at that site. Though we cannot yet distinguish between these alternatives, it may be pertinent that breast cancer cells can secrete growth factors²⁰ that can influence steroid metabolism in adjacent tissues.²¹ Whether appreciable variation in aromatase activity occurs in normal breasts is unknown, and we have been unable to obtain specimens from multiple sites of individual breasts of patients without cancer.

The increase in aromatase activity in quadrants containing tumours is unlikely to be due to increased cellularity. Histological examination of adipose tissue from different breast quadrants did not show appreciable differences; most quadrants seemed to be composed predominantly of adipocytes with fibrovascular stroma accounting for less than 15% of the sections examined. Increased cellularity might also be expected to result in a higher protein concentration of tissue extracts; the protein concentrations in samples were, however, fairly consistent within individual breasts and were not disproportionately higher in quadrants containing tumours. In addition, the activity of another enzyme that metabolised steroids, 17 β hydroxysteroid dehydrogenase, was measured in the same tissue samples and did not correlate with either the anatomical site or the presence of tumour in the quadrant.²²

Increased aromatase activity in quadrants containing tumours therefore does not reflect a non-specific increase in steroid metabolism in these quadrants.

In conclusion, these results establish an association between aromatase activity in adipose tissue and breast cancer and further strengthen the case for metabolism in mammary adipose tissue contributing to the natural course of breast cancer.

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ONE HUNDRED YEARS AGO

MR. HERRING, of Leeds, continues his efforts at Sheffield "for the special treatment of small-pox and the stamping out of the epidemic." The "stamping out" process is illustrated in the contents of the advertisements calling his meetings. At one it is advertised that "several cured patients will give their experience," and that at another "a number of persons benefited by their treatment will be present and testify." An advertisement in the daily papers of February 25th appeals to the public for funds to "meet the necessary expenses of a treatment which has done and is doing so much to rid the town of the disease." It states also that it is three weeks since the man Herring came to the town, so that the dangerous condition of at least some of the people advertised to attend and testify at his meetings on February 16th and the later one may be realised. Is it any wonder that infection spreads; especially when the character of the gatherings he calls together is considered? (*British Medical Journal* 1888;ii:479)