

EFFECT OF DIETARY GLA +/- TAMOXIFEN ON THE GROWTH, ER EXPRESSION AND FATTY ACID PROFILE OF ER POSITIVE HUMAN BREAST CANCER XENOGRAPTS

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Gamma linolenic acid (GLA) possesses a number of selective anti-tumour properties including modulation of steroid receptor structure and function. We have investigated the effect of dietary GLA on the growth, oestrogen receptor (ER) expression and fatty acid profile of ER+ve human breast cancer xenografts. Experimental diets A, B, C, D were commenced after subcutaneous implantation of 40 female nude mice with the MCF-7 BIM cell line (Group A = control diet; B = control diet + GLA supplement; C = control diet + tamoxifen; D = control diet + GLA + tamoxifen; 10 mice/group). The mice were terminated when tumour cross-sectional area reached 250 mm². ER H-scores were assessed by immunohistochemical assay and fatty acid profiles by gas-liquid chromatography of termination tumour samples. Groups C and D displayed significantly slower tumour growth ($p = .0002$, $p = .0006$) with trend for slower growth in B ($p = .065$) compared to control Group A. ER was significantly reduced in all groups compared to A ($p < .0001$) with Group D (combined therapy) displaying markedly lower ER expression than with either therapy alone ($p = .0002$). There were significantly raised levels of tumour GLA and metabolites in the two groups (B and D) receiving GLA ($p < .0001$). This xenograft model of ER+ve breast cancer has demonstrated significantly lower tumour ER expression in those groups receiving GLA, an effect which appears to be additive to the reduced ER expression resulting from tamoxifen alone. The effects of GLA on ER function and the possibility of synergistic inhibitory action of GLA with tamoxifen via enhanced down-regulation of the ER pathway require further investigation.

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Gamma linolenic acid (GLA) is a member of the n-6 family of essential polyunsaturated fatty acids (EFAs) found in evening primrose and borage oils. GLA is well established as an effective treatment for benign cyclical mastalgia where the mechanism of action is thought to involve attenuation of breast hormone receptor sensitivity to circulating oestrogens.¹ More recently GLA and other n-6 and n-3 EFAs have raised interest as novel anti-cancer agents as they have been shown to exert a number of selective cytotoxic and anti-proliferative effects on human cancer cells without affecting normal tissues.² *In vitro* experiments have identified a variety of EFA anti-tumour actions. These include direct cytotoxicity via liberation of free radicals and lipid peroxides; up-regulation of cell surface adhesion molecules; inhibition of angiogenesis; induction of apoptosis; interaction with secondary messenger and cell-signalling pathways and modulation of cellular receptor structure and function including steroid hormone receptors.³ A number of animal studies have demonstrated inhibitory effects of dietary supplementation of EFAs on experimental tumours.^{4–6} More recently, pilot clinical trials of GLA have achieved useful tumour regression and improved quality of life with negligible systemic toxicity in a variety of advanced solid malignancies.^{7–10}

GLA and other EFAs have been shown to enhance the *in vitro* anti-cancer actions of radiotherapy and of cytotoxic drugs and may have beneficial role in the reversal of multi-drug resistance.³ In the current work the effects of GLA combined with an endocrine agent in a hormonally responsive cancer have been explored. The xenograft study has been designed to complement a Phase II clinical trial in which the therapeutic and biological effects of oral GLA + tamoxifen as primary therapy for endocrine-sensitive breast cancer have been compared with the effects of tamoxifen alone. Combined treatment was found to have significant beneficial effects on both the clinical response and biological parameters of the tumours.¹¹ In the present study we have assessed the effect of dietary supplementation of GLA +/- tamoxifen on the growth, ER expression and fatty acid profile of ER+ve human mammary tumours in a murine host. The advantages of the xenograft model over the clinical trial have been the opportunity to investigate GLA as sole therapy as well as the inclusion of a control arm receiving no active cancer treatment.

Of particular interest in both the xenograft and clinical studies has been the effect of GLA on the expression of ER. Tamoxifen and other anti-oestrogens are known to down-regulate ER.^{12–14} Our own group has also recently shown that a greater extent of ER down-regulation was correlated with better clinical response on primary tamoxifen.¹⁵ EFAs have previously been reported to modulate the structure and function of steroid hormone receptors, including the ER.^{16–19} The findings from our recent GLA clinical trial¹¹ of greater reduction in the expression of ER, the ER-regulated protein bcl-2 and faster clinical response in patients receiving Tamoxifen + GLA compared to Tamoxifen only has prompted us to investigate the outcome of GLA alone, as well as in addition to tamoxifen, on the expression of ER in the xenograft setting. Our results from the latter have complemented those of our clinical trial.

MATERIAL AND METHODS

The ER+ve human breast cancer cell line MCF-7 BIM was maintained in serial passage in donor female MF1 nude mice (Cancer Studies, University of Nottingham). The mice were

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housed in pathoflex isolators (Elwyn Roberts Isolators, U.K.) at 26°C, on 12 hours light, 12 hours dark cycles. Irradiated RB2 diet (Special Diet Services, U.K.) and autoclaved water was provided *ad libitum*. For the experimental therapies, tumour was excised from the donor animals, finely minced and 3 mm³ pieces subcutaneously implanted into the left flanks of 40 juvenile females under anaesthetic (Hypnorm, Roche; Hypnovel, Jansen). (The method of tumour grafting from donor mice rather than direct tumour cell injection was employed as the former results in tumours that do not ulcerate and are more reproducible than the latter). Following tumour implantation the RB2 diet was provided for the first 3 weeks after which the mice were randomly assigned to the four dietary treatment groups (10 mice per group). Groups C and D additionally received tamoxifen as a subcutaneous pellet (5 mg of tamoxifen [free base] per 60 day release, resulting in blood levels of 3–4 ng/ml) on commencement of the experimental diet. Groups A and B received a subcutaneous placebo pellet at the same time point (the placebo pellets contained all the components of the tamoxifen pellets except the tamoxifen itself, the carrier-binder excipients of the matrix including cholesterol, lactose, celluloses, phosphates and stearates). Both the tamoxifen and placebo pellets were obtained from Innovative Research of America.

The experimental diets were made up as described below:

1. CONTROL = fat-free diet* + 5% corn oil** w/w (to provide essential fatty acids)
2. GLA = fat-free diet + 2% GLA*** w/w + 3% corn-oil w/w (to make 5% total fat)
3. TAMOXIFEN = fat-free diet + 5% corn-oil w/w + Tamoxifen
4. TAMOXIFEN + GLA (T + GLA) = fat-free diet + 2% GLA w/w + 3% corn-oil w/w + Tamoxifen

*irradiated fat-free diet containing: casein 21%, sucrose 69%, cellulose 5%, mineral mix 4% and vitamin mix 1% w/w (supplied by Special Diet Services, U.K.)

**corn-oil containing linolenic acid (18:2 n-6) 60% and GLA (18:3 n-6) 0.5% w/w

***GLA as 95% free fatty acid (supplied by Scotia Pharmaceuticals)

Preparation of diets

The corn oil and GLA were sterilized by 0.2-micron filtration in a laminar flow hood and mixed with the fat-free diet in the required proportions for the 4 different treatment groups, maintaining sterile conditions throughout. One week's supply was prepared at a time, sealed in dark air-tight containers in order to avoid peroxidation of the GLA and refrigerated at 4°C until use. Each mouse was allocated 5 gm feed per day, uneaten diet being removed. Additional feed and sterilized water were made available as described above.

Monitoring

The mice were weighed weekly and their clinical condition closely monitored by a trained observer. Tumour size was measured twice-weekly using calipers in two perpendicular dimensions. UKCCCR guidelines were adhered to in all experimental procedures. Each mouse was terminated when cross-sectional tumour area reached 250 mm². We have previously found that for most tumours 250 mm² corresponds to the limit of 10% of initial mouse weight allowed by the UKCCCR guidelines. The use of this tumour size as an end-point thus allows "survival type" data to be compiled and maximizes the information obtained from the study (termination at a set timepoint allows a direct comparison of tumour burden but does not give information about any slowing of tumour growth that has been achieved).

At termination, cardiac puncture was performed and the serum samples obtained were placed into EDTA tubes stored on ice

followed by centrifugation at 2500 rpm for 20 minutes. The plasma and red cells were then separated by pipette and stored at -20°C in separate tubes for subsequent transfer to the Efamol Research Institute, Nova Scotia, Canada, for fatty acid analysis. The tumours were excised, weighed and divided into 3 equal portions. Two of the portions were immediately snap-frozen in liquid nitrogen and stored at -70°C, one for subsequent transfer to Nova Scotia, the other for immunohistochemical assessment of ER expression. The remaining third was fixed in formalin.

Immunohistochemical analyses

ER immunohistochemical staining was performed using the Abbott H222 antibody kit (Abbott, Chicago, Illinois) which uses a sensitive PAP technique for the visualization of oestrogen receptors in frozen tissue sections. The methodology for this assay has been described elsewhere.²⁰ Certain modifications were made to the basic technique to accommodate for the xenograft nature of the tissue i.e. the primary antibody was incubated for 2 hours rather than 30 minutes and the goat-anti-rat bridging antibody was pre-adsorbed with 10% normal mouse serum for 30 minutes prior to adding to each slide. Nuclear tumour cell staining for ER was assessed in the xenograft sections by consensus agreement from two personnel (JMWG, FSK) using a dual-viewing attachment to a light microscope. Immunocytochemical analysis was performed in a blinded fashion without knowledge of the xenograft treatment information. An overall examination of nuclear immunostaining was first performed at an ocular magnification of ×10 in order to locate representative areas of tumour cells for further analysis and to exclude areas of native stromal tissue. These areas were then viewed at ×40 for more detailed assessment. The percentage nuclear immunopositivity and staining intensity (i.e. weak, moderate, strong) was noted for each sample, examining a minimum of 2000 cells. This was performed in order to assign an H-score value for every xenograft specimen. The H-score is a well established semi-quantitative immunostaining index measured on a 0-300 scale²¹ i.e. H score = (1 × % cells staining low intensity) + (2 × % cells staining moderate intensity) + (3 × % cells staining high intensity). A known ER-ve (BR 293) breast cancer xenograft sample was used as a control section and showed complete absence of nuclear immunostaining for ER.

Fatty acid analyses

The fatty acid content of frozen samples of tumour, RBC and plasma for each case was analysed by gas-liquid chromatography at the Efamol Research Institute, Nova Scotia, Canada. The results are expressed as mg/100 mg of total fatty acid content. Detailed methodology for the lipid extraction and chromatography procedures have been described elsewhere.²²

Statistical analyses

All statistical analyses were performed using SPSS for Windows, Version 6.1.3, SPSS UK. Growth rates of the tumours in the different treatment groups were analyzed as survival functions using life-tables with the Wilcoxon (Gehan) statistic. The time for the tumours in the different groups to reach 250 mm² was also compared with the median test. Differences in ER expression and fatty acid profiles were assessed non-parametrically using the Kruskal-Wallis test for overall significance and the Mann-Whitney U test for pairwise comparison of individual treatment groups. The relationship between termination ER expression and GLA level was analyzed with Spearman rank correlation. The data in Figs 2–4 are illustrated as "box-plots" where the upper and lower box limits are equal to the upper and lower quartiles and the central bar represents the median value of the marker.

RESULTS

All the mice thrived and put on weight with the experimental diets. The tumour of one host in Group C (Tamoxifen alone) was very slow-growing and had not reached 250 mm² by the time the remainder of the study had been completed. A decision was made

TABLE I – TIME TO REACH 250 MM² FOR EACH OF THE 4 EXPERIMENTAL DIETARY GROUPS¹

Dietary group	n	Mean	Median	Range (days)
A: Control	10	51	50	44–64
B: GLA	10	56	58	44–69
C: Tamoxifen	10	79	78	64–95
D: T + GLA	10	73	76	55–90

¹Median test: $\chi^2 = 24.4$; 3 d.f., $p < 0.0001$.

to terminate this mouse at day 95, the tumour at this time measuring 186 mm². A sample from this tumour was analyzed for fatty acid content. Assessment of ER expression was not performed.

Tumour growth rate

All treatment groups exhibited slower tumour growth compared to control Group A, $p < 0.0001$ overall with both the median test ($\chi^2 24.4$, 3 d.f.), Table I, and on life-table analysis (Wilcoxon [Gehan] statistic 25.8, 3 d.f.) Fig. 1. In Groups C (Tamoxifen alone) and D (T + GLA) the difference in growth was highly significant compared to Group A ($p = 0.0002$; $p = 0.0006$) however there was no statistical difference in the growth rate between these two active treatment arms ($p = 0.38$). In Group B (GLA alone) there was a non-significant trend for slower growth compared to the controls ($p = 0.065$).

ER Expression

The ER expression of the tumour samples at termination biopsy was significantly lower in all treatment groups compared to control Group A, $p < 0.0001$ overall (Fig. 2). GLA or Tamoxifen as sole therapy (Groups B and C) appeared to have an equivalent effect on reduction of ER (confirmed by 2×2 table analysis of effect of specific treatment against no treatment—data not shown) whereas combined T + GLA (Group D) resulted in markedly lower ER expression than with either therapy alone ($p = 0.0002$).

Fatty acid analyses

GLA and the downstream metabolite dihomogammalinolenic acid (DGLA) were found to be elevated in the xenograft tumours that had received GLA (Groups B and D) compared to the control and Tamoxifen arms (Groups A and C). In contrast, there were higher levels of the precursor linolenic acid (LA) in the control and Tamoxifen arms compared to the two groups receiving GLA. The differences in fatty acid levels in the GLA-containing dietary groups compared to the GLA-free arms were found to be highly significant overall $p < 0.0001$ (Kruskal-Wallis) and remained significant on pairwise comparison of individual treatment groups (Mann-Whitney U) (Fig. 3). A similar pattern of raised GLA and metabolites with lowered linolenic acid levels was found in the plasma (Fig. 3) and to a lesser extent the red blood cell phospholipid fraction (data not shown) of the murine hosts in Groups B and D.

Combined analysis of ER and GLA levels

As GLA is implicated to modulate the structure of hormone receptors we were interested to examine whether there was any direct relationship between the levels of GLA contained in the tumours and the ER expression of the tumours at termination biopsy. Indeed, we found there to be direct inverse correlations with GLA and ER, and DGLA and ER expression, those tumours with the highest levels of the fatty acids displaying the lowest levels of ER (GLA: $r_s = -0.51$, $p = 0.001$; DGLA: $r_s = -0.48$, $p = 0.004$).

DISCUSSION

Polyunsaturated fatty acids play key physiological roles within the body. They form essential structural components of cell, nuclear and organelle membranes, which are necessary for the maintenance of normal membrane integrity and fluidity.²³ More recently a range of other properties have been identified including

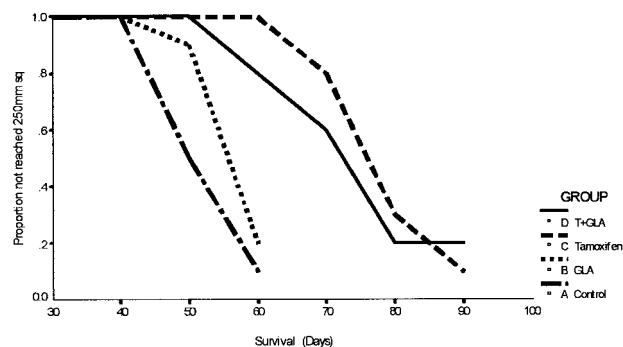


FIGURE 1 – Actuarial tumour growth rate, MCF-7 BIM human breast cancer xenografts: Overall comparison $p < 0.0001$ (Wilcoxon-Gehan statistic). Pairwise comparison A vs B $p = 0.065$; A vs C $p = 0.0002$; A vs D $p = 0.0006$; B vs C $p = 0.0002$; B vs D $p = 0.0042$; C vs D $p = 0.38$.

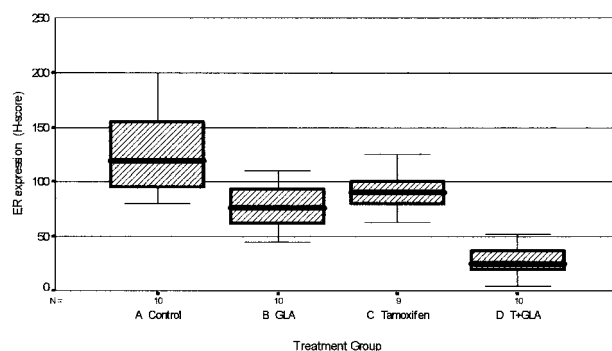


FIGURE 2 – ER expression (H-score) at termination biopsy, MCF-7 BIM human breast cancer xenografts. Median values: Group A = 119; Group B = 77; Group C = 90; Group D = 25. Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0032$; A vs C $p = 0.045$; A vs D $p = 0.0002$; B vs C $p = 0.16$; B vs D $p = 0.0002$; C vs D $p = 0.0002$ (Mann-Whitney U).

interaction with many secondary messenger systems, regulation of cytokines and modulation of the behaviour of membrane-bound proteins including receptors, ion-channels and ATPases.^{24,25} There are two families of essential polyunsaturated fatty acids (EFAs), the n-3 and the n-6 series, so named after the position of the first double carbon bond from the methyl end of the molecule. The parent fatty acids, linolenic (18:2 n-6) and α -linolenic (18:3 n-3) cannot be synthesized by the body *de novo* and must be obtained from the diet. The parent molecules are converted to downstream metabolites by a series of alternating desaturation and elongation reactions (mediated by desaturase and elongase enzymes) leading to the production of numerous metabolites with differing biological functions. The most important of these in the n-6 series are 18:3 n-6 gamma linolenic acid (GLA), 20:3 n-6 dihomogammalinolenic acid (DGLA) and 20:4 n-6 arachidonic acid (AA) and in the n-3 series 20:5 n-3 eicosapentaenoic acid (EPA) and 22:6 n-3 docosahexaenoic acid (DHA) (Fig. 4). Since the late 1980s it has been established that certain highly unsaturated EFAs beyond the first δ -6-desaturation step in the n-3 and n-6 pathways have selective toxic actions on malignant cells that differ from those of the parent fatty acids. This selective toxicity has been identified to be due in part to the production of lipid peroxides and free radicals.² More recent *in vitro* work has additionally shown EFAs to exert a regulatory action on tumour cell motility, invasion and metastasis through interaction with cell-cell adhesion mechanisms, tumour suppressor molecules and motility related signal transduction pathways.³

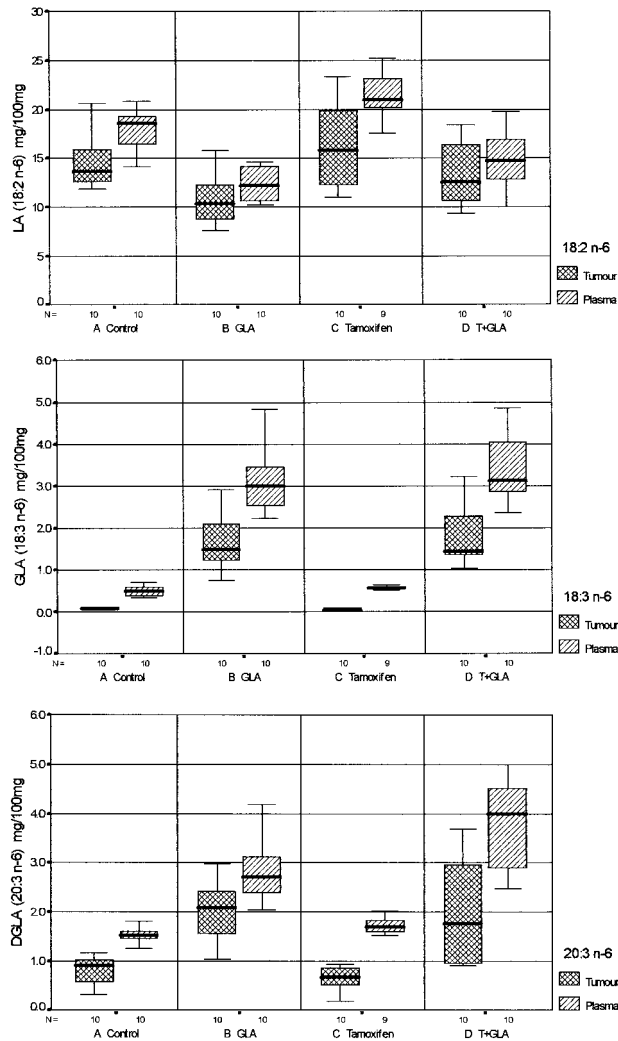


FIGURE 3 – Fatty acid content of tumours and plasma at termination biopsy, MCF-7 B1M human breast cancer xenografts: (a) Linolenic acid (18:2 n-6): – Tumours: Overall comparison $p = 0.017$ (Kruskal-Wallis); A vs B $p = 0.011$; A vs C $p = 0.47$; A vs D $p = 0.36$; B vs C $p = 0.0071$; B vs D $p = 0.086$; C vs D $p = 0.11$ (Mann-Whitney U). – Plasma: Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0004$; A vs C $p = 0.028$; A vs D $p = 0.041$; B vs C $p = 0.0002$; B vs D $p = 0.069$; C vs D $p = 0.0019$ (Mann-Whitney U). (b) Gamma linolenic acid (18:3 n-6): – Tumours: Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0002$; A vs C $p = 0.15$; A vs D $p = 0.0002$; B vs C $p = 0.0002$; B vs D $p = 0.62$; C vs D $p = 0.0002$ (Mann-Whitney U). – Plasma: Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0002$; A vs C $p = 0.25$; A vs D $p = 0.0002$; B vs C $p = 0.0002$; B vs D $p = 0.45$; C vs D $p = 0.0002$ (Mann-Whitney U). (c) Dihomo-gamma-linolenic acid (20:3 n-6): – Tumours: Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0004$; A vs C $p = 0.13$; A vs D $p = 0.013$; B vs C $p = 0.0002$; B vs D $p = 0.46$; C vs D $p = 0.0003$ (Mann-Whitney U). – Plasma: Overall comparison $p < 0.0001$ (Kruskal-Wallis); A vs B $p = 0.0002$; A vs C $p = 0.016$; A vs D $p = 0.0002$; B vs C $p = 0.0002$; B vs D $p = 0.023$; C vs D $p = 0.0002$ (Mann-Whitney U).

In the current study we found highly significant changes in tumour, red blood cell and plasma fatty acid profiles of the murine hosts receiving the different dietary treatments. Notably there was a large increase in GLA plus the downstream metabolite DGLA with a reduction in the precursor linolenic acid in the two dietary groups receiving GLA supplementation. Our fatty acid results are in agreement with those of Pritchard *et al.* who found and increased levels of GLA and DGLA in MCF-7 xenograft hosts

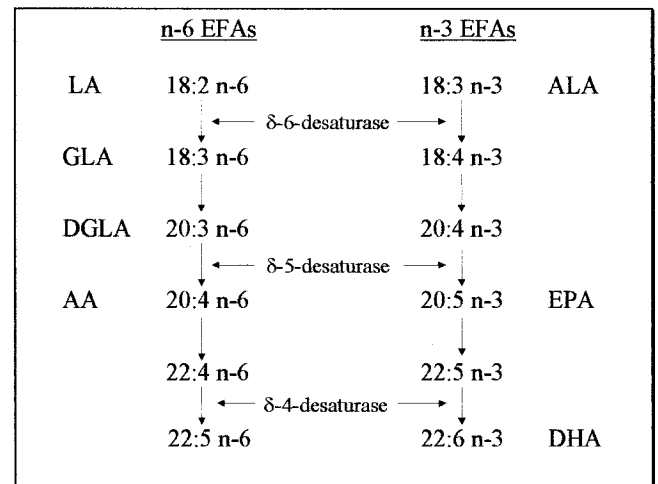


FIGURE 4 – N-6 and n-3 essential fatty acid metabolic pathways. N-6 series: LA = linolenic acid; GLA = gamma linolenic acid; DGLA = dihomogammalinolenic acid; AA = arachidonic acid. N-3 series: ALA = alpha linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

receiving diets containing evening primrose oil.⁴ Horrobin has previously hypothesized there to be a build up of LA in cancerous tissues due to lack of the δ -6-desaturase enzyme which catalyses the desaturation of LA to GLA. By providing dietary GLA he proposed that one can bypass the blockage in the n-6 pathway caused by the lack of δ -6-desaturase and hence restore the balance of anti-carcinogenic downstream metabolites to the pro-carcinogenic parent fatty acid LA with resultant suppression of tumour growth.²⁶ More recently a number of *in vitro* and animal studies have reported LA to be cancer-promoting whereas the downstream metabolites GLA and DGLA to be inhibitory.^{5,6,27,28} in keeping with Horrobin's original hypothesis.

In the present study whilst GLA as sole therapy (Group B) did appear to exert some slowing effect on tumour growth compared to the control Group A the difference was not statistically significant and was much smaller than that seen in Groups C and D receiving tamoxifen alone or tamoxifen in combination with GLA (Fig. 1). Nor did we find a significant difference between the growth rates of the latter two groups—one would have anticipated the growth of Group D (combined treatment) to be slower than C if the two therapies were working in an additive fashion. The lack of statistical significance in the above instances may have been partly attributable to the small number of mice per treatment group. Unfortunately due to financial constraints it was not possible to use a larger number of mice in our study.

Several other research groups have previously examined the effects of dietary GLA on the growth of chemically-induced or transplanted mammary tumours in rodents.^{4,5,29,30} These studies have in general found a significant reduction in tumour growth with GLA or GLA-containing evening primrose oil supplementation. The methodology of the experiments has varied, however, including the proportion of overall fat and EFAs used in the diets; the dosage, formulation and frequency of administration of the EFA; whether the dietary treatments were started before or after tumour induction/implantation; the number of animals used and differing end-points of the studies. These factors may have had a bearing on why we were not able to show a significant growth inhibitory effect in the current study. In particular the numbers of mice used were greater in the majority of the other studies and the dietary treatments were commenced before initiation of the tumours rather than afterwards as we have done. The rationale for commencing the EFA diet beforehand is to achieve the desired lipid environment at the stage of initial tumour proliferation. In the

present study it was decided to start the dietary treatment after tumour implantation as we considered this to be a more accurate reflection of the clinical situation i.e. commencement of treatment after the tumour is already established.

Whilst we have not been able to demonstrate a definite effect of GLA on the suppression of breast cancer growth in this study we have found marked biological changes in the expression of ER. The combined effect of GLA plus tamoxifen on ER expression of the xenograft tumours is particularly interesting. The partial oestrogen antagonist tamoxifen and the newer pure anti-oestrogens are known to down-regulate the expression of ER and ER-regulated gene-products in human breast carcinomas.^{12,13,31} In previous work from this unit we have shown a greater extent of ER down-regulation to be associated with better quality and longer duration of clinical response on primary tamoxifen.¹⁵ Given the experimental capacity for EFAs to modulate the structure and function of steroid hormone receptors we wished to examine the effect of GLA +/- tamoxifen on the expression of ER in this xenograft model of ER+ve breast cancer. In this study we have demonstrated dietary GLA to result in a highly significant reduction of ER expression in the MCF-7 BIM human breast cancer cell line. Our ER findings are consistent with those of Borras and LeClercq who have demonstrated a dose-dependent down-regulatory effect of n-6 EFA metabolites on growth and ER expression in cultured MCF-7 breast cancer cells.³² In addition, the xenograft findings of lower ER expression on combined Tamoxifen and GLA are in agreement with the ER results from our pilot clinical trial.¹¹ In the latter we also demonstrated a significantly faster clinical response on combined treatment compared to tamoxifen alone, raising the possibility of an additive or synergistic growth-inhibitory action of these two agents via enhanced down-regulation of the ER machinery. In contrast to our clinical study in the xenograft work we have not been able to show the greater down-regulation of ER on combined T + GLA to translate into slower tumour growth than on tamoxifen alone. A reason for this could be Type II error due to the small number of mice used per treatment group as discussed earlier. In the interpretation of these results it is also important to consider the inherent differences between a xenograft model of human breast cancer and human breast cancer in the clinical

setting. A xenograft tumour consists of clones of the same breast cancer cell rather than being a heterogeneous population of different cancer cells as are clinical breast tumours. Therefore whilst xenograft models are useful tools with which to investigate the behaviour of human breast cancer they may not be a direct reflection of what occurs in the clinical situation.

It is of further interest that in the xenograft study those tumours with the lowest expression of ER at termination contained the highest levels of GLA. This finding adds to the evidence for an interaction between the GLA molecule and the ER such to alter the structure and/or expression of the latter. Previous kinetic studies have suggested that EFAs bind to a separate entity on the hormone receptor rather than to the hormone binding site, thus inhibiting subsequent ligand binding via induction of a conformational change in the receptor molecule.^{18,19,33} Vallette and colleagues have demonstrated irreversible covalent binding of oestradiol to components of the ER protein in the presence of EFAs.³⁴ It is possible that in the presence of GLA a similar covalent bond is formed between tamoxifen and ER with resultant attenuation of receptor activity. Further research is required to confirm this hypothesis.

In conclusion, this xenograft model has shown dietary supplementation of GLA to result in marked biological changes in the ER expression and the fatty acid profiles of ER+ve human breast carcinomas, the effects on the former which appear to be additive to those induced by tamoxifen. These results, taken in conjunction with the encouraging findings of our recent Phase II clinical study, suggest GLA to be potentially valuable new agent in the treatment of endocrine-sensitive breast cancer and one that deserves further investigation in the form of randomized controlled trials.

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