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The DietCompLyf Study: A Prospective Cohort Study of Breast Cancer Survival and Phytoestrogen Consumption

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Abstract

DietCompLyf is a multi-centre prospective study designed to investigate associations between phytoestrogens - naturally occurring plant compounds with oestrogenic properties - and other diet and lifestyle factors with breast cancer recurrence and survival. 3159 women with grade I-III breast cancer were recruited 9-15 months post-diagnosis from 56 UK hospitals. Detailed information on clinico-pathological, diet, lifestyle and quality of life is collected annually up to 5 years. Biological samples have also been collected as a resource for subsequent evaluation. The characteristics of the patients and associations between pre-diagnosis intake of phytoestrogens (isoflavones and lignans; assessed using the EPIC-Norfolk UK 130 question food frequency questionnaire) and breast cancer (i) risk factors and (ii) prognostic factors are described for 1797 women who had complete data for all covariates and phytoestrogens of interest. Isoflavone intakes were higher in the patients who were younger at diagnosis, in the non-smokers, those who had breast-fed and those who took supplements. Lignan intakes were higher in patients with a higher age at diagnosis, in ex-smokers, those who had breast-fed, who took supplements, had a lower BMI at diagnosis, lower age at menarche and were nulliparous. No significant associations between pre-diagnosis phytoestrogen intake and factors associated with improved breast cancer prognosis were observed. The potential for further exploration of the relationship between phytoestrogens and breast cancer recurrence and survival, and for the establishment of evidence to improve dietary and lifestyle advice offered to patients following breast cancer diagnosis using DietCompLyf data is discussed.

Keywords: breast cancer, isoflavones, lignans, phytoestrogens, recurrence, survival

1. Introduction

Age-standardised incidence rates for female breast cancer in Northern Europe exceed those for Eastern Asia, with 84 and 25 cases reported per 100 000 population respectively [1]. Migration studies have shown that the risk of developing breast cancer increases to approximately that of the host nation within a few generations [2]. Considerable effort has been expended to identify dietary, lifestyle and environmental factors that may contribute to these geographic differences in breast cancer risk.

Phytoestrogens are naturally occurring plant compounds capable of eliciting oestrogen-like properties through binding to oestrogen receptors (ER) and other mechanisms. Intakes vary significantly between Eastern and Western diets. Eastern diets typically include soy-based foods which are rich in isoflavones, one of the main groups of phytoestrogens whilst Western diets more typically obtain isoflavones from legumes, coffee, nuts, bread and soya milk [3]. Lignans are the most common phytoestrogens found in UK foods (e.g., beverages, cereals, cruciferous vegetables and some fruits), yet isoflavones account for the greatest phytoestrogen intakes due to the use of soya in bread production [4].

The biological complexity of phytoestrogens is well described [5]. Understanding the in vivo effects of phytoestrogens is complicated by different forms and quantities of intake and individual metabolic differences. Despite these difficulties, several studies have investigated the relationship between phytoestrogen intake and breast cancer risk. Research findings are not yet conclusive; however early evidence suggests protective relationships, to varying extents and using different markers (diet, urine and serum concentrations) in Western [6] and Eastern populations [7]. The role of phytoestrogens on breast cancer recurrence rates and overall survival is less well studied. Early results show inverse associations between phytoestrogen intake and breast cancer recurrence [8] and mortality [9]. Biomarkers of phytoestrogen metabolism are associated with improved survival [10]. However more studies are needed to investigate the link between phytoestrogens and breast cancer recurrence as conflicting data continues to emerge [11].

2. Aims

The DietCompLyf study has been established to examine the relationship between dietary intake of lignans and isoflavones and breast cancer recurrence (primary outcome) and survival in pre- and post- menopausal women in the UK. Data on acknowledged risk factors has been collected alongside information on more novel factors which currently have suggestive evidence of an association with recurrent breast cancer. We outline here the methods used within the DietCompLyf study, describe the cohort and the evaluation procedures used to estimate phytoestrogen intake levels prior to diagnosis collected using the EPIC-Norfolk 130 question food frequency questionnaire (FFQ). Considering the relationship between this and other known prognostic factors for breast cancer, these results are used to inform the planned later analyses.

3. Study design

3.1. DietCompLyf - overview

DietCompLyf is a prospective, observational study based in the UK. With ethical approval from the University College London Hospitals Research Ethics Committee, recruitment was rolled out in two phases. The first wave recruited 582 patients from 4 hospitals (Feb 1997 – Feb 2005). In 2004, the study was adopted by the National Cancer Research Network and the protocol was expanded to obtain clinical information over a longer duration and from an increased number of centres. The second phase recruited 2808 patients (Dec 2004 – Aug 2010) from 56 NHS centres (Fig. 1). Recruitment rates are shown in Fig. 1. 3390 breast cancer patients were enrolled, of whom 3159 (93.2%) fulfilled all inclusion criteria and were considered evaluable for analysis of phytoestrogen intake levels (Fig. 2). Poor compliance with the 24 h urine collections in the initial protocol (52.3% completed a 24 h sample) and evidence of comparability between levels of enterolactone in spot and 24 h urine samples [12] prompted a protocol amendment to collection of spot samples.

3.2. Recruitment and follow-up

Female patients were invited to participate if they had a histologically confirmed invasive primary breast cancer (grade I to III), were 9 to 15 months post diagnosis (after completion of active breast cancer treatment) and were up to and including 75 years of age. Exclusion criteria were: previous cancer (except basal cell carcinoma); concomitant primary cancer; bilateral cancer of the breast; cognitive impairment; psychological difficulties; or a poor understanding of English. All patients provided written consent agreeing to participate in the study.

Recruitment and follow-up visits were designed to run in parallel with the patients' clinical follow-up schedule. Patients were assessed every 6 months for 2 years in the first recruitment wave and annually for 4 years when recruitment was extended. Clinico-pathological details and treatment was recorded at recruitment and follow-up (Supplementary Table S1). At each visit, blood and urine samples were collected and questionnaires were completed (Table 1). A summary of the information collected is illustrated in Fig. 3. A record was made if a course of antibiotics had been taken in the previous 3 months as this could disrupt the intestinal microflora and affect phytoestrogen metabolism [13]. Data quality checks were initiated at the coordinating centre and anomalies were checked and corrected in consultation with each centre. Data entry checks were performed on 5% of baseline data (median transcription errors 0.68%). Centre staff reported details of a new primary cancer, breast cancer recurrence, metastasis, withdrawal, if patients had become lost to follow up or had died. Updates on participant mortality were provided through the NHS Information Centre. Final follow-up data is expected to be collected by the end of 2015.

3.3. Sample collection

At recruitment, 6 ml of blood was collected in a Vacutainer containing EDTA (BD Bioscience) for plasma and 12 ml of blood was collected for serum at each study visit. All blood was allowed to stand for at least one hour

and centrifuged at approximately 1200 *xg* for 15 min and 0.5 ml of the supernatant was aliquoted. Buffy coats were removed and stored in a DNase / RNase-free cryovial (Nalgene) for future DNA extraction. Urine samples were centrifuged at approximately 1200 *xg* for 15 min and ten aliquots of 1.25 ml were stored frozen. Time of blood and urine collection, freezing and centrifugation were recorded. Samples were temporarily stored at centres at temperatures between -20°C and -80°C. After transfer to the coordinating centre, all samples were catalogued and stored at -80°C.

3.4. Questionnaires

A range of questionnaires, validated where possible, to evaluate diet, lifestyle, general health and quality of life were given to patients.

A lifestyle questionnaire was completed at recruitment. This provided information on ethnicity, occupation, education, family history of breast cancer, reproductive history, physical activity, alcohol use, smoking and CAM. The European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 v2.0 quality of life assessment [14] was used. A follow-up questionnaire comprising a condensed version of the lifestyle questionnaire assessed changes in lifestyle annually. A general health questionnaire (GHQ-12) was completed at every study visit [15].

Diet was evaluated annually using the 130 question EPIC-Norfolk FFQ (CAMB/PQ/6/1205) [16]. Patients reporting dietary changes post-diagnosis completed a retrospective FFQ at recruitment. The Compositional Analyses from Frequency Estimates (CAFÉ) program was used to estimate nutrient intake [17] from the FFQs following quality control checks on ≥10% of entered questionnaires (Adetiq, East Sussex). Individuals with ≥10 missing responses and those in the extreme 0.5% of energy intake: basal metabolic rate ratios were excluded [17]. At year 2 (and 4 in the second recruitment wave), a more detailed 7-day food diary was also completed [18].

3.5. Sample size calculation

Using the primary outcome – recurrence free survival – for individual or combined phytoestrogens, sample size calculations were based on 20 events per covariate for the primary analysis. The number of patients required to observe at least 200 events was estimated using the expected event rates observed from the ATAC [19], NEAT [20] and Early Breast Cancer Trialist' Collaborative Group [21] studies where 5-year survival was 70-86%. A sample size target of 2300-3000 was set after adjustment for recruitment approximately 1 year post-diagnosis; missing or non-analysable urine samples; missing data for pre-specified prognostic covariates; patients lost to follow-up, addition of further covariates and taking into account improvements in breast cancer survival rates.

3.6. Assessment of phytoestrogen consumption and breast cancer prognostic factors

The association between pre-diagnosis phytoestrogen intake and breast cancer risk/prognostic factors was assessed for 1797 patients who had complete data for all covariates and phytoestrogens of interest (Table 2). Log transformed phytoestrogen intake estimates were used in the univariate regression models to identify factors for inclusion in the final multivariate model, using a backward stepwise selection model; computed using STATA version 11 (Stata/IC 11.0, Texas, USA, StataCorp LP©).

Intakes of phytoestrogen-containing food groups were considered in age tertiles and by dietary preference (vegetarian, fish, meat or meat and fish diet). Foods were grouped as: meat; fish; bread and savoury biscuits; cereals; potatoes, rice and pasta; dairy products and fats; sweets and snacks; soups, sauces and spreads; non-alcoholic drinks; alcoholic drinks; fruit; vegetables; beans, lentils, nuts and peas; tofu, soya meat, textured vegetable protein and vegeburgers. Daily portions consumed per food group were calculated using a previously published method [17]. Differences between age and dietary preference groups were assessed using one-way ANOVA with a post-hoc Bonferroni adjustment for multiple testing in patients with evaluable data. Number of portions consumed between the lower and upper age quartile was assessed using an independent samples t-test. Data was analysed using SPSS version 19 (SPSS Inc., Chicago, IL, USA, IBM Company).

This evaluation has allowed us to determine the patterns of phytoestrogen consumption across the DietCompLyf cohort to assess the heterogeneity of consumption levels across all pre/peri and post-menopausal breast cancer patients.

4. Data reported at baseline

4.1. Recruitment and return rate

Flow chart of patient recruitment and their contribution at the various time-points up to where the visits are complete (up to year 3 post-diagnosis) is presented in Fig. 2. The return rate for the lifestyle questionnaire at recruitment was 94.7% and follow-up lifestyle questionnaires from active patients have remained above 80% throughout the study. Almost all the patients contributed biological samples at recruitment: 98.6% and 93.5% for urine and blood, respectively.

4.2. Baseline characteristics

The mean time from diagnosis to recruitment for the 3159 evaluable patients was 12.4 ± 1.75 months. The majority of patients were post-menopausal (64.9%), had a grade II (45.9%), ER positive (81.6%) tumour, less than 20 mm diameter (49.9%), and were lymph node negative (61.9%) (Table 2). At the time of writing (26/02/13), the mean follow-up on the study was 38.5 ± 15.7 months from recruitment (50.8 ± 15.8 months from breast cancer diagnosis).

4.3. Phytoestrogen intake

Pre-diagnosis phytoestrogen intakes in the analysed subgroup were similar to that of the whole cohort (Table 3a). The lignan, secoisolariciresinol was consumed in the greatest quantity, with genistein the predominant isoflavone consumed. Phytoestrogen consumption showed a skewed distribution. In the sub-group analysis, the lignan, secoisolariciresinol was consumed in the greatest quantity (281.0, 276.1 – 285.9 µg/day), with genistein the predominant isoflavone consumed (267.6, 254.6 – 281.2 µg/day). Consumption of isoflavones varied a 1000 fold, with an average consumption of 484.3 (462.2 – 507.4) µg/day and consumption of lignans varied 100 fold with an average of 322.7 (317.3 – 328.1) µg/day.

Patients with a vegetarian or fish-only diet consumed significantly more isoflavones than those with a meat containing diet. Meat eaters had a significantly lower intake of total lignans compared to those who had a vegetarian or a meat and fish diet but not a fish only diet (Table 3b). The intakes of the isoflavones genistein, daidzein and glycitein correlated with each other ($R>0.96$, $p<0.001$), and to a lesser extent with the lignan secoisolariciresinol (R 0.20 – 0.30, $p<0.001$) (Table 3c).

Univariate analyses (Supplementary Table S2) showed genistein, daidzein, glycitein and secoisolariciresinol were significantly associated with every variable tested ($p<0.001$). However, multivariate analyses showed no association with the pre-diagnosis phytoestrogen intake and tumour characteristics (data not shown; $p>0.05$). Intakes of genistein, daidzein and glycitein were significantly higher in patients who were younger at the time of diagnosis, those who took supplements, non-smokers and those who had breast fed ($p<0.05$) (Table 4). Genistein intake was also significantly higher in patients who had never taken hormone replacement therapy ($p<0.05$). Consumption of secoisolariciresinol pre-diagnosis was higher in older patients, those who reported supplement usage, ex-smokers, those who had breast fed, nulliparous women, those with a lower BMI at diagnosis and lower age at menarche ($p<0.05$).

Differences in phytoestrogen consumption were further investigated by grouping the patients into tertiles, according to age at diagnosis (mean ages: 43.4, 54.0, 64.2 years) (Table 5). Significantly more lignans were consumed by patients in the middle and upper age tertiles compared to the lower age tertile ($p<0.01$). More isoflavones were consumed by the lower and middle age tertiles than the upper age tertile ($p<0.01$). This variance is explained by the food groups consumed by these women. Breads, cereals, fish, soups, sauces and spreads, fruit and vegetables were preferred by older women and potatoes, rice and pasta, sweets and snacks, tofu/soya meat, by younger women. Other food groups: dairy, non-alcoholic drinks, alcoholic drinks, beans and lentils, were consumed in similar proportions by all age groups. There were significantly more vegetarian women in the younger compared to the older participant age group (tertile 1: 4.0%, tertile 3: 0.8%) ($\chi^2 = 12.4$, $p<0.01$).

5. Expected outcomes of DietCompLyf

DietCompLyf has been established to evaluate associations between phytoestrogen intake levels and breast cancer recurrence (primary outcome) and survival. To our knowledge, this is the largest, most detailed study of breast cancer patients' diet and lifestyle in the UK. The initial assessment of the reported phytoestrogen consumption levels show participants have a lower phytoestrogen intake than those observed in Asian populations [22], but were consistent with those of other UK populations [23]. Multicollinearity was evident with intakes of individual isoflavones genistein, daidzein and glycitein ($R>0.9$), showing that different isoflavones are found and/or eaten in foods at the same time. Isoflavones were consumed in a greater quantity than lignans, as previously shown in a UK population [24].

Food preferences which impacted total isoflavone and lignan intake levels were identifiable with age. Older women tended to favour lignan-containing, younger women isoflavone-containing, foods. The increased intake of isoflavones in younger women agrees with other UK data [3] and higher lignan consumption in older women has also been reported elsewhere [25]. However, the differences in lignan consumption across the different age groups has not (to our knowledge) previously been reported for UK breast cancer patients. Food group analyses attributed the isoflavone intake in the DietCompLyf population to tofu/soya meat consumption. Vegetarianism alone did not account for disparities in tofu/soya meal consumption; therefore it is hypothesized that this dietary change may reflect recent changes in health, diet and food availability and/or a combination thereof. It was expected that bread and soya milk consumption would provide the majority of isoflavones consumed in a UK diet [3], yet only 1% of the patients in this subset reportedly drank soya milk. The heterogeneity (bread containing soy and linseed for example being contained in the same group as standard brown bread; genistein contents of 6,807 and 246 µg/100g respectively) was not controlled for [26].

In other studies phytoestrogens have been shown to relate to breast cancer characteristics at diagnosis, for example an increase in dietary lignans [27] and isoflavones [28] have been found to be associated with ER positive tumours. Higher lignan consumption has been related to a reduction in lymphovascular invasion, and smaller tumour size [29], although the Australian population studied had an order of magnitude higher lignan intake levels compared with the DietCompLyf cohort (between 1,640 and 2,260 µg/d from an FFQ). Similarly, Buck et al., [10] reported that post-menopausal breast cancer patients with higher levels of serum enterolactone had smaller tumours of a lower tumour grade and were more likely to be hormone receptor positive. In the DietCompLyf cohort total phytoestrogen intake was associated with tumour characteristics (tumour grade, size, lymph node status, ER status, and vascular invasion) at diagnosis in univariate regression models (effects attenuated in multivariate models). In addition, there were significant associations between each of the dietary phytoestrogens measured and risk factors for breast cancer: age at diagnosis, age at menarche, breast feeding and parity. Phytoestrogen levels were also associated with lower BMI at diagnosis, consistent with either higher consumption of fibre containing foods and/or lower consumption of fatty foods.

Supplement use can potentially affect an individual's overall phytoestrogen consumption levels. An interim analysis of patients recruited onto DietCompLyf identified that 56% of patients were supplement users pre-

diagnosis [30]. With recruitment now complete estimates using data from all the participants who provided a pre-diagnosis FFQ highlights a reduced proportion of supplement users (47%). The underlying reasons for this difference is not clear, but – in light of adding centres to the study – it is expected that geographical, socioeconomic, educational status and so on may explain this variance, but this will require further investigation to elucidate. The difficulties in determining phytoestrogen levels in supplements led to this factor being assessed as a binary variable, accordingly, only a partial exploration of supplement usage has been undertaken. A database incorporating phytoestrogen levels in supplements is under construction as part of a longer-term research endeavour. The absence of an association between phytoestrogen consumption and prognostic factors for breast cancer reported here may reflect the paucity of information on intake of phytoestrogens from supplements and it is likely that phytoestrogen consumption has been somewhat underestimated. The availability of serum/urine samples from individual patients however will allow an exploration of supplement usage and phytoestrogen levels in later studies.

To our knowledge, DietCompLyf is the largest, most comprehensive evaluation of diet, phytoestrogen intake and lifestyle in women diagnosed with breast cancer across the UK. Dietary intake estimates are complemented by analyses of serum and urinary phytoestrogen levels; the cohort includes pre, peri and postmenopausal patients, and data are sampled over repeated time points. The continued reporting of recurrence and mortality rates will allow the progression of the disease to be mapped with respect to each of these factors. The DietCompLyf study will therefore provide an opportunity for strengthening of current scientific evidence to establish dietary recommendations for individuals diagnosed with breast cancer, which could lead to reduced recurrence rates.

Figure captions

Fig. 1 DietCompLyf recruitment. (a) Map showing the distribution of DietCompLyf centres across the UK. (b) Chart shows the rate of recruitment onto DietCompLyf displaying the number of participants (□) and the number of recruiting centres (◆). Data is given for August of each year.

Fig. 2 Consort diagram for patients eligible on the DietCompLyf study. The total number of returned questionnaires and samples collected are shown for the completed phases of the study.

Fig. 3 The design of the DietCompLyf study showing the multi-factorial nature of breast cancer and the diverse information collected in the DietCompLyf study is shown. The instrument used to capture the information is displayed in bold.

Supplementary data:
Supplementary data are available at Maturitas online

Conflict of interest: None declared.

Funding:
This work was funded by Against Breast Cancer (Registered Charity Number 1121258).

Acknowledgements:
We would like to thank Amber Gibney and Sally Hughes for data checking, the MRC CNC in Cambridge for their assistance with the FFQ and 7-day food diary processing and the expertise provided by the late Shelia Bingham of the MRC centre and the use of the FFQ and 7-day food diary.

We would like to thank all the DietCompLyf study patients and the following Principal Investigators funded through the National Cancer Research Network: Dr J Abraham, Mrs M Adams, Dr M Ah-See, Dr S Ahmed, Dr W Appel, Dr L Barraclough, Prof P Barrett-Lee, Mr R Bonomi, Mrs S Breakspear, Mrs S Bulley, Mrs J Clarke, Dr A Clayton, Prof N Davidson, Ms J Dodge, Mr H Dymond, Dr H Eldeeb, Prof O Eremin, Miss A Evans, Dr D Fermont, Mr C Gateley, Mr I Gouldbourne, Mr M Graham, Prof R Grieve, Mr D Hadjiminas, Dr M Hall, Dr A Hong, Dr S Houston, Dr R Hunter, Mrs S Jones, Mr M Keshgar, Mr I Laidlaw, Mrs A Lannigan, Dr B Lavery, Dr G Mazdai, Dr K McAdam, Dr J McAleer, Dr M Napier, Dr C Palmer, Dr U Panwar, Mrs J Pascoe, Mr A Paterson, Mrs C Reed, Dr P Riddle, Mr C Rogers, Dr A Sahu, Dr E Sawyer, Dr N Shah, Dr M Shere, Dr E Sherwin, Mr M Sibbering, Dr R Simcock, Dr D Spooner, Dr K Stepp, Dr A Stevens, Dr J Stewart, Ms H Sweetland, Prof S Tahir, Dr R Thomas, Prof A Thompson, Dr S Upadhyay, Mr R Visvanathan, Miss B Weber, Mrs B Williams-Yesson, Mr A Wilson, Prof J Yarnold, Mrs T Young.

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Competing Interests

The authors declare no conflict of interest

Funding Information

The work in this article was supported by the charity Against Breast Cancer (Registered Charity Number 1121258).

The authors received no funding for this article.

TABLES

Table 1 The schedule of participant follow up and contribution at each study visit.

	Time after diagnosis (years)						
	1	**1.5**	**2**	**3**	**4**	**5**	**6**
Spot urine	10 x 1.25 ml	10 x 1.25 ml[a]	10 x 1.25 ml*	10 x 1.25 ml[b]	10 x 1.25 ml[b]	10 x 1.25 ml[b]	
24 h urine			2 x 20 ml*[a]				
Serum	6 x 0.5 ml	6 x 0.5 ml[a]	6 x 0.5 ml	6 x 0.5 ml[b]	6 x 0.5 ml[b]	6 x 0.5 ml[b]	
Plasma	3 x 0.5 ml	3 x 0.5 ml[a]	3 x 0.5 ml[a]				
Buffy coat	1	1[a]	1[a]				
Lifestyle questionnaire	✓		✓[b]	✓	✓	✓	✓[a]
FFQ	✓[†]	✓[a]	✓	✓	✓	✓	✓[a]
GHQ	✓	✓[a]	✓	✓	✓	✓	✓[a]
7-day food diary			✓		✓[b]		

[a]Recruitment 1997-2004, [b]recruitment 2004-2010 only. *Either a 24 h or a spot urine was collected during first wave of recruitment. [†]Two FFQs were filled in at recruitment to reflect diet pre- and post-diagnosis.

Table 2 Descriptive covariates, showing the number of evaluable participants (n=3159) and those used in the sub-group (n=1797) analysis. Age at first full pregnancy was omitted from the analysis due to the association with parity.

Variable	Evaluable participants n=3159 n (%)	Sub-group n=1797 n (%)
Age at diagnosis mean ± SD	54.4 ± 9.7	53.9 ± 9.3
n	3159 (100.0)	1797 (100.0)
Tumour grade		
Grade I	500 (15.8)	294 (16.4)
Grade II	1451 (45.9)	823 (45.8)
Grade III	1176 (37.2)	680 (37.8)
Missing	32 (1.0)	-
Tumour size		
<20 mm	1575 (49.9)	916 (51.0)
≥20 mm	1285 (40.7)	746 (41.5)
Multifocal	234 (7.4)	135 (7.5)
Missing	65 (2.1)	-
Lymph node status		
Negative	1956 (61.9)	1083 (60.3)
Positive	1190 (37.7)	714 (39.7)
Missing	13 (0.4)	-
Histology		
Ductal	2492 (78.9)	1428 (79.5)
Ducto-lobular	102 (3.2)	58 (3.2)
Lobular	351 (11.1)	198 (11.0)
Other	193 (6.1)	113 (6.3)
Missing	21 (0.7)	-
ER		
Negative	551 (17.4)	313 (17.4)
Positive	2578 (81.6)	1484 (82.6)
No tested	30 (0.9)	-
Vascular invasion		
No	1815 (57.5)	1228 (68.3)
Yes	764 (24.2)	569 (31.7)
Missing	580 (18.4)	-
Supplement use		
No	1406 (44.5)	952 (53.0)
Yes	1248 (39.5)	845 (47.0)
Missing	505 (16.0)	-

Variable	Evaluable participants n=3159 n (%)	Sub-group n=1797 n (%)
Age at menarche mean ± SD	12.7 ± 1.6	12.7 ± 1.5
n	2646 (83.8)	1797 (100.0)
BMI at diagnosis (Kg/m^2) mean ± SD	26.8 ± 5.3	26.6 ± 5.2
N	3077 (97.4)	1797 (100.0)
Menopausal status		
Pre	609 (19.3)	343 (19.1)
Peri	464 (14.7)	301 (16.8)
Post	2049 (64.9)	1153 (64.2)
Missing	37 (1.2)	-
Smoking		
Never	1531 (48.5)	906 (50.4)
Current	294 (9.3)	168 (9.3)
Ex-smoker	1152 (36.5)	723 (40.2)
Missing	182 (5.8)	-
Parity		
0	360 (11.4)	236 (13.1)
1+	2181 (69.0)	1561 (86.9)
Missing	618 (19.6)	-
Age at first full pregnancy		
No pregnancy	360 (11.4)	236 (13.1)
≤ 20 years	457 (14.5)	326 (18.1)
20-30 years	1357 (43.0)	970 (54.0)
> 30 years	367 (11.6)	265 (14.7)
Missing	618 (19.6)	-
Breast fed		
No	1242 (39.3)	752 (41.8)
Yes	1407 (44.5)	1045 (58.2)
Missing	510 (16.1)	-
Oral contraceptive use		
No	416 (13.2)	273 (15.2)
Yes	2101 (66.5)	1524 (84.8)
Missing	642 (20.3)	-
HRT		
No	1647 (52.1)	1151 (64.1)
Yes	973 (30.8)	646 (35.9)
Missing	539 (17.1)	-

Abbreviations: ER, oestrogen receptor; HRT, hormone replacement therapy; SD, standard deviation.

Table 3 Average dietary intake of phytoestrogens from FFQs for (a) the whole cohort with FFQ data (n=2582) and evaluable participants used in sub-group analysis (n=1797). (b) Individuals in the sub-group are categorised by diet type. (c) Pearson correlation coefficients between phytoestrogen intake for evaluable participants with complete data (n=1797). [†]Untransformed data. The geometric mean is the back-transformed mean of the log transformed data. All correlations were significant p<0.001. Total isoflavones includes genistein, daidzein, glycitein, biochanin A, formononetin. Total lignans includes secoisolariciresinol, shonanin, and matairesinol. Total phytoestrogens includes genistein, daidzein, glycitein, biochanin A, formononetin, secoisolariciresinol, shonanin, matairesinol, coumestrol, enterolactone, equol, enterodiol.

a

	Total cohort with evaluable FFQ data n=2582			**Sub-group n=1797**		
	Geometric mean (95% CI)		**Range[†] µg/day**	**Geometric mean (95% CI)**		**Range[†] µg/day**
Genistein	266.3	(255.2 – 277.8)	14.8 – 31 025.7	267.6	(254.6 – 281.2)	19.7 – 29 754.4
Daidzein	153.0	(147.1 – 159.2)	5.9 – 13 456.4	153.8	(146.8 – 161.1)	9.9 – 13 351.0
Glycitein	32.5	(31.3 – 33.7)	2.6 – 2280.3	32.7	(31.3 – 34.1)	2.7 – 1623.0
Secoisolariciresinol	276.1	(272.0 – 280.3)	54.2 – 1105.5	281.0	(276.1 – 285.9)	75.1 – 1105.5
Total isoflavones	482.2	(463.4 – 501.8)	39.7 – 46 415.5	484.3	(462.2 – 507.4)	43.5 – 44 634.1
Total lignans	317.3	(312.8 – 321.8)	60.2 – 1178.2	322.7	(317.3 – 328.1)	84.5 – 1178.2
Total phytoestrogens	918.8	(891.7 – 946.7)	156.4 – 46 819.5	924.2	(892.5 – 957.1)	219.5 – 44 940.0

b

Diet	**n**	**Total isoflavones Geometric mean (95% CI)**		**Total lignans Geometric mean (95% CI)**		**Total phytoestrogens Geometric mean (95% CI)**	
Vegetarian	47	2788.3	(1829.9 – 4248.5)	360.7	(328.7 – 395.9)	3607.3	(2569.4 – 5064.3)
Fish only	44	2074.7	(1358.7 – 3168.2)	329.8	(293.7 – 370.4)	2775.2	(1987.7 – 3874.9)
Meat only	59	350.4	(269.4 – 455.7)	278.9	(253.6 – 306.7)	721.5	(594.0 – 876.4)
Meat and fish	1647	448.3	(429.2 – 468.2)	323.1	(317.6 – 328.8)	871.0	(843.4 – 899.4)

c

	Log genistein	**Log daidzein**	**Log glycitein**	**Log secoiso-lariciresinol**	**Log total isoflavones**	**Log total lignans**
Log daidzein	0.993	-	-	-	-	-
Log glycitein	0.976	0.963	-	-	-	-
Log secoisolariciresinol	0.211	0.204	0.300	-	-	-
Log total isoflavones	0.999	0.995	0.980	0.227	-	-
Log total lignans	0.208	0.202	0.295	0.994	0.224	-
Log total phytoestrogens	0.962	0.949	0.963	0.384	0.968	0.381

All p-values <0.001

Abbreviations: CI, confidence interval.

Table 4 Multivariate regression model for each phytoestrogen and variables included in the final model for evaluable participants with complete data (n=1797).

		Log genistein				Log daidzein				Log glycitein				Log secoisolariciresinol			
Variable	**Category**	**Coef.**	**t**	**p**	**95% CI**	**Coef.**	**t**	**p**	**95% CI**	**Coef.**	**t**	**p**	**95% CI**	**Coef.**	**t**	**p**	**95% CI**
Age at menarche	Years													-0.017	-2.9	0.004	(-0.028 – -0.006)
Age at diagnosis	Years	-0.008	-2.5	0.012	(-0.014 – -0.002)	-0.008	-3.3	0.001	(-0.013 – -0.003)	-0.009	-4.0	<0.001	(-0.014 – -0.005)	0.003	3.1	0.002	(0.001 – 0.005)
BMI at diagnosis	Kg/m^2													-0.004	-2.4	0.018	(-0.008 – -0.001)
Parity	0													Ref.			
	1+													-0.102	-3.4	0.001	(-0.160 – -0.043)
Breast feeding	No	Ref.				Ref.				Ref.				Ref.			
	Yes	0.144	2.8	0.005	(0.043 – 0.244)	0.145	3.0	0.003	(0.051 – 0.239)	0.129	2.9	0.004	(0.042 – 0.215)	0.090	4.5	<0.001	(0.050 – 0.130)
HRT	No	Ref.															
	Yes	-0.117	-2.0	0.048	(-0.234 – -0.001)												
Supplement use	No	Ref.				Ref.				Ref.				Ref.			
	Yes	0.156	3.1	0.002	(0.056 – 0.256)	0.135	2.8	0.005	(0.042 – 0.228)	0.159	3.6	<0.001	(0.074 – 0.245)	0.067	3.8	<0.001	(0.032 – 0.102)
Smoking	Non-smoker	Ref.				Ref.				Ref.				Ref.			
	Smoker	-0.247	-2.7	0.006	(-0.423 – -0.070)	-0.259	-3.1	0.002	(-0.424 – -0.094)	-0.247	-3.2	0.001	(-0.399 – -0.095)	0.014	0.5	0.650	(-0.047 – 0.076)
	Ex-smoker	-0.027	-0.5	0.607	(-0.132 – 0.077)	-0.049	-1.0	0.324	(-0.147 – 0.049)	-0.023	-0.5	0.622	(-0.113 – 0.067)	0.048	2.6	0.010	(0.012 – 0.085)

Abbreviations: CI, confidence interval; Coef., regression coefficient; p, p-value; t, t statistic.

Table 5 Average daily portions and phytoestrogen intake consumed for the different food groups by age tertile.

	Age tertile		
	1	**2**	**3**
Age (years) mean	43.4 ± 4.50	54.0 ± 2.80	64.2 ± 3.90
range	27.0 – 49.5	49.5 – 58.7	58.7 – 75.3
Food Groups	**Mean number of portions per day ± SD**		
Meat	1.22 ± 1.13	1.08 ± 0.63	1.17 ± 0.75
Fish	0.36 ± 0.28	0.35 ± 0.28	0.39 ± 0.27
Bread and savoury biscuits	1.92 ± 1.40	2.11 ± 1.62	2.18 ± 1.56
Cereals	0.69 ± 0.56	0.71 ± 0.52	0.78 ± 0.64
Potatoes, rice and pasta	1.27 ± 0.49	1.14 ± 0.48	1.13 ± 0.48
Dairy products and fats	3.26 ± 1.97	3.18 ± 1.66	3.31 ± 1.87
Sweets and snacks	3.78 ± 3.54	3.20 ± 2.97	3.09 ± 3.00
Soups, sauces and spreads	1.16 ± 0.88	1.21 ± 1.02	1.27 ± 0.95
Non-alcoholic drinks	6.13 ± 2.54	6.17 ± 2.49	5.94 ± 2.49
Alcoholic drinks	0.84 ± 1.04	0.88 ± 1.13	0.78 ± 0.99
Fruit	2.50 ± 2.12	3.03 ± 2.41	3.26 ± 2.61
Vegetables	4.36 ± 2.15	4.65 ± 2.27	4.82 ± 2.38
Beans, lentils, nuts and peas	0.34 ± 0.40	0.36 ± 0.44	0.31 ± 0.39
Tofu, soya meat, TVP and vegeburgers	0.03 ± 0.12	0.03 ± 0.11	0.01 ± 0.05
Phytoestrogen consumption µg/d	**Geometric mean (95% CI)**	**Geometric mean (95% CI)**	**Geometric mean (95% CI)**
Total isoflavones	522.1 (480.1 – 567.9)	502.2 (460.0 – 548.2)	433.1 (404.1 – 464.2)
Total lignans	309.2 (299.6 – 319.1)	330.2 (321.0 – 339.7)	329.0 (320.3 – 337.9)
Total phytoestrogens	957.3 (897.8 – 1020.7)	966.3 (905.2 – 1031.6)	853.4 (811.3 – 897.7)

Abbreviations: CI, confidence intervals; SD, standard deviation; TVP, textured vegetable protein.

Figure

Figures

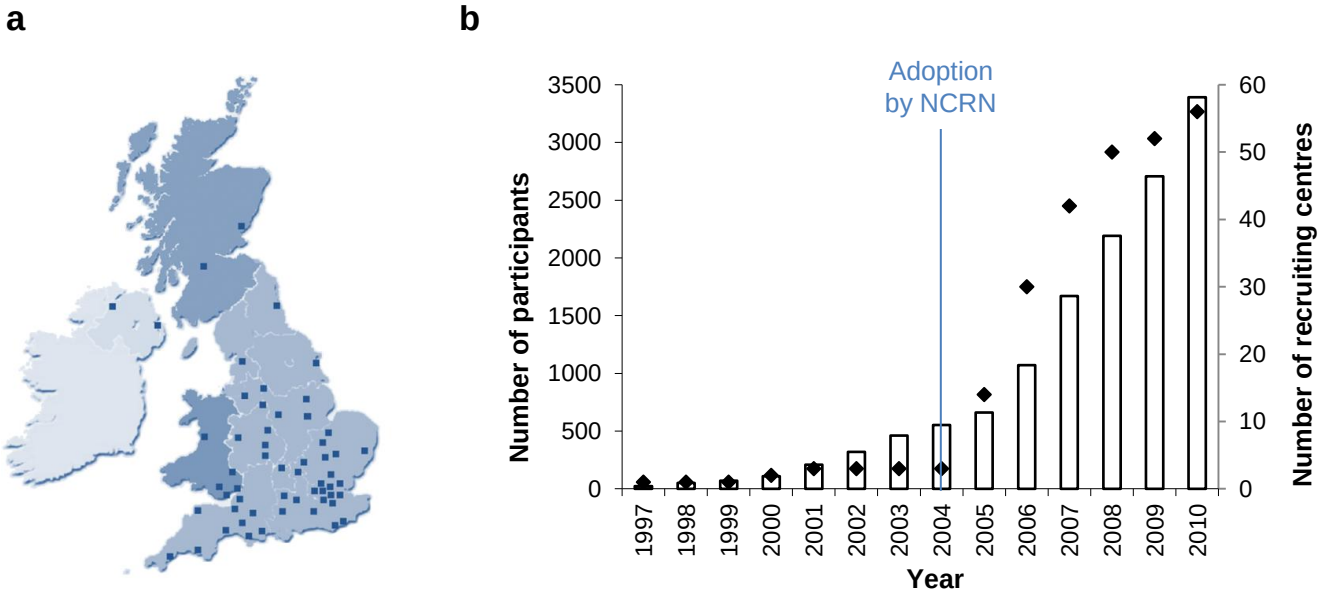
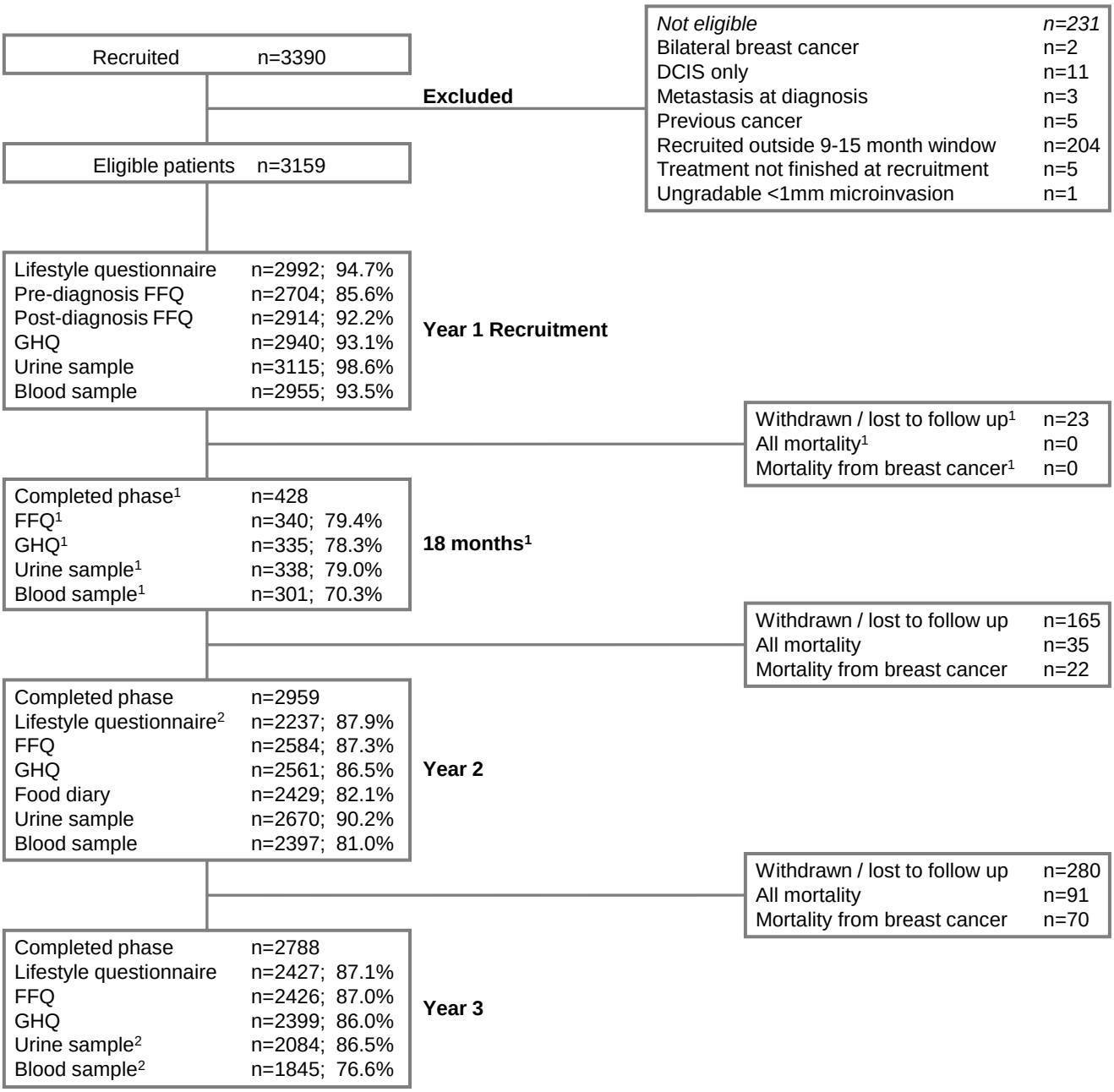


Fig. 1



[1]Recruitment 1998-2004, [2]recruitment 2004-2010. Percentages are from active patients at each stage. Data is shown up to for all completed visits (up to year 3). Follow-up is still on-going.

Fig. 2

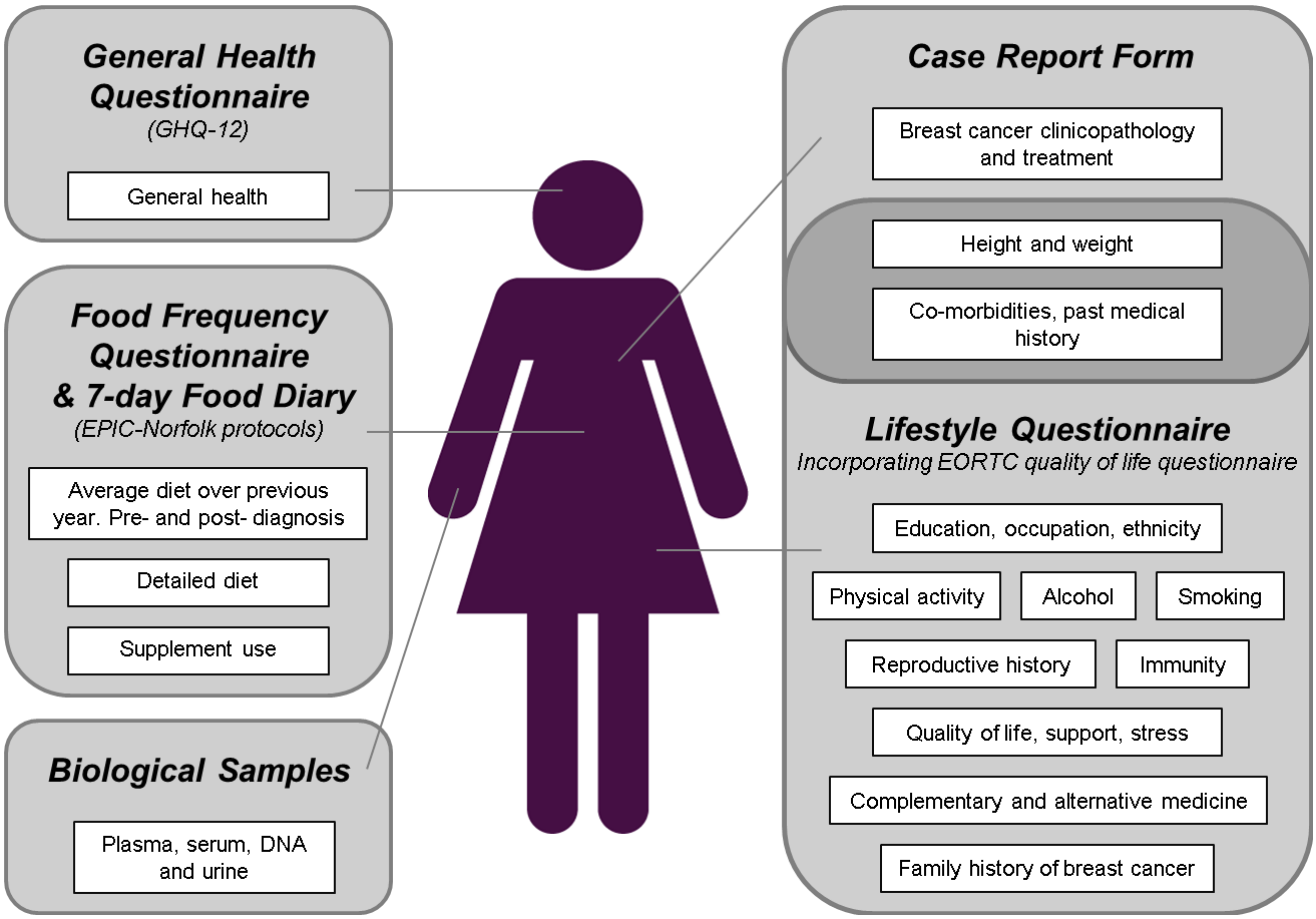

General Health Questionnaire
(GHQ-12)
General health
Food Frequency Questionnaire & 7-day Food Diary
(EPIC-Norfolk protocols)
Average diet over previous year. Pre- and post- diagnosis
Detailed diet
Supplement use
Biological Samples
Plasma, serum, DNA and urine
Case Report Form
Breast cancer clinicopathology and treatment
Height and weight
Co-morbidities, past medical history
Lifestyle Questionnaire
Incorporating EORTC quality of life questionnaire
Education, occupation, ethnicity
Physical activity
Alcohol
Smoking
Reproductive history
Immunity
Quality of life, support, stress
Complementary and alternative medicine
Family history of breast cancer

Fig. 3

Supplementary Tables

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