A RETROSPECTIVE STUDY EXPLORING CLINICAL, BIOLOGICAL AND BEHAVIOURAL FACTORS CONTRIBUTING TO CHANGES IN BODY WEIGHT FOLLOWING EARLY BREAST CANCER DIAGNOSIS

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Copyright Statement

Abstract

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Title: A retrospective study exploring clinical, biological and behavioural factors contributing to changes in body weight following early breast cancer diagnosis.

Background

Changes in body weight and adiposity levels can follow breast cancer (BC) diagnosis and treatment, which could result in poor long-term BC outcomes. The factors associated with these changes and the biological mechanisms underlying them remain unclear.

Aims, methods, sample and data analysis

A longitudinal retrospective study was conducted to explore weight change post-diagnosis and its association with BC treatment, disease characteristics and biological and behavioural factors. A second aim was to examine factors associated with adiposity and metabolic parameters measured at the end of the follow up. 239 women diagnosed with BC one to seven years prior to study entry attending a specialist BC centre were recruited. Weight from BC diagnosis to the study entry, BC treatments received, disease characteristics, menopausal status, smoking status and age were collected from medical notes. Genetic profile (FTO, Mc4R), body fat and fat free mass, waist circumference and fasting glucose and insulin levels were measured once, at study entry only. Associations were examined using t-tests, correlations, regression and multilevel modelling.

Results

Participants were followed for a mean of 47.46 months (SD: 20.45).

Average weight change across the sample from diagnosis to 12, 24, 36 and 48 months post-diagnosis was + 1.30 kg, + 0.85 kg, + 1.59 kg and + 0.42 kg

respectively (increased mean weight). At 12 months, 61% gained weight, 32% lost weight and 7% remained the same. Nearly all participants had weight changes at 24, 36 and 48 months post-diagnosis. Based on evidence, large weight changes (over 10% of weight at diagnosis) were considered clinically relevant. At 24 months post-diagnosis, 6% of participants had an important decrease of weight, 8% had an important weight gain and 86% of participants had no potentially relevant weight change (less than 10% weight change). Notably, 62.9% of the sample was overweight or obese (BMI larger than 25 kg/m^2) at the time of diagnosis.

Chemotherapy was not associated with statistically significant weight change post-diagnosis. Tamoxifen contributed significantly to weight change at 24 and 36 months, whereas smoking status and weight at diagnosis were significant predictors of weight change in the first 12 months of diagnosis. Time-invariant multilevel models fitted showed differences in rate of weight change across women. The differences were predicted by tamoxifen used. Moreover, time-varying models indicated that weight post-diagnosis was larger among tamoxifen users compared to non-users (estimated magnitude of the difference 1.09 kg, 95% CI: -1.63 to -0.56, $p<0.01$) and among anastrozole users (difference 0.85 kg, 95% CI: -1.48 to -0.21, $p<0.01$).

Body adiposity parameters were significantly associated with larger body weight and older age at the time of diagnosis (both $p<0.01$), whereas glucose and insulin levels were predicted by higher weight at diagnosis ($p=0.05$ and $p<0.01$) and the presence of the risky A-allele of the FTO gene ($p=0.03$ and $p=0.01$ respectively).

Conclusions

Findings confirmed that on average weight increased post BC diagnosis. The magnitude of the changes in body weight and the levels of body fat and waist circumference found among participants could compromise their BC prognosis and increase risk of obesity-related diseases, such as type-two diabetes. Understanding the factors associated with weight changes or adiposity and metabolic levels can help health professionals to identify patients at risk. The study has found that hormone therapy contributed to differences in weight and rate of weight change post-diagnosis and that weight at the time of BC diagnosis was a predictor of body adiposity and

metabolic parameters at the end of the follow up. Nonetheless the effects of other variables (i.e. smoking status, genetic profile and other variables not included in this study) cannot be ruled out. Because of the potential negative effects that large body weight at diagnosis, weight changes post diagnosis, excessive adiposity and high insulin levels have, it is imperative to assess those parameters following BC diagnosis and to implement care plans to control them and help newly diagnosed BC patients to achieve optimal weight, body adiposity parameters and a healthy metabolic status.

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Abbreviation List

Abbreviation	Meaning
ABCPP	After Breast Cancer Pooling Project
ABCSG-6	Austrian Breast and Colorectal Cancer Study Group Trial 6
AC	Doxorubicin and Cyclophosphamide
AINs	Aromatase Inhibitors
ANOVA	Analysis of the Variance
ARC	Arcuate Nucleus
AUC	Area Under the Receiver Operating Characteristic Curve
B	Regression beta Coefficient
ATAC	The Arimidex, Tamoxifen Alone or in Combination Trial
BC	Breast Cancer
BRCA	Tumour Suppressor Gene
BIA	Bioelectrical Impedance Analysis
BIC	Schwarz's Bayesian Criterion
BMI	Body Mass Index
C	Degrees Celsius
CFA	Cyclophosphamide, 5-fluorouacil and Doxorubicin
CI	Confidence Intervals
Cm	Centimetres

CT	Computed Tomography
CMF	Cyclophosphamide, Methotrexate and 5-fluorouacil
d.f.	Degrees of Freedom
DNA	Deoxyribonucleic Acid
DXA	Dual Energy X-ray Absorptiometry
EC	Cyclophosphamide and Epirubicin
ER	Oestrogen Receptors
Et al.	And Others
Etc.	Et Cetera
ERK1/2	Extracellular Signal-regulated Kinases
F	F-statistic resulting from the F-Test ANOVA Test.
FEC	Fluorouracil, Cyclophosphamide and Epirubicin
FM	Fat Mass
FFM	Fat Free Mass
FTO	Fat Mass and Obesity-associated Gene
HbA1c	Glycated Haemoglobin
HEAL	Health, Eating, Activity and Lifestyle Study
HER-2	Human Epidermal Growth Factor Receptor-2
HOMA	Homeostasis Model Assessment

HOMA IS	Homeostasis Model Assessment of Insulin Sensitivity
HOMA IR	Homeostasis Model Assessment of Insulin Resistance
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
IBIS	International Breast Cancer Intervention Study
IBM	International Business Machines
I.e.	In Example
IGF	Insulin Growth Factor
IR	Insulin Resistance
Kg	Kilogram
LACE	Life After Cancer Epidemiology Study
MAPKs	Mitogen-activated Protein Kinase
MAP4K4	Mitogen-activated Protein Kinase Kinase Kinase Kinase Four
MAR	Missing at Random
Mc4R	Melanocortin-4 Receptor
ML	Maximum Likelihood
Mmol/l	Milimol per Litre
MPOA	Medial Preoptic Area
NHS	National Health System
OR	Odds Ratio
PI3K/Akt	Phosphatidylinosito-3-kinase

PhD	Doctor of Philosophy
Pmol/l	Picomole per Litre
PVN	Paraventricular Nuclei
Q-Q plot	Quantile-quantile Plot
r	Pearson's Product Moment Correlation Coefficient
R	Multiple Correlation Coefficient
R2	Coefficient of Determination
R&D	Research and Development Department
REC	Research Ethics Committee
REE	Resting Energy Expenditure
RR	Risk Ratio
SBCSS	Shanghai Breast Cancer Survival Study
SD	Standard Deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
U	Statistic resulting from the Mann-Whitney U test
UK	United Kingdom
USA	United States of America
VMH	Ventral Medial Hypothalamus
Vs.	Versus

WHEL	Women's Healthy Eating and Living Trial
WHR	Waist to Hip Ratio
$\chi 2$	Chi Squared Value for the Kruskal Wallis Test
Z	Statistic resulting from the Mann-Whitney U test

CHAPTER 1: INTRODUCTION TO BREAST CANCER AND WEIGHT CHANGE

1 Introduction

It is not uncommon that women diagnosed with breast cancer (BC) will experience changes in weight and body composition (i.e. increases in body fat). These changes have been reported for many years and are the focus of on-going investigations to understand the causes.

The first chapter of this dissertation offers the reader a brief introduction to general aspects of BC, such as epidemiology, diagnosis and current treatments offered in the United Kingdom (UK). The attention then moves to the focus of the study: weight change after BC diagnosis, exploring how common it is and its magnitude. The chapter concludes with an exposition of current knowledge about the impact that this common and undesired phenomena may have on women's health. In particular, its effects on BC recurrence and survival. This highlights the importance of understanding the factors associated with changes in body weight and the underlying mechanisms, to ultimately identify women at risk and to develop interventions to help them to avoid potentially harmful changes in body weight following BC diagnosis.

1.1 Breast cancer epidemiology

Statistics from 2012 show that BC was the most common cancer affecting women in the world and constituting the first cause of cancer death in women in both developed and in developing regions (World Cancer Research Fund International 2014). In the UK, incidences have increased since the mid-1970s up to mid-2000s. In 2011, 50,285 new cases of BC were diagnosed, 99% of them in women,

representing a female/male ratio of 43/1; it is expected that one in eight women will develop BC during their lifetime (Cancer Research UK 2014b).

The aetiology of BC is not completely understood and therefore, it is difficult to explain the BC incidence trends over these decades (World Health Organization Regional Office for the Eastern Mediterranean 2006). Biological (i.e. ageing, increased adiposity), behavioural [alcohol intake, use of hormone replacement therapy (HRT), or oral contraceptives] and genetic factors [mutations in tumour suppressor genes one and two (BRCA1 and BRCA2)] can increase the risk of BC (Cancer Research UK 2014d). Therefore, changes in incidence or prevalence of those factors over the past few decades might provide plausible explanations for the growing incidence of BC (Kumle et al. 2002). On the other hand, survival rates have improved dramatically, probably due to improvements in BC treatment and use of the BC screening programme. Currently, around 90% of women will survive for at least five years after diagnosis and 80% are expected to survive for more than ten years. Nonetheless, cancer can recur, most likely within the first two years of diagnosis, although it can happen even ten or 20 years after first diagnosis (Cancer Research UK 2014f).

1.2 Types of breast disorders and diagnostic tests

BC can be classified in non-invasive (ductal or lobular carcinoma in situ) and invasive carcinoma [National Health System (NHS) 2014a; Cancer Research UK 2015]. Ductal carcinoma is the most common malignant invasive carcinoma and accounts for around 80% of diagnosed BCs (National Health System 2014a). Other less common types of BC are inflammatory BC, Paget's disease and finally those included under the broad name of "special type of BC" (Cancer Research UK 2015).

Currently, the BC diagnostic journey includes a triple assessment involving a clinical examination, imaging tests and a biopsy (National Institute for Health and Care Excellence (NICE) 2014). The results of these tests will determine how aggressive the cancer is (grade from 1-3) and will provide information about tumour size and

whether it has spread (cancer stage) (Breakthrough breast cancer 2013; NHS 2014b). The two methods used to describe staging are the TNM staging system, which looks at tumour size (T), whether the tumour has spread to the lymph nodes (N), or whether the tumour has metastasised (M) (Cancer Research Uk 2014). The second method is the number system, which classifies cancer into stages cero to four (Cancer Research UK 2014e). Early BC comprises stages I, II and III, whereas stage IV denotes advanced BC (National Collaborating Centre for Cancer 2009). In addition to grade and stage, clinically important subsets of BC can be identified according to the expression of two main types of receptors: oestrogen receptor (ER) and human epidermal growth factor receptor-2 (HER-2) (Burstein 2011). ER and HER-2 are growth factor receptors (Cancer Research UK 2014g; NHS 2014b), therefore, upon ligand binding they are able to transmit signals from outside the cell to the inside, promoting tumour cells. HER-2 is a tyrosine kinase receptor involved in the proliferation and survival of BC cells, via receptor activation of different signalling pathways, including the phosphatidylinosito-3-kinase (PI3K/Akt), or the mitogen-activated protein kinase (MAPKs) signalling pathways (Roy and Perez 2009). The level of HER-2 protein present in the tumour can be graded between cero and three positive (Cancer Research UK 2014g), an its overexpression has been associated with a more adverse prognosis (Roy and Perez 2009).

On the other hand, ER belongs to the family of nuclear receptors and mediates the effects of oestrogens. A tumour that has ER is classified as ER positive. ERs are activated upon ligand binding, leading to transcriptional activity, which can be promoted by coactivators, or by contrast, can be suppressed by corepressors. Furthermore, ERs' role on breast carcinogenesis and BC progression can mediated by the interaction with other growth factors and their downstream signalling proteins, as well as by the interaction between signalling pathways emerging from ERs and other growth factors receptor pathways (i.e. the PI3K/Akt, or the MAPKs) (Murphy et al. 2003).

ERs are present in two thirds of BCs (ER-positive cancers), whereas HER-2 is found in about 25% of breast tumours (Cancer Research UK 2014g). The tumour expression of these receptors shows a differential response to different types of drugs

(Hsu and Hung, 2016), hence the presence of those receptors in the breast tumour will help doctors to determine the BC treatment for each individual.

1.3 Breast cancer treatments

BC treatment is largely determined by cancer characteristics (Cancer Research Uk 2014), although other factors including age, menopausal status, potential benefits, acceptability of the diagnosis and treatment, as well as patients' needs and preferences should also be accounted (National Collaborating Centre for Cancer 2009).

The main treatments currently offered in the UK for early BC are surgery, radiotherapy, chemotherapy, hormone therapy and biological therapy, singularly or in combination (NHS 2014c). Local treatments (surgery and radiotherapy) aim to remove the tumour from the breast and axilla, whereas systemic treatment (chemotherapy, hormone therapy and biological therapy) reach the blood systems to prevent the growth of microscopic tumour cells that might have remained in the tissue or the spread of breast tumour to other parts of the body (Breakthrough breast cancer 2013). Mastectomy and breast conserving surgery are the two types of surgery used for BC (Breakthrough breast cancer 2013; Cancer Research UK 2014g). Currently, it is recommended that further treatment with adjuvant chemotherapy or radiotherapy should start within 31 days of completion of surgery (NICE 2009), although in some cases, systemic therapy can also be offered before surgery (neo-adjuvant therapy) in order to reduce the size of the tumour so the subsequent surgery is less extensive (National Collaborating Centre for Cancer 2009).

Chemotherapy uses one or a combination of different cytotoxic agents that disrupt the growth of tumour cells, by killing dividing cells. Consequently, in addition to tumour cells, it also affects cells in healthy tissues where the cells are constantly growing and dividing, like the skin, bone marrow, hair follicles or the digestive system (Cancer Research UK 2014a). That explains the numerous side effects of

different cytotoxic agents (Breakthrough breast cancer 2013), including anaemia, neutropenia, fatigue, nausea and vomiting, loss of appetite, sore mouth or mouth ulcers (Cancer Research UK 2014a). Chemotherapy can also have endocrine effects, by interrupting ovarian oestrogen production, which leads to a temporary or permanent infertility accompanied by amenorrhea (Zhao et al. 2014) and other menopausal symptoms (i.e. hot flushes or sleep disturbances) (Cancer Research UK 2014c; Zhao et al. 2014). Currently, NICE recommends taxane-based chemotherapy regimens that include docetaxel (NICE 2009). The lengh of treatment depends on the agents and the number of cycles prescribed (usually four to eight cycles), which are usually administered at intervals of three to four weeks (Cancer Research UK 2014a).

Hormone therapy aims to reduce the availability of endogenous oestrogen to tumour cells (Goel et al. 2009), as it is well known the ability of oestrogen to stimulate BC cells growth (Jonat 2001; Folkerd and Dowsett 2013). It is therefore, the mainstay treatment for ER positive tumours (Burstein 2011) and it is recommended for about five years (Cancer research uk 2014). One variant of hormone therapy acts on ER, aiming to block the activation of oestrogen-stimulated signalling pathways in the cells. Tamoxifen is a drug that does that. It competes with oestrogens and binds and phosphorylates ER in breast tumour cells (Burstein 2011). A second way to reduce oestrogen availability is by stopping oestrogen production in the tissues synthesising oestrogen (i.e. ovaries in premenopausal women and adipose tissue). In premenopausal women, this can be achieved mainly via surgical or chemical ovarian ablation (Burstein 2011). Chemical agents to reduce oestrogen levels are gonadorelin analogues, such as goserelin, an injectable gonadotropin releasing hormone supeagonist (Cancer research uk 2014), which is able to suppress circulating oestrogen to postmenopausal levels (Jonat 2001). On the other hand, in postmenopausal women, oestrogen synthesis can be reduced with the use of aromatase inhibitors (AINs), which block the action of aromatase, a crucial enzyme in the process of oestrogen production in the adipose tissue, the main source of oestrogen in postmenopausal women. Three AINs used to treat early BC are: exemestane, anastrozole and letrozole (Burstein 2011). At present, AINs are used

singularly or in combination with tamoxifen to treat postmenopausal women with ER positive tumours (NICE 2009; Burstein 2011).

Finally, biological therapy with trastuzumab is the standard treatment for patients with HER-2 positive invasive BC (National Collaborating Centre for Cancer 2009), as it targets the HER-2 signalling pathway in BC cells (Moja et al. 2012). Currently, it is administered every three weeks for one year or until tumour recurrence (European Medicines Agency 2008).

1.4 Problem: weight change after breast cancer diagnosis

Weight change, in particular weight gain, following BC diagnosis is an undesired phenomenon documented in several papers with large and small sample sizes and with short and long follow ups, from all around the world, including Australia (Vagenas et al. 2015), Brasil (Costa et al. 2002), Canada (Kutynec et al. 1999), China (Chen et al. 2011), France (Tredan et al. 2010), Italy (Del Rio et al. 2002), the Netherlands (Heideman et al. 2009), UK (Lankester et al. 2002; Harvie et al. 2004; Sestak et al. 2012) and United States of America (USA) (Camoriano et al. 1999; McIness and Knobf 2001; Nissen et al. 2011; Reddy et al. 2013; Saquib et al. 2007) (Table 1, Appendix I).

In the general population, changes in weight during adulthood are not unusual (Williamson et al. 1990) (more details in Chapter 2). Nonetheless, although weight change can occur in absence of BC diagnosis, the amount of weight change observed after BC diagnosis seems to be larger than what would be expected in the health population (Vance et al. 2011). This is a worrying finding because there is evidence of a link between weight change post BC diagnosis and poorer BC outcomes, as it is explained below in this chapter.

The following lines present a summary of findings from previous studies reporting weight changes following BC diagnosis. Table 1 (Appendix I) contains more details of the characteristics of these studies (i.e. length of follow up, sample size, frequency and magnitude of weight change and factors related to it).

1.4.1 Magnitude of weight change after breast cancer diagnosis

Most studies in the field have shown changes in weight following BC diagnosis. Mean weight changes from + 1.6 kilograms (kg) to + 5.0 kg have been documented across the samples within the first year of diagnosis (Goodwin et al. 1999; Demark-Wahnefried et al. 2001; McInnes and Knobf 2001; Harvie et al. 2004; Kumar et al. 2004; Makari-Judson et al. 2007; Heideman et al. 2009; Basaran et al. 2011; Chen et al. 2011) (Table 1, Appendix I). Post-diagnosis weight changes in the long term have been less frequently explored, but evidence indicates weight changes are also present. For instance, mean weight changes of + 1.7 kg or even + 2.4 kg within the first two and three years' post-diagnosis have been reported (Irwin et al. 2005; Kroenke et al. 2005; Caan et al. 2006, 2008; Chen et al. 2011; Caan et al. 2012b).

1.4.2 Frequency of weight change after breast cancer diagnosis

Most research has reported positive weight changes following BC diagnosis. Nonetheless, their findings also revealed that some women maintain their pre-diagnosis weight, or in other words, do not experience weight changes, whereas others lose weight post diagnosis. Caan et al. (2012b) published data from nearly 13,000 BC survivors included in the After Breast Cancer Pooling Project (ABCPP), a large international collaboration of four prospective cohort studies from USA and from China: The Shanghai Breast Cancer Survival Study (SBCSS), the Life After Cancer Epidemiology (LACE), the Women's Healthy Eating and Living (WHEL) and the Nurses' Health Study. Findings showed that two years post-diagnosis, 34.7% of the participants gained weight (more than 5% of initial body weight) and 14.7% lost weight. The remaining participants maintained their weight (changes within 5% of their initial body weight) (Caan et al. 2012b). These figures are consistent with several studies that reported that 22% to 37.7% of all participants experienced weight gain and 14.96% to 27.1% participants lost weight post-diagnosis (Rock et al. 1999; Kroenke et al. 2005; Caan et al. 2006, 2008; Gu et al. 2010; Chen et al. 2011; Nissen et al. 2011; Bradshaw et al. 2012; Caan et al. 2012b).

It is worth noting that the incidence of weight change post diagnosis depends on the categorization of weight change. Several studies have defined weight change as changes larger than 5% of baseline weight (Han et al. 2009; Nissen et al. 2011; Bradshaw et al. 2012, Caan et al. 2012b). Unfortunately, most of these studies did not report mean weight change within the 'weight stable' (or not weight change) category and it is possible that other studies might have been identified that unreported magnitude of mean weight change as weight gain or weight loss, depending on the direction of the change. For instance, previous published data from the SBCSS (Gu et al. 2010; Chen et al. 2011), the Arimidex, Tamoxifen Alone or in Combination trial (ATAC) (Sestak et al. 2012) and the LACE (Wayne et al. 2014) defined weight change those changes larger than 2 kg.

Despite the methodological differences in the definition of weight change, most studies showed changes in weight across their sample. Nonetheless, it has being acknowledged that the magnitude of weight change post BC diagnosis seems to have decreased since the mid 1990s (Vance et al. 2011; Makari-Judson et al. 2014). Unfortunately, this post-diagnosis weight change still seems to be larger than what would be expected in the general population in the same period of time (Vance et al. 2011). For instance, a study demonstrated that 50% of BC patients put on weight and 80% of them gained more weight than the average weight gain observed in a healthy age-matched sample (Vagenas et al. 2015). Furthermore, larger weight change (on average a positive weight change) was reporded in a study with women treated with BC compared to women at risk of BC (Sestak et al. 2012).

As noted above, the exact magnitude of, and the frequency of weight change are difficult to ascertain due to differences in the way weight change was analysed (i.e. definition of weight change, relative and absolute terms, weight change as a continuous variable or as a categorical variable) and the timing of the follow up measurements. Furthermore, the starting point, or baseline weight, has been measured at different times, which ranges from one or two years before diagnosis (i.e. Kroenke et al. 2005; Caan et al. 2006; Bradshaw et al. 2012), to six months post diagnosis (Irwin et al. 2005) (Table 1, Appendix I). For instance, Bradshaw et al. (2012) explored weight change from one year before diagnosis to a year post diagnosis in a sample of 1,436 women diagnosed with early BC. Overall weight

change was not reported. Instead, it was categorised, and findings showed that most participants (60%) maintained weight (± 5% of baseline weight), whereas 26% lost weight (more than 5%), and 14% gained weight (more than 5%). On the other hand, Irwin et al (2005) investigated changes in body fat and body weight in 514 breast cancer survivors enrolled onto the Health, Eating, Activity and Lifestyle (HEAL) study, a population based prospective cohort study conducted to determine whether weight, physical activity, diet, sex hormones, and other exposures affect breast cancer prognosis. Baseline weight was recorded an average of six months from diagnosis. Mean weight change two years post-baseline weight measurement was + 1.7 kg [Standard deviation (SD): 4.7 kg], with 68% of the sample gaining weight (mean weight gain + 3.9 kg, SD: 3.7 kg). In a previous study, Caan and colleagues (2006) published data from 3,215 BC survivors enrolled either in the LACE, or in the comparison group of the WHEL, a dietary intervention trial to prevent breast cancer recurrence. Similar to Bradshaw et al. (2012), baseline weight was measured a year before diagnosis. Overall change in body weight two years post diagnosis was +2.4 kg (SD: 7.5). The percentage of participants that gained weight (more than 5%), maintained their weight (± 5% of baseline weight), and lost weight (more than 5%), was 39.6%, 45.4% and 14.9% respectively. The authors noted that weight gains were of smaller magnitude than other studies, and discussed that participants taking part in the WHEL study might have already change their eating habits. As Caan and colleagues (2012b) note, the characteristics of the sample could influence the magnitude and frequency of weight changes, if for instance, the prevalence of factors that potentially influence weight change (i.e. eating habits, physical activity, chemotherapy use, or menopausal status, more detailed in Chapter 2) is larger in some studies compared to others.

1.4.3 Trajectory of weight change after breast cancer diagnosis

At the earlier stages of this project, most of the studies reported changes in body weight between two evaluation points: around diagnosis and at the end of follow up (Rock et al. 1999; Wayne et al. 2004; Irwin et al. 2005; Kroenke et al. 2005). This provided limited information on what happened in between. Only the study

conducted by McInness and Knob (2001) presented weight change at different points in between, and noted after two and three years post-diagnosis, 68% and 40% of those who gained weight during the first year maintained a significant weight gain. This finding is consistent with more recent reports which indicate that the average increase in weight peaks and that some breast cancer survivors are able to reverse the weight gained. For instance, Gu et al. (2010) found significant weight change from diagnosis to six months (+1.0 kg), 18 months (+2.0 kg) and 36 months (+1.0 kg). Makari-Judson et al. (2007) found average weight changes of +1.5 kg and +2.7 kg from diagnosis to the first and second year post-diagnosis respectively, and an average weight increase of + 0.1 kg from the second to the third year post-diagnosis, suggesting that weight change stabilises three years post-diagnosis. Findings also revealed that 15% and 20% of those who gained weight during the first year of diagnosis lost it by year two and three respectively. Data from the WHEL trial study showed that 10% of those who gained weight returned to their pre-diagnosis weight nearly two years later (Saquib et al. 2007). Recently, a study reported that body weight of Taiwanese women diagnosed with BC experienced a cubic trajectory post-diagnosis. Their body weight was a mean of 56.9 kg before chemotherapy, it increased to a mean of 59.4 kg at eight months after chemotherapy, and decreased to 58.5 kg at 21.5 months. Finally, body weight slightly increased in the last two months. Interestingly, body weight never returned to the initial level (Liu et al. 2014).

For most women, the journey following BC diagnosis is characterized by multiple phases: surgery, radiotherapy, adjuvant therapy. It is possible that these therapeutic periods have a different impact on the factors that might contribute to weight changes observed post-diagnosis. For instance, the common fatigue, nausea and vomiting side effects accompanying chemotherapy treatment (Cancer Research UK 2014a) could reduce physical activity and modify eating behaviours during the length of treatment, which could ultimately, have an impact on body weight (more details in Chapter 2). The emerging literature reporting fluctuations in weight change following diagnosis highlight the need of studies to investigate the trajectory of weight change in the long term and to identify factors that might contribute to different patterns of weight change.

1.4.4 Changes in body adiposity after breast cancer diagnosis

Two large cohort studies have reported individual variations in body adiposity levels following BC diagnosis. Data from the HEAL study showed that in addition to the + 1.7 kg weight increase observed across the sample (see previous paragraphs), there was a rise in fat mass (FM) percentage (mean + 2.1%, SD: 3.9%), which seemed to be negatively correlated with both body mass index (BMI) (p for trend 0.050) and age (p for trend: 0.060). Most participants (74%) gained FM (Irwin et al. 2005). Arpino et al. (2015) documented changes in waist and hip circumference (both are indicators of body fat) in a sample of patients receiving adjuvant therapy for BC. Over a median follow-up of 14 months, there were increases in weight and waist circumference (+ 0.72 kg/year, 95 % CI: 0.32 to 1.11 and +1.53 cm/year, 95 % CI: 0.85 to 2.22 respectively). Hip circumference levels only increased among participants treated with chemotherapy (+ 3.16 cm/year, 95 % CI: 1.60 to 4.73).

Interestingly, changes in body fat levels in the absence of weight gain among women treated for BC have been reported by at least two studies (Kutynec et al. 1999; Freedman et al. 2001). Kutynec et al. (1999) explored prospective changes in weight and body FM, and in non-bone fat free tissue, or fat free mass (FFM), in a sample of 18 women treated with either chemotherapy or radiotherapy (n=10). The magnitude of body weight from initiation of adjuvant therapy to 12 weeks later did not change. Nonetheless, in both groups there were significant increases in FM percentage (mean + 1.3%, p=0.04) and a significant decrease in FFM (mean -0.4kg, p=0.02). Freedman et al. (2001) compared weight change in a sample of 20 women receiving chemotherapy for BC and a group of 51 healthy controls, and could not find significant changes in weight, in either of the groups at the end of chemotherapy or six months later. Nonetheless, the group treated for BC experienced an increase in the percentage of FM and a reduction of FFM.

This phenomenon of gaining FM and losing FFM is consistent with sarcopenia (Demark-Wahnefried et al. 2001), and has been reported by several other small studies (Cheney et al. 1997; Demark-Wahnefried et al. 1997a; Demark-Wahnefried et al. 2001; Freedman et al. 2004; Harvie et al. 2004; Nissen et al. 2011). Nonetheless, gains in lean body mass have also been reported (Del Rio et al. 2002;

Genton et al. 2006). Villasenor et al. (2012) explored the prevalence of sarcopenia, assessed by lean mass in arms and legs relative to height, and the association with overall and BC cancer-specific mortality in 471 participants in the HEAL study. Results indicated that 16 % of the sample were sarcopenic, and 38 % of these women with sarcopenia were classified as obese (total body fat percentage ≥38 %) and 62 % as not obese (<38 %).

The repercussions of abnormal levels of FM or FFM are described later in the chapter.

1.4.5 Metabolic status after breast cancer diagnosis

Post-diagnosis changes in weight and body fat levels can predispose women to develop chronic illnesses commonly associated with obesity such as diabetes or cardiovascular disease (Vagenas et al. 2015).

At the planning stages of this study, little data was available on the metabolic status of women treated for BC. In 2009, Makari-Judson et al. published data on body weight, waist and circumferences, insulin and glucose levels in a sample of 95 women diagnosed with BC. Measurements were collected before adjuvant therapy, and at six and 12 months later. At six months, waist measurement and waist to hip ratio were unchanged. However, the Homeostasis Model Assessment (HOMA) of insulin resistance (IR) (HOMA IR) and glucose/insulin ratio tended to increase ($p=0.06$ and $p=0.05$ respectively) across the sample. A cross-sectional study with data collected a mean of 44.8 months post BC diagnosis showed that glucose, insulin and glycoslyated haemoglobin levels were all at the upper end of the normal range (Thomson et al. 2009). Worth noting, all participants (n=42) were overweight (BMI more than 24.9 kg/m^2). Two further studies were also published that year looking at the effect of BC treatments on glucose levels and on IR. A local study carried out by Hickish et al. (2009) measured changes in blood glucose levels with each cycle of chemotherapy in 39 non-diabetic women diagnosed with BC. Glucose levels increased as the chemotherapy cycles progressed, and an increasing number of

participants developed degrees of glucose intolerance. Results from paired t-tests showed statistically significant increases in blood glucose levels with later cycles among women who received the higher dose of dexamethasone in combination with docetaxel (cycle 5: $p < .001$ and cycle 6: $p = 0.002$). Later on, in 2012, Erickson et al. analysed data from the WHEL study examining the association between weight change (from around one year pre-diagnosis to two years post-diagnosis) and incident diabetes over six year follow up, in BC survivors (n =1,617). Higher BMI was statistically significantly associated with incident diabetes ($p \leq 0.05$). Overall, 43.8% of women had stable weight post-diagnosis (± 5% of baseline weight), 23.5% of the sample gained more than 10% of baseline weight, 19.1% of participants had a moderate weight gain, and 13.6% of participants lost more than 10% of baseline weight. Both weight loss and major weight gain increased the risk of incident diabetes compared to stable weight ($p < 0.05$ for both). A plausible explanation for the association found regarding weight loss and diabetes was that participants in that category had the highest pre-diagnosis weight (mean BMI= 27.1, SD =5.3 kg/m^2) and returned to that weight by the year six follow-up visit. Finally, in the previously cited cohort study published by Arpino and colleagues (2015), there were no statistically significant changes in glucose and insulin levels over a median follow-up of 14 months, despite the observed increases in body weight and waist circumference levels described earlier on.

1.5 The negative effects of weight change, high adiposity levels and abnormal metabolic status after breast cancer diagnosis

Current evidence suggests that changes in body weight, or FM and FFM levels post BC diagnosis might not benefit women. Slight changes in weight post-diagnosis might not be problematic. However a large magnitude of weight change (in both directions) can have a negative impact on BC prognosis. For instance, each 5 kg/m^2 increment of BMI before 12 months post-diagnosis increased risk of all-cause mortality [RR: 1.08, 95% Confidence interval (CI): 1.01 to1.15] and BC specific mortality (RR: 1.29, 95% CI: 0.97 to 1.72) (Chan et al. 2014). Caan et al. (2012b) found that the risk of death was only associated with substantial weight gain (more

than 10% of baseline weight, mean + 10.5 kg) during the first two years of diagnosis. Kroenke et al. (2005) also found an adverse prognosis among non-smoking women who gained a mean of 6.6 lb (around 3 kg) within the first three years post-diagnosis [risk ratio (RR) of all cause mortality: 1.59, 95% CI: 1.07 to 2.51 and RR of BC recurrence: 1.53, 95% CI: 1.04 to 2.24]. Weight gains of 5% after BC diagnosis have also been associated with worse physical functioning (Imayama et al. 2013).

Similarly, large weight losses (more than 10% of the baseline weight) occurring by two years post BC diagnosis have been associated with increased risk of all cause mortality among women who ever smoked [hazard ratio (HR): 1.58, 95% CI: 1.20 to 2.09], among underweight women with no comorbidities (HR: 2.16, 95% CI: 1.25 to 3.75), and among overweight/obese women with comorbidities (HR: 1.89, 95% CI: 1.25 to 3.75) (Caan et al. 2012b). Weight losses of smaller magnitude might be also negative for health. In Caan and colleagues' study (2012b), a moderate weight loss of 5 to 10% (mean: - 4.9 kg) in normo-weight (BMI: 18.5-24.9kg/m^2) women was associated with an increased risk of all cause mortality. The association was not found among overweight women (BMI more than 24.9 kg/m^2). A previous study found that women who lost more than 1 kg by 18 months post-diagnosis had higher risk of all cause mortality (HR: 2.41, 95% CI: 1.62 to 3.58) and BC recurrence (HR: 1.60, 95% CI: 1.03 to 2.48) (Chen et al. 2010). As Jackson et al. (2017) discusses, it is worth noting that the association between weight loss and mortality could have been the result of methodological limitations. Most studies did not control for tumour stage (so might have included participants with advanced stages of BC), nor for comorbidities. These might be confounding factors that could have biased the association found between weight loss and BC mortality. Interestingly, a further limitation found in most literature exploring the impact of weight changes post BC diagnosis is that they did not distinguish between intentional and disease-related (unintentional) weight loss. This is an important issue as research in non-cancer populations indicates that unintentional weight loss is associated with increased risk of mortality whereas intentional weight loss has an overall neutral effect on survival (Harrington et al. 2009). Moreover, weight loss in a healthy population leads to improvements in biomarkers that are plausibly linked with cancer-related outcomes (Jackson et al. 2017). Therefore, the fact that this finding contrasted with results

from those studies suggesting a detrimental effect of weight loss post BC diagnosis suggests that the weight loss observed among BC survivors in those studies was not intentional (Jackson et al. 2017). Consequently, more research is needed in the area.

Although there is no agreed definition of clinically significant weight change (Paige et al. 2014), the figures above reported suggest that weight changes larger than 10% of the diagnosis weight, or weight gains of more than 3 kg could have clinical relevance. Nonetheless, smaller figures could also affect different subgroups of BC survivors (i.e. normo-weight women).

In addition to the potentially harmful impact of large weight changes post-diagnosis, abundant literature has also underlined the link between high adiposity levels (as seen in obesity) at the time of BC diagnosis, and BC prognosis (Protani et al. 2010; Druesne-Pecollo et al. 2012; Niraula et al. 2012; Jain et al. 2013; Azrad and Demark-Wahnefried 2014; Chan et al. 2014). A systematic review and meta-analysis of 82 studies indicated that a pre-diagnosis BMI of more than 30.0 kg/m^2 was associated with an increased risk of total mortality (RR: 1.41, 95% CI: 1.29 to 1.53) (Chan et al. 2014). Nonetheless, whether obesity affects all biologic subtypes of BC, or whether there is a direct or indirect causal relationship between obesity and BC is unclear (Goodwin 2015b).

Similarly, sarcopenia has been associated with higher risk of developing dose-limiting toxicity in treatment of advanced BC and other cancers (Prado et al. 2009; Antoun et al. 2013). Data from the HEAL study revealed that sarcopenia was associated with an increased risk of overall mortality following BC after adjusting for adiposity (HR: 2.86, 95 % CI: 1.67 to 4.89), suggesting that sarcopenia could be an independent predictor of BC outcomes (Villasenor et al. 2012).

Remaining weight stable post-diagnosis has been associated with the lowest risk of mortality, and hence, avoiding weight change appears to constitute the most favourable target for newly diagnosed BC patients (Caan et al. 2012b). Worth noting, in Caan's et al. (2012b) study, weight stable was defined as weight changes within 5% of the baseline weight. This recommendation is supported by the American Cancer Society, which advises patients to maintain and achieve a healthy

body weight during and after cancer treatment through diet and physical activity (Rock et al. 2012).

The role of insulin in cancer is well documented. Being resistant to the actions of insulin has been described as one of the most significant metabolic disturbances in cancer (Hursting et al. 2007). Pre-diagnosis elevated fasting glucose has been associated with shorter overall survival after BC diagnosis (HR: 3.50, 95% CI: 1.87 to 6.54) (Monzavi-Karbassi et al. 2016). Similarly, higher insulin levels and IR have been associated with adverse BC prognosis, even after adjusting for BMI and other prognosis factors (Goodwin et al. 2002; Borugian et al. 2004; Duggan et al. 2011; Goodwin et al. 2012). Women with fasting insulin in the highest versus (vs.) lowest quartile had a HR of 2.32 (95% CI, 1.39 to 3.86) for distant BC recurrence and a HR of 2.85 (95% CI: 1.48 to 5.50) for death after BC (Goodwin et al. 2012). And a systematic review and meta-analysis concluded that diabetic women diagnosed with BC have an increased risk of all-cause mortality post diagnosis (pooled HR: 1.49, 95% CI: 1.35 to 1.65) (Kimberly et al. 2010). These findings suggest that insulin and glucose levels post BC diagnosis should be taken into consideration.

1.6 Potential mechanisms explaining the association between weight change, obesity and insulin with breast cancer outcomes

The link between obesity and BC outcomes might be non-causal due to numerous facts: First, it is possible that the diagnosis of BC occurs at more advanced stages in obese women. Second, less aggressive treatment might be given to obese patients. And third, obesity might affect the efficacy of hormone therapy (Goodwin 2015a). However, a biological plausible explanation might also exist. Physiological changes arising from a metabolically active adipose tissue can increase serum levels of insulin, oestrogens and other inflammatory substances both locally at the breast and systemically in the whole body (Goodwin 2015b). Insulin itself enhances aromatase activity and oestrogen production in the adipose tissue (Rose et al. 2015), and down-regulates plasma concentrations of sex hormone binding globuline, resulting in an elevation of available bioactive oestrogens (Vance et al. 2011), which altogether, can

activate oestrogen, insulin and insulin growth factor (IGF) signalling pathways related to carcinogenesis, tumour progression, invasion and metastasis (Goodwin 2015b). Supporting this plausible explanation, the previous section in this thesis has shown the growing body of literature providing evidence on the negative effect of high insulin levels on BC (Goodwin et al. 2002; Borugian et al. 2004; Duggan et al. 2011; Goodwin et al. 2012), which is worrying as an increase in body weight and fat levels could rise baseline insulin levels (Makari-Judson et al. 2009).

1.7 Conclusions

BC has usually good prognosis; nonetheless, post-diagnosis weight change is a common phenomenon, which seems to be greater than for women in the general population. Changes in body weight, as well as the variations in body adiposity levels might be accompanied by metabolic disruptions (i.e. elevation of insulin and glucose levels). Increasing evidence is highlighting the fact that both positive and negative weight changes, as well as abnormal adiposity or high insulin and glucose levels could have a negative impact on BC prognosis, and could put women at risk of other comorbidities associated with obesity.

Therefore, since weight change post-diagnosis remains an area of concern for clinicians as well as for patients, the main aim of this study is to explore the magnitude, the frequency and the trajectory of weight change following BC diagnosis. Furthermore, as the next chapter will show, there is a need to identify the factors involved in weight change post-diagnosis. This study will address that need by exploring the association between weight change and different factors that could potentially affect body weight. Finally, the study will also look at adiposity parameters and the metabolic status of participants, which is a relevant and relatively unexplored area.

It is expected that the findings from this study will increase current knowledge of this undesirable weight change phenomena and of the metabolic status following BC diagnosis. Findings will also generate hypothesis to understand the biological

mechanisms underlying changes in body weight and in metabolic and adiposity parameters. Finally, the knowledge generated from this study may inform the development of weight management interventions that could potentially benefit women diagnosed with BC.

CHAPTER 2: LITERATURE REVIEW ON WEIGHT CHANGE AFTER BREAST CANCER DIAGNOSIS

2 Introduction

This chapter shows what is known and what still remains unclear about weight changes after BC diagnosis. It details the link between BC treatments and the potential mechanisms that could mediate their association with weight change post-diagnosis. It also introduces two genes that could have an impact on body weight post BC diagnosis, an area that has only started to be explored. All this will precede the last section of the chapter: the statement of the aims of the study.

2.1 Body weight regulation

Body weight is the result of the difference between energy intake (derived from food intake) and energy expenditure (resulting from a combination of physical activity, resting metabolic rate and thermogenesis): when energy intake exceeds energy expenditure, fat accumulates increasing body weight. Conversely, a reduced energy intake accompanied by high energy expenditure would lead to weight loss (Flier and Maratos-Flier 2007).

The observations that humans tend to keep a relatively constant level of body weight over prolonged periods of time, as well as the fact that body weight is likely to return to previous levels after marked increased or decreased of weight (i.e. after an illness or diet-induced weight loss), led to the idea that body weight is subject to regulation, what is known as energy homeostasis (Havel 2000). Nowadays, it is accepted that energy homeostasis is a complex process orchestrated by the hypothalamus and other parts of the brain; they process signals related to feeding, glucose and body fat status, and translate this information into short and long-term actions to regulate food intake and energy expenditure to keep body energy and fat levels within the desired range

(Flier and Maratos-Flier 2007). These signals emanate from organs like the adipose tissue, the gastro-intestinal system and the pancreas (Mayer 1955), and include peptides, which are released after nutrient ingestion, neuronal signals resulting from gastro-intestinal distension, metabolic signals reflecting the availability of glucose levels and fat stores, as well as adipose signals (i.e. insulin and leptin) (Schwartz et al. 1999; Dulloo 2005). The hypothalamus regulates anabolic pathways, which are activated when energy balance is negative, and as a consequence, food intake increases and energy expenditure decreases. It also regulates catabolic pathways, which are triggered when energy balance is positive, so they inhibit appetite and promote energy expenditure (Frank et al. 2014).

ER-alpha are expressed in different regions of the brain involved in energy homeostasis, such as the ventral medial hypothalamus (VMH), the arcuate nucleus (ARC), the medial preoptic area (MPOA), or the paraventricular nuclei (PVN). A reduction in ER in the VMH can increase body weight gain and visceral fat deposition, and can reduce energy expenditure. Similarly, a decrease of oestrogen levels in the ARC can lead to an inhibition of a group of neurones that reduce food intake and increase energy expenditure (Frank et al. 2014). Oestrogens can also affect cholecystokinin receptors, a substance that increases satiation, and can also decrease the appetite-promoting effects of ghrelin and melanin-concentrating hormone. Furthermore, a reduction of oestrogen levels has been associated with an increase of IR (Lipscombe et al. 2012) and with a decrease sensitivity to leptin in the brain (Frank et al. 2014). Leptin is a substance that operates in different areas of the brain to regulate energy balance (Trayhurn and Beattie 2001). Alteration in leptin signalling can lead to leptin resistance, a primary risk factor for obesity (Su et al. 2012). Leptin production can be also stimulated by insulin (Trayhurn and Beattie 2001) and circulating glucose levels, increasing leptin's effects on appetite and energy expenditure (Su et al. 2012) Insulin signalling in the brain controls body weight and also regulates peripheral glucose and fat metabolism (Konner et al. 2011). Insulin is therefore considered an adipose signal that promotes uptake and storage of glucose and lipid in the adipose tissue (Dullo 2005; Szablewksi 2011). Activation of insulin receptors in the ARC, PVN and in the lateral hypothalamus decreases food intake and decreases body weight (Frank et al. 2014). In line with

that, the resistance to the actions of insulin, or IR, has been linked to weight gain, probably due to attenuation in the response to insulin in areas of the brain relevant to eating behaviours (Anthony et al. 2006).

There are a number of factors that can also influence food intake and energy expenditure, and therefore, can determine body weight. These include behaviour (i.e. level of physical activity, diet,or smoking), psychological and socio-cultural aspects (i.e. availability and abundance of food, or meal patterns) (Jequier and Tappy 1999; Flier and Maratos-Flier 2000; Drewnowski and Bellisle 2003), as well as biological factors (genes, gender and age) or pharmacological agents (Institute of Medicine 2004). Weight changes in adulthood seem to be common among the healthy population, and seem to be the consequence of changes in modifiable factors (i.e. food intake), as well as in changes in factors that individuals cannot control, for instance, the ageing process. The National Health and Nutrition Examination Survey found a gradual increase in body weight in early and middle adulthood (25 to 55 years of age), whereas weight loses were common after that (Williamson et al. 1990). The rationale for these weight changes is little understood, but it could involve reduced physical activity and a decrease in metabolically active tissues (i.e. skeletal muscle) (Institute of medicine 2004). Furthermore, a critical period in women's weight is the transition to menopause, which is commonly associated with weight gain and with a redistribution of body fat to the abdominal area, although it is not clear whether such changes are the result of a physiological reduction of sex steroids (oestrogen and progesterone), or due to the ageing process (Toth et al. 2000; Boonyaratanakornkit and Pateetin 2015). In addition to that, research has pointed to the importance of genetic and environmental factors, and their interaction, in determining body weight (Xi et al. 2012a). Studies conducted with pairs of monozygotic twins reared apart have shown that one-third of the variability in BMI is attributable to non-genetic factors and two-thirds to genetic factors (Galgani and Ravussin 2010). This indicates that some individuals have a genetic predisposition to obesity, which is particularly relevant in an environment where high-fat food is available at low cost, and where there is little need for physical activity (Galgani and Ravussin 2010).

The fat mass and obesity-associated gene (FTO) and a common polymorphism near the melanocortin-4 receptor (Mc4R) gene are two genes most robustly associated with common forms of obesity. In 2007, the FTO was identified as the first gene that contributes to common forms of obesity (Loos and Bouchard 2008). A study of 38,759 Europeans identified a high-risk allele of the FTO gene. People homozygous for the high-risk single nucleotide polymorphism (SNP) rs9939609 A-allele of the FTO associated gene tended to weigh 3 kg more and had higher risk of obesity [odds Ratio (OR): 1.67, 95% CI: 1.47 to 1.89, $p<0.001$] compared to those homozygous for the low risk FTO T-allele (Frayling et al. 2007). Furthermore, this A-allele has also been associated with type-two diabetes (OR 1.13, $p=4.5 \times 10$ (-8) (Frayling et al. 2007; Hertel et al. 2011; Xi et al. 2012a). A year later, common genetic polymorphisms near the Mc4R were also associated with common forms of obesity (Loos et al. 2008). Each copy of the high risk rs17782313 C allele was associated with a difference in BMI of around 0.22 kg/m^2 (Loos et al. 2008). A more recent meta-analysis confirmed the association between the rs17782313 polymorphism near the Mc4R gene with obesity (Xi et al. 2012a) and also with type-two diabetes (OR: 1.10, 95% CI: 1.07 to 1.13, $p=2.83 \times 10$ (-12) (Xi et al. 2012b).

Little is known about the molecular bases of the relationship between these two genes and obesity (Olszewski et al. 2009, Xi et al. 2012a; Arrizabalaga et al. 2014). Nonetheless, evidence has suggested that both genes are highly expressed in the central nervous system which regulates the energy metabolism (Xi et al. 2012a). The FTO gene is expressed in hypothalamic sites related to hunger/satiation control, and low hypothalamic FTO levels have been associated with an increase in food intake but not with an increase in body weight (Olszewski et al. 2009). Consequently, FTO seems to control appetite and feeding behaviour, and also seems to reduce insulin secretion (Fan et al. 2015). In addition, evidence indicates that the FTO rs9939609 polymorphism is associated with lower resting energy expenditure (REE), and with serum thyroid-stimulating hormone's levels, suggesting that the influence of FTO on the increased adiposity might be mediated by the endocrine system that depends on the hypothalamus, pituitary and thyroid gland (Arrizabalaga et al. 2014). On the other hand, the gene Mc4R seems to activate both satiety and hunger signals by integrating an anorexigenic (satiety) signal provided by the alpha-melanocyte-

stimulating hormone and an orexigenic (hunger) signal provided by the agouti-related peptide (Valette et al. 2013). The presence of the risky allele at rs17782313 polymorphism might affect the leptin-melanocortin signalling system, whose downstream peptides bind to Mc4R and inhibit food intake (Valette et al. 2013). On the other hand, most evidence concludes that the Mc4R does not affect body adiposity through its effects on REE (Kring et al.2009; Arrizabalaga et al. 2014).

Lesions in the hypothalamus can produce weight changes by disrupting the balance of appetite and metabolic rate (Flier and Maratos-Flier 2007). Hence, from a scientific point of view, it is logical to think that any interference of the neurological regulation of energy homeostasis or factors that produce metabolic/endocrine disturbances or behavioural changes could lead to weight following BC diagnosis.

As pointed out in Chapter 1, although weight change is common among the general population, evidence suggests that the weight change observed among BC patients is larger than the weight change experienced by age-matched healthy controls. For instance, Gross' et al. (2015) study, BC survivors gained significantly more weight [β = + 3.06 pounds (lb), 95% CI: 0.94 to 5.17] than cancer-free women. Vagenas and colleagues (2015) found that 56% of BC patients gained weight (mean + 5.3 kg) between six and 72 months post- BC surgery, and nearly 80% of them experienced greater gains than the average weight gain seen in sex- and age-matched controls.

2.2 Factors related to weight change after breast cancer diagnosis

The interest in uncovering the factors that could explain weight changes after BC has a long history. Most of the research conducted on weight change after BC has looked at chemotherapy and less frequently at hormone therapy and at energy intake and expenditure. Only recently research has broadened its scope by looking at genes associated with obesity. The following lines summarise the results of these investigations.

2.2.1 Breast cancer treatments and their association with weight change after breast cancer diagnosis

2.2.1.1 Hormone therapy and weight change post diagnosis

In clinical practice, many women diagnosed with BC complain of gaining weight in the abdominal area, and they believe that is a consequence of using tamoxifen, a drug that blocks the actions of oestrogens in BC cells (Burstein 2011). This is a reasonable belief, as oestrogens can reduce food intake and ultimately body weight, possibly through its alpha receptor in the hypothalamus (Roesch 2006). Unfortunately, the effects of tamoxifen in the hypothalamus are still unknown (Aquino et al. 2016), and the biological mechanisms that could link tamoxifen with a potential weight changes post-diagnosis are not understood.

Research conducted with women diagnosed with BC has provided inconsistent results on the association between weight change post-diagnosis and tamoxifen use. Several large cohort studies with long follow up have failed to provide empirical evidence to support the link between tamoxifen and significant weight gain post BC diagnosis (Rock et al. 1999; Irwin et al. 2005; Saquib et al. 2007; Gu et al. 2010; Chen et al. 2011; Arpino et al. 2015) (Table 1, Appendix I). For instance, the HEAL study did not find statistically significant associations between tamoxifen use and absolute changes in body fat and body weight from diagnosis to within three years after diagnosis, in a population of 514 breast cancer survivors (Irwin et al. 2005). Arguably, baseline measurements were taken within their first year after diagnosis (mean six months from diagnosis), and some weight change might have occurred prior that. Similarly, data from 2,972 BC survivors taking part in the WHEL study revealed that tamoxifen was not associated with significant weight gain (>5% of baseline weight) from a year prior BC diagnosis to two years post-diagnosis (OR= 1.03, 95% CI: 0.71 to 1.51) and it did not modify the effect of chemotherapy use (OR= 1.65 vs. OR = 1.69) (Saquib et al. 2007). These findings support previous results from the same trial (Rock et al. 1999). On the other hand, data from a cohort of 445 women with newly diagnosed breast cancer showed that those who received tamoxifen had a mean weight change of + 1.26 kg (95% CI: 0.7 to 1.8) one year post-diagnosis, a magnitude larger than those who did not received adjuvant

treatment (+ 0.6 kg, 95% CI: 0.01 to 1.3). Nonetheless, chemotherapy users had the largest amount of weight change (+2.5 kg, 95% CI: 1.8 to 3.2), and multivariable analysis indicated that the onset of menopause and chemotherapy use, not tamoxifen, were independent predictors of weight change (all p<0 .05) (Goodwin et al. 1999). Results from the Chinese SBCSS cohort study with more than four thousand participants also failed to find a significant association between tamoxifen use and weight change (more than 2 kg weight change), from one year prior diagnosis to 18 months post-diagnosis (Chen et al. 2011), or to six months post-diagnosis (Gu et al. 2010).

Conversely, findings from other studies did advocate for a possible role of hormone therapy in weight changes. A smaller study (n=50) conducted by McInness and Knobf (2001) suggested that women who took tamoxifen had a greater weight change than those who did not take tamoxifen from diagnosis to 12, 24 and 36 months post-diagnosis, although the differences were not statistically significant (data not reported). Nonetheless, it is possible that they might have been with a larger sample size. A more recent study published by Heideman et al. (2009) with data from 271 women treated for BC followed for a median of three years post-diagnosis showed a significant weight change, with an average change of + 2.4 kg. In that study, women treated with chemotherapy and hormone therapy experienced the greatest weight change (+ 4.7 kg, SD: 6.3 kg), whereas women who were treated with chemotherapy only had an overall change of +1.7 kg (SD: 5.1 kg), and those who received hormone therapy only (mainly tamoxifen) and those who did not received adjuvant treatment had a mean weight change of + 0.9 kg (SD: 5.0 kg and SD: 4.0 kg respectively). Combined adjuvant therapy was strongly related to larger weight change during longer follow up (Heideman et al. 2009). In addition, findings from the Nurses Health Study showed that women with the largest weight changes post BC diagnosis tended to be more likely to have received treatment with chemotherapy or tamoxifen (Kroenke et al. 2005). Similarly, Reddy and colleagues (2013) found larger weight change 18 months post-diagnosis among women who took tamoxifen, although the results were not statistically significant.

A randomise control trial comparing women diagnosed with BC treated with tamoxifen with women diagnosed with BC without receiving tamoxifen could

provide stronger evidence on the impact of tamoxifen on weight change. Unfortunately, due to ethical implication of not treating BC patients with hormone treatment if it can benefit them makes it difficult to conduct a study with those characteristics. The closest data was presented by Sestak et al (2012). They reported data on weight change from three large clinical trials on endocrine therapy for the treatment of BC (ATAC study), or for the prevention of BC [International Breast Cancer Intervention Studies (IBIS-1 and 2)]. Participants in the ATAC were postmenopausal women with early BC randomly assigned to receive anastrozole alone (n=3092), tamoxifen alone (n=3094), or the combination (n=3097) for five years after surgery. The other two trials involved women without BC. In the first one, postmenopausal women without BC were randomly allocated to either five years of tamoxifen or matching placebo, and were followed up every six months during the five years of treatment (IBIS -1). In the second one, high risk postmenopausal women without BC received either anastrozole or placebo for five years (IBIS-2). In the ATAC study, after 12 months of follow-up, the mean weight change was + 1.4 kg, and at 60 months there was a mean negative weight change (-0.35 kg). There were no significant changes in weight observed between 12 months and 60 months in either treatment arm. The IBIS-1 showed comparable weights at baseline, at 12 and at 60 months of follow-up between tamoxifen users and placebo users. In the IBIS-2 study, women in the anastrozole group had a mean weight change of + 0.8 kg (5.3) compared with a + 0.5 kg (7.3) weight change seen in the placebo group at 12 months of follow up. No statistically significant differences were found at any point between the groups (Sestak et al. 2012).

The reasons for the discrepancy of the findings in the previous studies looking at tamoxifen are unclear. The percentage of participants that took tamoxifen in the studies varied considerably. For instance, in Heideman's et al. (2009) study and in McInness and Knobf's (2001) study were 44.3% and 46% respectively, whereas in the studies conducted by Saquib et al.(2007), and by in Goodwin's et al. (1999), the percentages were 67.3% and 82.9%. Furthermore, the approach used to analyse weight change in relation to tamoxifen use (and also to other factors of interest) might have also influenced the findings. Paige et al (2014) demonstrated that in a healthy sample of participants the association of weight change with different

variables (i.e. education, gender) varied when weight change was measured as the average of a continuous weight change vs. categories of weight change (weight gain, loss, maintenance). Moreover, the associations were also different when different cut-points for defining the categories of weight change were used. Therefore, Paige's and colleagues' (2014) findings could explain inconsistencies in the literature exploring the association of weight change with tamoxifen and other factors.

Interestingly, although the studies exploring the association between tamoxifen and weight change present mixed results, several studies suggests that the effect of tamoxifen might be reflected in body adiposity. Cross-sectional data from 32 postmenopausal women taking tamoxifen for BC for a mean of 30 months were compared with data from a convenience sample of 39 women without BC. Visceral adiposity levels measured with a computed tomography (CT) scan were statistically significantly higher among women taking tamoxifen (Nguyen et al. 2001). Nonetheless, causal relationship between tamoxifen and increased adiposity should not be concluded from this cross-sectional study, as it is possible that those with higher adiposity levels were more likely to have BC and therefore, were treated with tamoxifen. In fact, other observational studies did not find a similar association (Freedman et al. 2004; Irwin et al. 2005; Francini et al. 2006). Nonetheless, interventions conducted with BC survivors support a possible impact of tamoxifen on body fat. Nissen et al (2011) conducted a small trial to evaluate the effects of a physical activity intervention vs. bisphosphonate (a drug to prevent bone mass loss) on bone mineral density in women undergoing treatment for breast cancer. Findings showed that although tamoxifen use did not affect weight change, it was associated with higher body fat levels. Women who took tamoxifen following chemotherapy gained FM whereas those who took AINs or had no hormone therapy following chemotherapy did not. Nonetheless, the association vanished when other variables (i.e. BMI, age) were taken into account. Knobf et al. (2008) conducted a study to determine the feasibility of a one-group pre-post-test exercise intervention on bone mass, weight and body adiposity, in 26 midlife breast cancer survivors with early menopause. There were no significant changes in weight over the course of the intervention. However, there was an interaction effect for hormone therapy over time ($p=0.04$), and FM increased significantly more for women on tamoxifen ($p=0.006$)

and women on AINs (p=0.05) compared to women who were not on hormone therapy.

Consistent with a potential role of hormone therapy, there is evidence that AINs can produce weight gain in mice (Kubatka et al. 2008a; Kubatka et al. 2008b; Sadlonova et al. 2009). However, those findings have not been mirrored in women diagnosed with BC (Francini et al. 2006; Sestak et al. 2012).

Given these inconclusive but inviting findings, it is clear that the role of hormone therapy deserves further investigation.

2.2.1.2 Chemotherapy and weight change post-diagnosis

Several studies have reported positive weight changes associated with chemotherapy use following BC diagnosis (i.e. Demark-Wahnefried et al. 1997; Aslani et al. 1999; Costa et al. 2002; Del Rio et al. 2002; Basaran et al. 2010). Unfortunately, they did not include a comparison group, making it difficult to ascertain whether other women who did not received adjuvant chemotherapy might have also experienced changes in weight. Nonetheless, data from the WHEL cohort study (Rock et al. 1999; Saquib et al. 2007) and from other smaller studies (Demark-Wahnefried et al. 2001; Makari-Judson et al. 2007; Heideman et al. 2009) that included a comparision group found a statistically significant association between chemotherapy and weight change.

Weight changes have been observed during chemotherapy or in the first year of diagnosis (i.e. Camoriano et al. 1990; Goodwin et al. 1999; Demark-Wahnefried et al. 2001; Costa et al. 2002; Del Rio et al. 2002; Lankester et al. 2002), but also in the long term, as demonstrated for example by Caan et al. (2006), who found that almost two years post-diagnosis, women who received chemotherapy had an mean increase of 4.6% (SD: 10.0) of their initial weight, a magnitude that was higher than those who did not receive chemotherapy (+ 2.5%, SD: 9.5). Interestingly, data from the SBCSS showed that chemotherapy was associated with weight change 18 months post-diagnosis (Chen et al. 2011), but the association vanished in the long term (i.e.

36 months post-diagnosis) (Gu et al. 2011), suggesting that the effect of chemotherapy on body weight might not be sustained over time. Findings from a smaller study conducted by Makari-Judson and colleagues (2007) are in the same line. Chemotherapy use was associated with weight changes at 12 and 24 months post-diagnosis, however, chemotherapy was not a predictor of weight change from 24 to 36 months post-diagnosis.

Importantly, large cohort studies including the ATAC showed that chemotherapy use was not associated with weight changes (Irwin et al. 2005; Sestak et al. 2012; Reddy et al. 2013; Arpino et al. 2015). Results from the HEAL showed greater weight change (mean positive weight change) among women who received chemotherapy than women who did not receive systemic treatment. However, when adjusted for potential confounders (menopausal status and physical activity), average weight change was no longer associated with chemotherapy use (Irwin et al. 2005). It has been hypothesised that the lack of association in these recent studies could be due to the new chemotherapy regimens used, which tend to contain anthracycline agents, and seem to lead to lower amounts of weight gain (Rock et al. 1999; Saquib et al. 2007; Makari-Judson et al. 2014). Arguably, other treatment characteristics (i.e. duration of chemotherapy treatment, or steroids use could influence the effect of chemotherapy on weight change, although some studies found that these factors did not have a significant effect (Lankester et al. 2002; Saquib et al. 2007; Makari-Judson et al. 2014). Therefore, the effect of current chemotherapy regimens on weight change post-diagnosis requires further research.

2.2.1.3 Potential mechanisms accounting for suspected treatment effects on weight change

Potential mechanisms accountable for the hypothetical association between BC treatment and weight change post-diagnosis include endocrine changes due to treatment-induced menopause, as well as changes in energy intake (food intake) and/or decreases in energy expenditure (i.e. alterations in metabolic rate, thermogenesis or physical activity) (Demark-Wahnefried et al. 1993; Partridge et al.

2001; Vance et al. 2011). In addition, the liver and the muscle might be affected by chemotherapy drugs, leading to disruptions of glucose metabolism and IR (Makari-Judson et al. 2014).

2.2.1.3.1 Transition to menopause

Many studies found that post-diagnosis weight gain appears to be more frequent among premenopausal women than among postmenopausal women (Camoriano et al. 1990; McInnes and Knobf 2001; Freedman et al. 2004; Caan et al. 2006, 2008; Heideman et al. 2009; Basaran et al. 2011; Chen et al. 2011; Caan et al. 2012b). Nonetheless, the opposite has also been reported (Cheney et al. 1997; Aslani et al. 1999; Rock et al. 1999; Costa et al. 2002; Lankester et al. 2002; Megale et al. 2002; Irwin et al. 2005; Kroenke et al. 2005; Makari-Judson et al. 2007; Saquib et al. 2007; Gu et al. 2010; Tredan et al. 2010; Sestak et al. 2012; Reddy et al. 2013).

Most premenopausal women experience chemotherapy-induced menopause (Vance et al. 2011). The transition to menopause is commonly associated with weight gain and with a body fat redistribution, possibly due to a physiological reduction of oestrogen and progesterone (Toth et al. 2000; Boonyaratanakornkit and Pateetin 2015). A reduction of oestrogens can cause a decrease in insulin sensitivity and glucose metabolism, as well as a reduction in cellular metabolism and energy expenditure, which could promote weight gain (Boonyaratanakornkit and Pateetin 2015). Nonetheless, this biological explanation has not been fully supported by empirical data in the context of this study. In Goodwin's et al. (1999) cohort study, 535 women with BC underwent anthropometric measurements at baseline and one year later. Menopausal status at diagnosis ($p=0.02$) and change in menopausal status post-diagnosis ($p=0.002$) predicted weight change in univariable analysis. When other variables were included in the analysis, onset of menopause and chemotherapy use remained significant predictors of weight change post-diagnosis (all $p<0.05$). Nonetheless, other studies found no significant differences in the magnitude of weight change between those who had a treatment-induced amenorrhea and those who remained premenopausal (Camoriano et al. 1990; Lankester et al. 2002; Makari-

Judson et al. 2007). Similarly, in other studies, menopausal status at the time of diagnosis was not associated with changes in body adiposity in women diagnosis with BC (Irwin et al. 2005; Arpino et al. 2005).

Lack of consistent findings might be due to the different classification of menopausal status, or the different duration of transition to menopause and follow ups when weight changes were assessed. On the other hand, some studies have postulated that age, rather than menopausal status, is a more significant risk factor for weight change (Saquib et al. 2007; Gu et al. 2010; Sestak et al. 2012; Reddy et al. 2013; Makari-Judson et al. 2014). For instance, multivariable analysis from the SBCSS showed that menopausal status was not associated with weight change post-diagnosis, however, age at diagnosis was associated with the positive weight change observed from diagnosis to 36 months post-diagnosis (all $p<0.001$) (Gu et al. 2010). Interestingly, age and menopausal status were both relevant in Irwin's et al. (2005) study: younger postmenopausal women (those aged 40-49 years) experienced the greatest weight changes [+5.2 kg, standard error (SE): 1.4], when compared with older postmenopausal women (+2.1 kg, SD: 0.4) (p for trend 0.002).

2.2.1.3.2 Insulin and glucose levels

The role of insulin in glucose metabolism and body weight regulation has been described earlier. Alterations in insulin and glucose levels can contribute to weight gain and obesity (Su et al. 2012). In fact, metabolic abnormalities have been associated with alterations in REE (Merritt et al. 2010), which constitutes the largest component of energy expenditure (Guinan et al. 2013). Unfortunately, the research investigating the metabolic effects of BC treatments is very limited. Makari-Judson and her team (2009) investigated the relationship between markers of IR, BC treatments, and weight change in a prospective study of 95 women receiving adjuvant systemic treatment for BC. Mean weight change at six and at 12 months were + 0.4 kg (95% CI: 0.3 to 1.0, p=0.23) and + 0.9 kg (95% CI: 0.4 to 1.8, p=0.04) respectively. Six-months HOMA IR tended to increase by mean + 0.34 (p=0.34). Chemotherapy-treated patients exhibited adverse changes in HOMA-IR compared

with non-treated patients (mean change + 0.53 vs. -0.64, p=0.005). Conversely, hormone therapy, age or BMI were not associated with significant changes in HOMA IR (Makary-Judson et al. 2009).

Further evidence exists showing that taxane-based chemotherapy to treat BC can raise insulin (Alacacioglu et al. 2016) and glucose levels (Hickish et al. 2009) and a population based study showed that BC survivors had an increased risk of diabetes (HR for chemotherapy users: 1.24, 95% CI: 1.12 to 1.38, and HR for non-chemotherapy users: 1.11, 95% CI: 1.05 to 1.16) (Lipscombe et al. 2013). These findings support the hypothesis that chemotherapy drugs could affect the liver and the muscle, leading to disruptions of glucose metabolism and to IR (Makari-Judson et al. 2014), which potentially could explain weight change post-diagnosis (Makari-Judson et al. 2007).

Despite the fact that Makari-Judson et al. (2009) failed to find a relationship between hormone therapy and IR, findings from a case-control study with old BC survivors revealed that tamoxifen use was associated with an increased incidence of diabetes (Lipscombe et al. 2012). Furthermore, results from a randomise control trial with women at risk of BC exposed to either low-dose tamoxifen or fenretinide (a drug investigated for its potential use to treat cancer) showed that in overweight women, tamoxifen was associated with a reduced insulin sensitivity (Johansson et al. 2008). Finally a case-study described a patient who developed tamoxifen-induced severe hypertriglyceridemia and steatohepatitis, both of which are features of IR and glucose intolerance (Elisaf et al. 2000).

The previous lines indicate that the literature on the impact of BC treatment on metabolic parameters is emerging. Similarly, the role of insulin or IR on BC treatment-related weight gain is unclear (Makari-Judson et al. 2009), and further research is needed to explore whether BC treatments affect insulin and glucose levels, as these might potentially explain weight change observed.

2.2.1.3.3 Energy intake and energy expenditure

BC treatment-related side effects, such as fatigue, nausea and vomiting, and the demands of multiple treatment days, as well as psychological distress can all affect eating and physical activity behaviours, leading to weight changes post diagnosis (Kayl and Meyers 2006; Vance et al. 2011). Consequently, the effect of lifestyle on weight change post-diagnosis is an area of active research (Vance et al. 2011). Most evidence suggests that there are little or insignificant changes in food intake following BC diagnosis and these could not explain the reported magnitude of weight change (Nissen et al. 2011; Vance et al. 2011) or increases in FM (Goodwin et al. 1999; Kutynec et al. 1999; Demark-Wahnefried et al. 2001; Del Rio et al. 2002; Harvie et al. 2004; Kumar et al. 2004; Wayne et al. 2004; Irwin et al. 2005; Francini et al. 2006; Genton et al. 2006).

Similarly, the cohort study conducted by Goodwin et al. (1999) also found that physical activity levels could not explain weight change observed during the first year post-diagnosis. In their sample, physical activity levels (assessed as the number of hours devoted to mild, moderate and strong physical activity at work, at home, or during leisure activities), increased during the first year of diagnosis, particularly among women who received chemotherapy. This increase did not correlate with the weight change observed. Physical activity was self-reported and measured at baseline and one year later, and the authors recognised that an association of physical activity over the course of that year with weight change might have been missed. Conversely, results from the HEAL study found a statistically significant trend of increasing weight gain with decreasing hours of sports/recreational (i.e. walking) and household activities (i.e. gardening) per week, from baseline to within three years after diagnosis (p for trend: 0.03) (Irwin et al. 2003, 2005). This finding is consistent with a report from the WHEL study stating that physical activity predicted weight stability in 1,116 BC survivors (Rock et al. 1999). Further small studies have provided additional evidence on the association between physical activity with weight and body fat gains following BC diagnosis (Demark-Wahnefried et al. 1997a; Kutynec et al. 1999; Demark-Wahnefried et al. 2001; Harvie et al. 2004; Genton et al. 2006; Broderick et al. 2014).

Other components of energy expenditure might also contribute to weight changes. Oestrogen withdrawal can reduce energy expenditure (Boonyaratanakornkit and Pateetin 2015). However, most studies found no significant changes in REE after BC diagnosis that could account for the increase of mean weight reported (Demark-Wahnefried et al. 1997a; Kutynec et al. 1999; Demark-Wahnefried et al. 2001; Harvie et al. 2004). Only in Del Rio's et al. (2002) study, REE increased significantly as body weight increased, but chemotherapy use was not associated to that increase in REE, rather, the raised levels of REE were thought to be the result of the concomitant increase in FFM also observed. Consequently, changes in FFM might obscure the association between REE and weight gain. Measuring components of energy balance is difficult, and it is possible that a number of methodological issues in the previous studies (i.e. small sample, or timing of data collection) might have prevented authors to finding changes in eating and physical behaviours, or an association between them and weight change. Subjective methods of data collection could also result in systematic errors. Social desirability bias might lead to over-reporting physical activity (Adams et al. 2005) or underreporting food intake (McNeill 2000).

These lines suggest that further research with a large sample of women diagnosed with BC, and using adequate validated and reliable data collection instruments, could help to clarify the impact of those behaviours and REE on weight change post-diagnosis. This could provide a clearer picture on their effect on weight change observed in this population.

2.2.2 Genes associated with common forms of obesity and their effect on weight change after breast cancer diagnosis

FTO and Mc4R, two genes associated with common forms of obesity, can determine the predisposition of individuals to gain weight (Loos et al. 2008). Hence, it is possible that these genes could play a role in weight changes post BC diagnosis. Reddy and colleagues reported for the first time data on the impact of genetic variables on weight change after BC diagnosis. At 18 months post-diagnosis 36% of the 459 participants had a BMI increase of more than 5kg/m^2 from baseline and an

average weight change of + 5.1 kg (SD: 3.76kg). Weight change was best predicted by a model that incorporated age and BMI and diagnosis, as well as data on all 14 SNPs explored from FTO and adiponectin genes which gave area under the receiver operating characteristic curve (AUC) value of 0.85 for 18-month weight gain. The model, as the authors argued, had a high discriminatory power to identify those at risk of weight gain post-diagnosis (Reddy et al. 2013).

On the other hand, to the research knowledge, no study has explored the effect of Mc4R on weight change post BC.

2.3 Summary and aims of the study

The previous lines draw attention to the fact that despite considerable research being conducted, the causes of changes in weight and in body adiposity parameters (i.e. FM, waist circumference) post-diagnosis are not understood. Weight change patterns seem to be heterogenic across the population of women diagnosed with BC, as both weight gains and weight losses have been reported, whereas some women manage to maintain their body post BC diagnosis. In addition, it is not clear from previous research whether weight change is permanent or temporary or whether weight change fluctuates during BC treatment. Finally, also of concern, is the little research dedicated to explore the metabolic disturbances (i.e. changes in glucose and insulin metabolism) that might occur post-diagnosis.

Therefore, the main aim of this study is to explore weight change following BC diagnosis and to identify factors associated to the change. A secondary aim is to determine body adiposity parameters and the metabolic status of BC survivors at the end of the follow up.

In order to meet the first aim, the objectives of this longitudinal retrospective study are:

- To review participants' medical notes to collect data on weight from the time of the BC diagnosis to the end of follow up (the time they accepted to take part in this study).
- Quantify the magnitude of weight change from diagnosis to different evaluation points after BC diagnosis (i.e. 12, 24, 36 and 48 months post-diagnosis).
- Quantify the frequency of weight change from diagnosis to those evaluation points.
- Explore the association between the magnitude of weight change post-diagnosis with:
    - Clinical factors: BC treatment and tumour characteristics.
    - Biological and behavioural factors: menopausal status at diagnosis, transition to menopause, age, genetic profile, weight at diagnosis and smoking status at diagnosis.
- Create a model to describe patterns of weight change from the time of BC diagnosis to end of follow up that will:
    - Summarise how each participant's weight changes over time.
    - Identify groups of participants that experience different patterns of change.
    - Identify which of the following factors are associated with which patterns:
        - Clinical factors: BC treatment and tumour characteristics.
        - Biological and behavioural factors: menopausal status at diagnosis, transition to menopause, age, genetic profile, weight at diagnosis and smoking status at diagnosis.

The specific objectives to meet the second aim of the study are:

- To measure participants' body adiposity parameters (FM percentage, FM/FFM ratio and waist circumference) at study entry (end of follow up).

- To measure participants' metabolic parameters (levels of fasting glucose and fasting insulin) at the time of study entry.
- To test the association between those body adiposity and metabolic parameters with:
    - Clinical factors: BC treatment and tumour characteristics.
    - Biological and behavioural factors: menopausal status at diagnosis, transition to menopause, age, genetic profile, weight at diagnosis, post-diagnosis weight change and smoking status at diagnosis.

CHAPTER 3: RESEARCH METHODS

3 Introduction

This chapter explains the design of the study and the methods used to meet the study's aims described in Chapter 2.

It starts with a description of the study design, a retrospective cohort study, explaining the adequacy of this kind of design to meet the research questions and its limitations. The chapter then introduces the target population and provides a rationale for the expected sample size chosen. The next section explains the participants' recruitment procedures, data collected from them and the instruments used in data collection. It follows with a description of the peculiarities of the dataset, providing useful information to understand the steps of data analysis. The last section of the chapter is devoted to ethical aspects taken into account when conducting this research.

3.1 Study design, its advantages and limitations

The main aim of this study was to explore weight change post BC and factors associated with it. The best approach to find a cause-effect relationship between two factors would be an experimental design, where the randomisation of participants to an intervention or to a control group is likely to prevent the introduction of confounding variables. Consequently, this would allow researchers to conclude that any change in the outcome can be attributed to the intervention (Black 1999). An experimental design like that was not appropriate to meet the aims of this study, as it would not have been ethical or possible to manipulate the variables under observation (i.e. BC treatment, menopause status, genes) to observe changes in the outcome (participants' weight following BC diagnosis). Therefore, a non-experimental, longitudinal design constituted the best alternative to explore potential cause-effect relationship (Black 1999) when looking at weight change post-diagnosis. More importantly, it could be used to observe patterns of weight change in

the target population (Singer and Willett, 2003), which was one of the aims of this study. Among the different types of observational longitudinal studies, a retrospective cohort study was adopted to guide this research project.

3.1.1 Retrospective cohort study

Retrospective cohort studies, as opposed to prospective cohort studies, are relatively quick to conduct and less costly, as both independent and outcome variables are already recorded (De Vaus 2001). In this project, data on weight (at diagnosis and during follow up) and other variables (i.e. BC treatment received) were documented in participants' medical notes as part of the routine care, prior to this research being planned. The only exception were weight at study entry and the metabolic and adiposity parameters, which were all measured by the researcher at study entry. Due to the limited time to conduct this research project, the fact that most weight records were already recorded in the medical notes was a very attractive and useful advantage. Other appealing attributes of retrospective cohort studies that were taken into account were firstly, its temporal dimension. It allows the observation of changes in the outcome over the time by collecting data at different follow up points. This temporal quality enables researchers to explore also different aspects of the outcome, including frequency of the outcome, when it occurs, whether it has a short or long term effect, or to observe patterns of occurrence (De Vaus 2001). Secondly, retrospective cohort studies also allow researchers to compare participants exposed to a predictor (independent variable) with an internal comparison group (a group of non-exposed participants from the same cohort without the predictor), in relation to the outcome under exploration, so as to observe the impact of different predictors on the outcome (i.e. differences in weight change between chemotherapy users vs. non-chemotherapy users) (Boston University. School of Public Health 2015). Thirdly, it is also possible to observe the temporal order of exposures preceding outcome (De Vaus 2001). Temporality is an important feature when looking at factors that are associated with and could predict the outcome in the model to be created and is one of the criteria of causality proposed by Hill (1965). Nonetheless, it is important to acknowledge that with a retrospective cohort study, the researcher would not able to

probe a causal relationship between a factor and weight change, but only to get an idea of the association between the variables explored (De Vaus 2001). Nonetheless, with this design, it could be possible to observe the presence of other criteria suggested by Hill (1965) that would add support for a causal relationship, including the assessment of factors that could plausibly cause weight change, the strength or effect size of the association between those factors and weight change, or the consistency of findings with results from previous studies (Hill 1965). Finally, another advantage that was taken into account in the planning stages of this study was that retrospective cohort studies allow researchers to examine multiple outcomes (i.e. weight change, FM, insulin levels) potentially associated with an independent variable under examination (i.e. chemotherapy use) (Boston University. School of Public Health 2015).

On the other hand, the fact that participants' weight (except the weight at study entry) and independent variables were already recorded in the medical notes as part of the routine care prior this study was conducted could pose some challenges: it may be detrimental to the internal validity of the study if the quality of data collected is poor (i.e. it might be inaccurate or collected with different degrees of precision). Moreover, different instruments might have been used to collect the same type of data, which might introduce measurement errors (De Vaus 2001). Nonetheless, the magnitude of these variations was assessed calculating the measurement error and the intra-class correlation (Peat et al. 2002). A further issue common in retrospective studies is missing data, as often data were collected in the past, outside the research context (Boston University. School of Public Health 2015). This study faced this problem as weight values were not always recorded, or were not always recorded at the same points following BC diagnosis (as explained later). Furthermore, in retrospective cohort studies it is important to account for the effect of other variables that could have an impact on the outcome (confounding variables). Once again, there is a chance that there was not accurate recorded information on confounding variables that could have been used in the research context (Boston University. School of Public Health 2015). These two issues were partly addressed with the data analysis approach proposed (see next section).

Finally, the validity of the results can be affected if the sample is not representative of the target population (De Vaus 2001). Due to the lack of randomised selection of the sample in this particular retrospective study, there was a risk of different types of bias, for example survival bias, as women who had died could not have been recruited into the study. Self-selection bias might be also present in this retrospective study if some subgroups of BC survivors were not interested in taking part in the study, for instance, women who gained weight could have been reluctant to agree take part in the study, or women who did not experienced weight change might have thought that their participation in a study looking at weight change was not relevant. These biases can lead to false conclusions because people with the outcome are overlooked and that can threaten the validity of the study (Lane et al. 2008). The way these biases were tackled in this study was by using different recruitment procedures: Firstly, trying to make target participants not feel ashamed by the weight gained. Secondly, by highlighting that it is a common event and that both weight changes and no weight changes are informative, and finally, by including women diagnosed as early as one year after BC diagnosis, so their chances of surviving that period were higher. In addition, the limitation imposed by lack of randomisation in relation to the effect of an independent variable on the outcome was overcome by collecting more independent variables and using multivariable analysis that accounted for the impact of these variables on the outcome. All these could help to improve the strength of evidence (De Vaus 2001).

3.1.2 Cross-sectional data nested within the study

Another aim of the study was to quantify body adiposity parameters (FM percentage, FFM/FM ratio and waist circumference) and metabolic parameters (insulin and glucose levels) at the time of study entry. These parameters are not measured routinely as part of the BC care, so they were measured only for the purpose of this research project, on one occasion only, at the time of study entry. Therefore, it was considered cross-sectional data (De Vaus 2001). As a consequence, it was not possible to analyse changes in these parameters following BC diagnosis.

Cross-sectional studies are also quick and cheap and allow researchers to describe the prevalence (not the incidence) of an outcome and explore associations with other variables that could be used to generate hypothesis about a causal relationship, rather than to test it (Centre for Evidence-Based Medicine 2014).

3.2 Target population

Weight change post-diagnosis has been particularly reported in women diagnosed with early stages of BC. As mentioned in Chapters 1 and 2, this group of patients have a good prognosis (Cancer Research UK 2014f), but unfortunately, it might be threatened by post-diagnosis weight changes (Caan et al. 2012b; Jackson et al. 2017). Therefore, this study targeted women diagnosed with BC (stages I-III) (Cancer Research UK 2015), regardless of body weight at diagnosis or weight changes post-diagnosis. Participants were recruited from two main hospitals: Royal Bournemouth Hospital and Poole Hospital. They were diagnosed with BC from 2003 up to a year before study entry. Women were not approached to enter the study if at the time of diagnosis:

- Were under 18 years old, as the incidence of BC in that group of women is very low (Cancer Research UK 2014b) and all published research on this area included only women over 18 years old.
- Had a history of BC recurrence, as their treatment for this recurrent BC could be different from that received by women diagnosed for the first time.

Excluded were also women who at the time of diagnosis had health conditions that could have influenced body weight, such as:

- Endocrine problems associated with the development of obesity.
- Inflammatory bowel disease or malabsorption syndrome.
- A history of invasive cancer other than completely resected non-melanoma skin cancer or successfully treated in situ carcinoma of the cervix.
- Mental disorders directly related to food intake (i.e. anorexia and bulimia nervosa) (World Health Organization 2007).

Finally, following the Mental Capacity Act 2005 Code of Practice, (Department for Constitutional Affairs 2007), women who lacked the capacity to make informed decision, or were unable to take part in the study as a result of an impairment or a disturbance (i.e. dementia) defined according to the International Classification of Diseases (World Health Organization 2007) were also excluded.

3.3 Sample size

The sample size issue was considered from a variety of different angles, both practical (within the context of a PhD) and scientific. From a practical point of view, recruitment of women over a one-year period was achievable. Every year, a minimum of 500 women are followed up for early BC in Royal Bournemouth Hospital and in Poole Hospital. Assuming a recruitment rate of 50%, this meant it was likely that around 250 participants could be recruited into the study.

To ascertain the magnitude of effect size that could be detected with this sample, a number of sample size/ power calculations were performed using nQuery Advisor (Statistical Solutions 2014), whilst looking at a variety of scenarios that are pertinent to the stated aims of the project.

All the calculations conducted in this study and described in the following paragraphs assumed a two-tailed significance level of 0.05 (equivalent to 5%). Two-tailed significance level is used in most research studies (Suresh and Chandrashekara 2012). Under the null hypothesis that the mean is equal to x (i.e. no weight change), a two-tailed test will test both if the mean is significantly greater than x (i.e. weight gain) and if the mean significantly less than x (i.e. weight loss). Since there is evidence that weight post-diagnosis can either increase or decrease, or even no change, a two-tailed significance test was preferred over a one-tailed significance test, which only accounts for one scenario (i.e. weight gain).

The statistical power was set up at 80%, which is appropriate for research studies (Martinez-Gonzalez 1998). The power represents the probability that the test performed will find a statistically significant difference between the two groups

compared, when there is a genuine difference in the population from where the samples were drawn. Increasing the power from 80% to 90% would reduce the probability of making a type-two error, but it will require increasing also the sample size by about 30%, something that could not be feasible due to time restrictions to conduct this research (Martinez-Gonzalez 1998). This could be a limitation for this study, as it is known that the bigger the sample size, the greater the power of the study to find smaller statistical differences. Nonetheless, attention should be given to the fact that despite smaller differences in weight change being statistically significant, they may not be clinically meaningful (Suresh and Chandrashekara 2012). Therefore, the magnitude of the effect of interest, in this case weight change, will be reported.

Standardised effect size is a scale-free measure that quantifies the magnitude of a difference in outcome between groups in relation to its SD. It emphasises the size of the effect of an independent variable, rather than the statistical significance of the effect (Coe 2002). Table 2 (Appendix I) summarises standardised effect sizes calculated from previous published research in the field, using the provided group's means, SD, or SE converted to SD and other relevant data. The calculations were assisted with nQuery Advisor (Statistical Solutions 2014). Cohen's d, an effect size measurement used to indicate the standardised difference between two means, was calculated after applying this formula (Thalheimer and Cook 2002):

$$d = \frac{\text{mean group 1} - \text{mean group 2}}{\text{pooled SD}}$$

Pooled SD was calculated as follow:

$$\text{Pooled SD} = \sqrt{[(n_t - 1)SD_t^2 + (n_c - 1)SD_c^2] \div (n_t + n_c)}$$

Where n denotes the number of participants, or sample size, and the subscripts refer to each of the comparison groups.

If SD was not listed, SD was calculated from SE using this formula:

$$SD = SE \sqrt{n}$$

Using the calculations and the assumptions stated above, it was thought that with a sample of 250, this study would be powered to detect a minimum standardised effect size of 0.35 when using an independent sample t-test to compare mean weight change between two groups according to an independent variable (i.e. chemotherapy use). This assumed an equal sample size in each group compared. In this hypothetical scenario, the 0.35 standardised size effect would indicate that weight change (either weight gain or weight loss) of the average participant in the chemotherapy group would be higher than 64% of the participants in the non-chemotherapy group (Coe 2002).

A sample size of 250 could be adequate to find effect sizes of that magnitude when comparing mean weight change between two groups, which due to the aims of this study, was the most likely scenario to be faced when conducting data analysis (Table 2, Appendix I). A high standardised effect size of 0.969 for the impact of chemotherapy was elicited from Makari-Judson's et al. (2007) modest study and a study with a much larger sample size (n=4561) found a standardised effect size of 0.395 (Chen et al. 2011). Standardised effect sizes for tamoxifen on weight change were higher than 0.35 in Nissen's et al. (2011) study, however, in the rest of the studies looking at tamoxifen, the size of the effect ranged from -0.021 to 0.246. Finally, regarding menopausal status, several studies (Chen et al. 2011; Heideman et al. 2009; Makari-Judson et al. 2007) have provided evidence of standardised effect sizes higher than 0.35 (Table 2, Appendix I).

In a second scenario, a sample size of 250 would allow researcher to detect a standardised effect size of that magnitude (0.35) when comparing the mean of an interval scaled independent variable (i.e. age or weight at diagnosis) between those whose weight changed (i.e. more than 5% of their weight at diagnosis) and those whose weight did not change. This would assume that 50% of women's weight would change and that the comparison would be made using the independent sample

t-test. In addition, it was expected that the study would be powered to detect a RR of weight change of 1.5 when comparing the proportion of participants that experience excessive weight change (i.e. changing more than 5% of their weight at diagnosis), between two groups (i.e. those who received chemotherapy and those who did not). This 1.5 RR would assume that the proportion of participants with the explored predictor would range between 0.3 to 0.6, and that between 58% to 64% of them experienced excessive weight change compared to 38% to 44% of participants without the predictor that also had their weight changed. Unfortunately, previous studies did not report effect sizes or enough data to calculate it, when weight change in their studies was treated as a dichotomous dependent variable.

Finally, when looking at correlations between an interval scaled independent variable (i.e. age at diagnosis) and weight change measured in interval scale, it was expected that the study would have 80% power to detect correlation coefficient of 0.16 and over. Table 2 (Appendix I) shows correlations of bigger magnitude (in absolute terms) for the effect of BMI on weight change (Makari-Judson et al. 2007). Nonetheless, correlations between age and weight change tended to be of a smaller magnitude (Goodwin et al. 1999; Makari-Judson et al. 2007).

3.4 Research procedures

3.4.1 Protocol amendments

Recruitment and data collection started in August 2008 following the original protocol (approved in May 2008) and the successive first, second and third protocol amendments granted in October 2008, October 2009 and March 2011. The first protocol amendment sought to collect more outcome data (metabolic parameters and waist circumference) and to add new recruitment procedures. The second protocol amendment requested an extension of the recruitment period and due to that extension, a third protocol amendment was required to modify the definition of inclusion criteria to allow the recruitment of women diagnosed with breast cancer up

to one year before study entry (±2 months), rather than to December 2007, as previously specified.

3.4.2 Participants' recruitment

Participants' recruitment was initially planned to commence in August 2008 and last twelve months. Recruitment of patients from Royal Bournemouth Hospital started as planned, however, recruitment in Poole Hospital started 10 months later. Due to this delay and the slow recruitment rate, the initial twelve months recruitment period was extended until June 2011.

Initially, participants were given oral and written information about the study (Appendix VI) in the follow up clinics. Women treated for BC with chemotherapy are followed up yearly by the Oncology and Surgery team, whereas those who did not received chemotherapy are seen yearly by the Surgery team only. All women that potentially met the inclusion criteria and were seen in the BC follow up clinics in both hospitals were supposed to be invited to participate in the study by the clinician (oncologist, surgeon or nurse specialist). These women were previously identified by the researcher, so as to keep a record of the number of women seen and to facilitate the role of the clinicians in identifying candidates. The targeted women received oral information regarding the study and were handed an invitation envelope to take home which contained an information letter with further details about the study, the research procedures, relevant contact numbers, a consent form, a decline form and a return pre-paid envelope. Those who were interested in taking part in the study signed the consent form enclosed in the information letter and posted it to the researcher in the prepaid envelope. The researcher then contacted them to answer any possible questions, to verify that they were happy to take part in the study and to arrange a convenient date for data collection other than that collected from the medical notes. At arrival to the clinic for data collection, participants confirmed that they had signed the consent form and that they were happy to take part in the study.

In order to reach the target sample size quicker these new recruitment procedures were put in place:

- By post: an invitation envelope was posted to women who met inclusion criteria but were not approached in the follow up clinics due to the fact they were already discharged from the BC follow ups clinics before starting the recruitment (August 2008), or because they attended the BC follow up clinics in Poole Hospital, from August 2008 to June 2009 (during the 10 months when women seen in the other hospital were recruited).
- By hand: key cancer groups (i.e. Poole and Bournemouth Breast Cancer Support Group, or the Patient Partnership Panel), were contacted and were happy to give an invitation envelope to women who met the inclusion criteria.

Finally, a reminder letter was sent to those women who had been invited to take part in the study and had not sent the consent or the deny form indicating whether they want to take part in the study.

3.4.3 Data collection: variables, procedures and instruments

Data collection took place in an office space that was located in the diabetes centre at Royal Bournemouth Hospital and in Poole Hospital.

3.4.3.1 Outcome variables

The main outcome was weight change. Therefore, any body weight recorded in the hospital medical notes at the time of BC diagnosis up to the study entry was collected. If weight at diagnosis was not available, weights available in the medical notes taken within two months prior BC diagnosis were included in the dataset. These weights taken from the medical notes could have been recorded during the BC follow up clinics, or by any staff in other departments or clinics at the hospital, that

the participant could have visited after being diagnosed with BC or near the time of diagnosis [i.e. cardiology reports, chemotherapy reports, et cetera (etc.)].

Three months after initial data collection, the first protocol amendment was approved and the following data were collected from the participants recruited from that date:

- Metabolic parameters (fasting insulin and glucose) at study entry only.
- Body adiposity parameters (FM, FM/FFM ratio and waist circumference), at study entry only.

3.4.3.2 Independent variables

Independent variables were retrieved from the hospital medical notes and also were self-reported in a brief interview conducted at the time of study entry which included:

- Biographic and behavioural data: date of birth, nationality, education level, ethnic origin, number of biological children, smoking status and number of cigarettes per day at diagnosis.
- Clinical data: height (at diagnosis and study entry), past and current medical history, menopausal status at diagnosis and at each year following diagnosis.
- FTO and M4CR genetic profile at study entry only.
- BC related data: diagnosis date, tumour characteristics, treatments received and dates of treatment's commencement and conclusion.

Data collected from fasting participants were taken in the morning and participants were offered some breakfast after taking blood samples and measuring body adiposity parameters.

3.4.3.3 Procedures and instruments of data collection

The instruments used for data collection for this study were blood samples, Tanita machine and a metric tape.

Blood samples were collected to measure genetic profile and metabolic parameters. One sample was collected to measure genetic profile. A second sample was used to determine fasting insulin, and a third sample was taken to read fasting glucose levels. The samples were collected early morning, from fasting participants (nothing to eat or drink for at least eight hours before the test), from a vein in the arm. Samples were taken by qualified and experienced nurses working in the diabetes department and were taken to the hospital laboratory. In there, they were handed to the laboratory team for the immediate analysis of fasting glucose.

Blood samples for genetic profile (FTO/Mc4R) were stored at -20 degrees Celsius (C), prior to deoxyribonucleic acid (DNA) extraction. DNA was extracted using QIAamp DNA Blood Mini Kit and was stored at -70C until further analysis. The two SNPs genotyped (FTO associated SNP rs9939609 and Mc4R associated SNP rs17782313) were selected based on their significant associations with obesity (Frayling et al. 2007; Freathy et al. 2008; Loos and Bouchard 2008; Loos et al. 2008). The genotyping of the two SNPs was performed using pyrosequencing with the QiagenPyroMark Q24 pyrosequencer using pyromark gold reagents. Results were automatically called using the proprietary software supplied with the analyser. All assays were performed in duplicate in a single run and where results were discordant/ambiguous the analysis was repeated in a separate run.

Fasting insulin were spun in the centrifuges at the hospital laboratories, at 4000 revolutions per minute for 10 minutes to obtain the serum, which was then stored at -40C until analysis. The samples were measured by automated chemiluminescence immunoassay. For insulin, the Siemens Immulite assay (Siemens Immulite assay kit, on a Siemens Immulite immunoassay analyser) was used. All analysis and calculation of results were automated. The lowest level that could be detected for insulin levels was 13.89 picomole per litre (pmol/l). The HOMA insulin sensitivity (HOMA IS) and HOMA IR were calculated using the HOMA Calculator developed by the Diabetes Trials Unit at the University of Oxford (Diabetes Trials Unit 2013).

On the other hand, glucose levels were determined using the standard protocol used in the hospital where the sample was drawn.

Body weight at study entry and FM and FFM levels were measured using an eight polar Electrodes Tanita BC-418MA Segmental Body Composition Analyser. The Tanita is a machine that measures body fat percentage, FM, FFM, total body water and predicted muscle mass on the basis of data obtained by Dual Energy X-ray Absorptiometry (DXA) using Bioelectrical Impedance Analysis (BIA) and information on current weight, height, age and impedance. The Tanita was graduated to 0.1 kg (Tanita Corportation 2014). The performance of the Tanita has been compared to other impedance systems (i.e. Bodystat) and it has been demonstrated to be an accurate (Jebb et al. 2000) and reliable method to measure body fat (Tanita Corportation 2014). Participants were asked to stand in the machine with bare feet onto two contact electrodes and to hold a pair of handgrips. Participants were asked to remove heavy clothes and to empty their urinary bladder before the measurement. The measurements were taken in less than 30 seconds.

Finally, a metric tape was used to record height and waist circumference. Height was measured by participants standing with bare feet against a wall. The measure contained one decimal. A waist circumference record had two decimals and was measured in centimetres (cm). Its value was obtained from the measure at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest, as recommended by the World Health Organization (World Health Organization 2008b), which also advised on other aspects when taking the measurement (i.e. participants' posture, respiration phase, or fasting state). There are difference protocols regarding the place of the tape when measuring waist circumference, i.e. using umbilical level or the minimal waist level. At the time of setting up this research protocol, the effects of the different methods of measurement used on measurement error or the prediction of abdominal fat had not been explored (World Health Organization 2008a), however, a systematic review concluded that the method of measuring waist circumference does not affect substantially the association between waist circumference values and health outcomes (i.e. mortality, risk of diabetes) (Ross et al. 2008).

With the emphasis lying on reducing the chances of measurement error, initially data collection was exclusively performed by the researcher. Nonetheless, as the study went on, a nurse researcher joined data collection and in order to reduce the likelihood of errors occurring and to preserve the integrity of the research, she received training and instructions on the correct use of instruments used.

The researcher entered all data collected onto a spread sheet in the same program used to analyse data: the International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics 20 computer software. Researcher also performed a data cleaning process: data entries were reviewed twice to verify that there were not entry errors (i.e. unusually large or small body weight value, unusual menopausal status for a giving age, etc.) or missing data (i.e. missing weight, or missing information about treatment received, or treatment dates, tumour characteristics, etc.). In the event of suspicious or missing data, the researcher reviewed the medical notes for an answer, and if the issue was unresolved, she contacted the participant (Web Center for Social Research Methods 2006).

3.5 Definition of the variables

Diagnosis date was defined as the date of the first histopathology or cytology report. For 236 participants (99.15%) of the sample, this report was available one month before BC surgery or the start of other BC treatments (mean 1.51 months, SD: 2.12). If date of the first histopathology or cytology report was not available (n=24), the date used as diagnosis date was the date of the first surgery. If both dates were not available (n=1), diagnosis date was reported as missing in the dataset.

The closest weight recorded around the BC diagnosis date was defined as weight at diagnosis. If there was no weight recorded within two months prior diagnosis and four months post-diagnosis date, weight value at diagnosis was considered missing. The definition of weight at diagnosis emerged after exploring the dataset and missing data (Appendix II), which showed that the window of margin chosen (± four months

post-diagnosis) decreased the number of participants with missing data for this variable.

Menopausal status at diagnosis was retrieved from the medical notes and was corroborated by the participant. Menopausal status in the years following diagnosis was self-reported and when possible, contrasted with the information on the BC treatment received, as some treatments are not relevant for premenopausal women (i.e. AINs) or postmenopausal women (i.e. gonadorelin analogues).

The guidance from the Faculty of Family Planning and Reproductive HealthCare (Faculty of Sexual & Reproductive Healthcare. Clinical Effectiveness Unit 2007) was used to assist classification of menopausal status when unsure, using age and menstrual cycle information, as these are the most useful factors to determine menopausal status (Faculty of Sexual & Reproductive Healthcare. Clinical Effectiveness Unit 2005). Accordingly, participants who fall into one of these conditions were considered postmenopausal:

- Women 50 years old or older with no menstrual periods in the last 12 months.
- Women younger than 50 years with no menstrual period in the last 24 months.
- Women with bilateral oophorectomy.

Furthermore, due to the fact that most women (95%) in the general population are menopausal by age 55 years and the perimenopausal period last for an average of five years (Faculty of Sexual & Reproductive Healthcare. Clinical Effectiveness Unit 2005), participants with hysterectomy were also classified as postmenopausal if they were either:

- Fifty-five years of age or older.
- Less than 55 years old and were using HRT for more than five years.
- Less than 55 years old and had symptoms of menopause for more than five years.

Participants were classified as premenopausal if:

- They had a uterus and no changes in the menstrual cycles from previous years.
- Women with hysterectomy, less than 55 years old, without HRT and without symptoms of menopause.

Finally, participants were considered to be perimenopausal if they fell under one of these situations:

- Younger than 55 years of age, who had a uterus and had experienced menstrual changes from previous years (i.e. period become irregular) and had at least one period in the past 12 months.
- Women with hysterectomy who are either:
    - Less than 55 years old with less than five years use of HRT
    - Less than 55 years old, who have symptoms of menopause for less than five years.

Participants who became either perimenopausal or postmenopausal in the interval between diagnosis to the evaluation point under examination were categorised as experiencing a change in menopausal status. Conversely, postmenopausal women at diagnosis and those pre or perimenopausal participants that did not experience any change in menopausal status were categorised as having no change in menopausal status.

3.6 Features of the dataset

The information described in this sub-section is useful to understand the terminology and the steps conducted for weight change modelling.

Variables collected were organised in two formats. The first one, a "person-level dataset" arrangement, was suitable to explore the magnitude and prevalence of weight change as well as to explore the body adiposity parameters and the metabolic status of BC participants. In this format, each participant had one record and multiple

variables: one for every weight record hold. This person-level dataset arrangement was then converted into a “person-period dataset”, a format in which each participant had multiple records, one for each time her weight was recorded. This person-period arrangement was most suitable for the modelling of weight change post-diagnosis.

3.6.1 Outcome, metric of time and predictors

The main outcome of the study was weight change, measured in kg. In order to quantify the frequency and magnitude of weight change post-diagnosis, weight was classified as weight at the time of BC diagnosis and weight post-diagnosis. Only the weights records at the desired evaluation points were used. On the other hand, all the weights available for each participant since the date of BC diagnosis to the study entry were used to model weight change. The metric of time used in the weight change modelling was months passed since BC diagnosis.

The dataset contained time-varying and time-invariant predictors. Time-invariant predictors’ value could not be changed during the follow up. Conversely, time-varying predictors could take different values during the follow up.

3.6.2 Follow up

Follow up starting point was set up as the time of BC diagnosis and ended at the time of study entry. The length of follow up differed from one participant to another ranging from 10.25 months to a maximum of 91.17 months post-diagnosis.

3.6.3 Waves of data collection and timing of weight measurement

Another peculiarity of data was the total number of waves of data collection (the number of observations or weights records available for each participant). This

number varied across participants. Eight participants (3.3% of the sample) had only one wave of data collection and that was their weight measured by the researcher at the time of study entry. One participant had 43 weight records. Thirty nine participants (16.3%) had a second weight available and 27 participants (11.3%) had three weights recorded from diagnosis to the end of the follow up. The remaining 165 participants (69.0%) have more weight records available since they were diagnosed with BC (Table 3, Appendix I). This number of weights recorded included those weights retrieved from the medical notes as well as the one weight taken as part of this research project at the time of study entry.

The timing at which participants' weights were recorded varied within each participant and between participants. The total number of weights recorded across all participants was 2,172 (Table 3, Appendix I). Half of these weights were recorded during the first 12.5 months of diagnosis, there were 266 weights recorded during the second year, 219 weights recorded during the third year and so on. This indicated that the timing of the weights records was erratic. The different and decreasing number of weights records held by participants seemed to be related to the use of chemotherapy, as it is explained in Appendix II.

3.6.4 Missing data

The variation in the number of weight records across participants raised questions about the completeness of the dataset and the presence of missing weight values. Fourteen participants (5.9% of the available 239 participants) did not have weights recorded in the first 12 months post-diagnosis. In the second and third year post-diagnosis, the proportion of participants with missing weights jumped to 44.5% and to 44.1% respectively. These figures highlighted the need to explore the reasons for the variations in the number of weight records across participants to assist decision making for data analysis. Could that variation represent missing records that were intended to be taken but were not? The following paragraphs answer that question and explain the potential mechanisms that led to the missing data, its impact and how missing data were dealt with during data analysis.

3.6.4.1 Are there missing data? Reasons for missing data

Yes, there are missing data in this dataset. All participants should have had a weight record in their medical notes around the time of diagnosis, as following the clinical protocol, they should have been weighed before surgery. However, some participants had missing weight values at BC diagnosis. Conversely, there were no missing values at study entry, as this was the only planned measurement occasion in this study.

A closer look at the dataset suggested that weight values might have been missing at any other point from the time of BC diagnosis to the end of the follow up. There were situations when it was obvious that a participant's weight was missing, for instance, if when reviewing the medical notes the place allocated to write down patient's weight was empty (i.e. in the chemotherapy drug record or anaesthesiology record). This was easily observed among participants treated with chemotherapy (more details in Appendix II). Unfortunately, the researcher did not record information on whether the weight was missing or not. Conversely, among non-chemotherapy-treated participants, the reduced number of weight records held was not considered missing data, as to the researcher's knowledge, this group of non-treated participants were not meant to be weighed up routinely as per clinical protocol, hence, it was difficult to ascertain when a participant's weight failed to be recorded (Appendix II).

3.6.4.2 Missingness mechanisms

There were two possible reasons that could have led to missing weight values. Firstly, a weight value was actually recorded in the medical notes, but the researcher failed to retrieve it from there. Nonetheless, this was unlikely as the researcher reviewed the medical notes twice. Secondly, the weight value was not recorded in the medical notes, due to at least one or more of the following reasons: 1) the hospital staff forgot to weigh the patient attending the BC clinic or any other department, 2) the staff did weigh the patient, but forgot to record her weight in the

medical notes, and 3) a patient might have refused to be weighed. Nonetheless, this was highly unlikely as usually weight is a parameter used for clinical decisions (i.e. to adjust the doses of chemotherapy treatment or anaesthesia). Consequently, although missing values in this dataset appeared to be linked to chemotherapy use, which was one of the predictors under exploration, it was concluded that the missingness records was at random (MAR), which implied that the probability of a missing value did not depend on the unobserved weight value (Singer and Willett 2003).

Similarly, any missing secondary outcomes (body adiposity and metabolic parameters) were regarded as MAR. The measurements were taken at study entry. None of the participants refused to be weighed and have their body adiposity parameters measured, however, the metric tape was not available to measure four participants' waist circumference, and the Tanita machine did not provide all the requested readings in 24 participants, leading to missing data. One participant did not want to have a blood test. The remaining 238 participants gave consent to have a blood test, although a number of different issues led to missing data: 1) the researcher failed to obtain a blood sample from four willing participants, 2) insulin levels could not be measured in seven haemolysed samples, 3) six blood samples were lost in the laboratory before any analysis was conducted on them, 4) fourteen samples were not stored properly and were lost, hence, some but not all the blood tests were conducted on them, and 5) there was insufficient funding to run the FTO/Mc4R tests on 96 samples.

Worth noting, 55 participants were recruited before the first protocol amendment, therefore, data on metabolic parameters and waist circumference were not available for them.

3.6.4.3 Dealing with missing data

Exploring the presence of missing weight values was important as they could affect the parameter estimators (Graham 2009). A complete case analysis, a procedure that

excludes participants with missing data, was the approach used to explore weight change at particular evaluation points, as it could be a useful way to deal with missing data (Graham 2009). Sensitivity analysis conducted (Appendix III) showed the validity of using complete-case analysis for the results presented in Chapter 4.

3.7 Data analysis

Data analysis was conducted in two main parts, defined by the two aims of the study. The first part of the analysis explored weight change. The second part of data analysis focused on the analysis of the body adiposity and metabolic parameters collected at the time of study entry. Before these two parts, data were initially analysed descriptively to get familiar with the features of data, to present the biological, behavioural, clinical and other relevant characteristics of the participants and to determine whether or not any unusual or missing values existed.

Weight change was explored from different angles and using different techniques that were considered more advantageous after taking into account the features of the dataset. The analysis started by looking at the magnitude of weight change between two evaluation points (i.e. from diagnosis to 12 months post-diagnosis, from diagnosis to 24 months post diagnosis, etc.). Most literature in the field explored weight change between two points. Therefore, this approach enhanced comparability with other studies, for instance with that of Makari-Judson and colleagues (2007) who also used paired t-tests to explore weight changes at 12, 24 and 36 months post-diagnosis.

The analysis then examined the frequency of weight change. Body weight change was defined as using different categories according to the direction of the change (i.e. weight gain, weight loss, no change). The analysis looked at the frequency of participants within each category of weight change from diagnosis to a particular evaluation point (i.e. diagnosis to 12 months). That would inform the reader of the heterogeneity in the phenomena of weight change across the sample.

Finally, the last section of this analysis examined the trajectory of weight change using multilevel modelling. This is a very advantageous approach because as opposed to other repeated measures techniques such as repeated measurement ANOVA, it is a very flexible technique that could deal with the unplanned unbalanced design of this study, and is not affected by missing data to the same extent, which allowed the researcher to maximise all (or most of) the 2,172 weight records collected across participants (Singer and Willet 2003).

All data analyses, including the multilevel modelling, were conducted with IBM SPSS Statistics 20 computer software.

3.7.1 Exploring the magnitude and frequency of weight change after breast cancer diagnosis

Weight change was analysed as difference in weight from diagnosis to each of the four evaluation points chosen: 12, 24, 36 and 48 months. Weight changes at 60, 72 and 84 months were not analysed as the number of participants with weight measure available at those periods was small (n=39, 27 and 16 respectively). Magnitude of weight change and the frequency of weight change from diagnosis to the chosen evaluation points were both explored as absolute weight change (kg) as a continuous variable and also as relative to the diagnosis weight (%). This increased comparability with other studies in the field.

The characteristics of the sample were summarised with means and SD for continuous variables and frequencies for categorical variables. Ninety-five percent CI were provided when exploring differences in the variable analysed.

The magnitude of weight change was explored using the weight record closest to each specific evaluation point, within a window period of +/- four months. If there was no weight recorded at those desired points (+/- four months), weight at that particular point was considered missing. When there were two weights recorded on the same date, or equidistant from the desired month, the mean weight of those dates was used. The ± 4 months range was arbitrarily chosen and thought to be appropriate

as this period window raised the percentage of participants with weight available at diagnosis and at 12 months, 24 months, 36 and 48 months post-diagnosis (Appendix II).The magnitude of weight change at those evaluation points was calculated as the difference between weight at each evaluation-point and the weight at BC diagnosis.

3.7.1.1 Analysis on the magnitude of weight change at 12, 24, 36 and 48 months post breast cancer diagnosis and factors related to it

Paired t-tests were used to assess whether weight change from diagnosis to each of the defined evaluation points (12, 24, 36 and 48 months post-diagnosis) was statistically significantly different from zero. Paired t-tests offered researcher a good initial look at the data before taking a more sophisticated approach to exploring weight change (i.e. using multilevel modelling analysis). Due to the presence of missing data, paired t-tests were preferred over other techniques that used list wise deletion (i.e. repeated measured ANOVA), as it allowed researcher to maximise the amount of participants included in the analysis. Furthermore, the use of paired t-tests was considered a reasonable choice as the sample sizes in each of the periods explored were larger than 50 and because this robust test could handle moderate departures from normality, such as the small one observed in weight change distribution (Appendix III) (Lund and Lund 2013). This small departure of normality suggested that the analysis of weight change should be conducted using distribution-free non-parametric tests. Unfortunately, non-parametric tests are less powerful compared to parametric tests, and therefore, they require a larger sample size to detect statistically significant differences. They also require that data from all groups to be compared in the analysis have the same dispersion. Conversely, parametric tests can deal with groups that have non-equal variances (Frost 2015). Most studies in the field have commonly used a parametric approach to explore weight change post-diagnosis. Parametric tests can work well with continuous variables that are non-normally distributed if the sample sizes are larger than 15 (Frost 2015), as it is the case of most of the analysis conducted in this study. Therefore, a parametric approach was used to analyse participants' weight change. Nonetheless, in order to verify the soundness of this decision, a sensitivity analysis was conducted and

weight change analysis was repeated using non-parametric tests (Appendix III). Furthermore, a sensitivity analysis was also conducted to explore the presence of outliers. Outliers were identified visually with boxplots (or scatter/dots for numerical variables). As routinely performed by the SPSS software used to analyse data, any value departing 1.5 or 3 box-lengths from the edge of the box was classified as outliers and was illustrated as a circular dot and as an asterisk respectively (Lund and Lund 2013). The outliers were dealt with according to the nature of the error: Data entry errors were corrected and the genuinely unusual values were not considered invalid and therefore, were initially included in the analysis (Lund and Lund 2013). In order to assess the impact of the outliers, the weight change analysis was conducted with and without outliers, as they can affect significantly the results of the parametric tests (Frost 2015). The results of the parametric tests were similar to the results of the non-parametric analyses, and the results of the tests conducted with outliers were comparable to the results conducted without outliers (Appendix III). Therefore, it was considered that both the use of parametric test and the inclusion of the outliers within the main analysis of weight change were decisions methodologically valid (Lund and Lund 2013a; Frost 2015). Weight change will be summarised with means and SD, as the visual inspection of the histograms of weight change suggested that the departure from normality was small (Figure 4, Appendix III).

Parametric independent sample t-tests were used to test the association of weight change with biological (i.e. menopausal status, genes) and clinical factors (i.e. BC treatment). As many studies in the field, and according to the aims of the study, weight change was analysed as a continuous variable and was measured in absolute terms (kg).

When exploring weight change in relation to BC treatment, participants were classified into one of the comparison groups (received treatment vs. not-received treatment) if they had been treated with the inspected treatment at any time from BC diagnosis to the evaluation point explored. As an example, if a participant started the treatment at 30 months post-diagnosis, she was included in the non-treatment group for the analysis of weight change at 12 and 24 months post-diagnosis and shifted to the treatment group for the analysis of weight change beyond 30 months (i.e. at 36

and 48 months post-diagnosis), as at those point, she had been or was being treated with that particular treatment.

When looking at the association between weight change post BC diagnosis and menopausal status at diagnosis, premenopausal and perimenopausal participants were grouped together because the sample size of the premenopausal and perimenopausal groups in each of the period explored was small (Table 4, Appendix I). Change in menopausal status was updated for the analysis of weight change at each of the evaluation point explored and participants were grouped accordingly, as occurred in the analysis of weight change in relation to treatment.

Due to small sample sizes in one of the three categories of the FTO and Mc4R genes (Table 5, Appendix I), the genetic variables were transformed into dichotonomous. The new categories were based on the presence vs. not presence of the risk allele A for the FTO gene and C for the Mc4R gene.

The Pearson's product moment correlation test was used to assess the association between weight change at 12, 24, 36 and 48 months post-diagnosis with the following covariates: weight at diagnosis, age at diagnosis and invasive tumour size.

One-way analysis of the variance (ANOVA) was used to explore stage of disease and weight change. Finally, smoking status at diagnosis and its impact of weight change post-diagnosis was analysed using independent sample t-tests.

Standard multiple linear regressions were conducted to look at the combined effect of two or more predictors of weight change at each particular evaluation point (12, 24, 36 and 48 months post-diagnosis). The variables of interest tested in the model were those mentioned in the aims of the study (Chapter 2): Clinical factors (BC treatments and tumour characteristics), biological and behavioural factors (menopausal status at diagnosis, transition to menopause, genetic profile, age, smoking status at diagnosis). All the variables were included into the models fitted. New models were created removing and adding new variables. Variables whose estimated coefficients were not statistically significant were removed from the analysis. The assumptions of homoscedasticity, linearity, independence of residuals, multicollinearity, normality of the residuals and the presence of unusual values of the

final model were assessed (data not shown) (Lund and Lund 2016). A scatter/dot plot of the studentized residuals and the unstandardised predicted values was used to check the assumption of linearity between the outcome and independent variables collectively, as well as the assumption of homoscedasticity. The linear relationship between the outcome and each independent variable was also analysed visual using partial regression plots. Independence of residuals was assessed using the Durbin-Watson statistic. A value close to 2 indicated that residuals were not correlated (Lund and Lund 2016). The presence of outliers was examined looking at the studentized deleted residuals (greater than ± 3 SD). The leverage score for each case was also inspected and those cases with high leverage value (more than 0.5) were considered unusual values. Finally, cases were considered influential points, and therefore unusual values, if the Cook's Distance values were above 1.0. A repeated analysis was conducted without the unusual values identified and it is detailed in Chapter 4, and in Appendix III (Lund and Lund 2016). The assumption of normality of the residuals was checked with a Normal Q-Q Plot of the studentized residuals (Lund and Lund 2016). Finally, multicollinearity between the variables included in each model fitted was assessed with an inspection of correlation coefficients and tolerance values provided by the software (Lund and Lund 2016).

Menopausal status at diagnosis and change in menopausal status post-diagnosis were correlated. Therefore, a new variable combining these two variables was created, with three categories: 1) pre and perimenopausal at diagnosis and change post-diagnosis, 2) remained pre or perimenopausal and 3) remained postmenopausal. The variable was updated at each evaluation point explored (12, 24, 36 and 48). This new variable created representing menopausal status was also correlated with age at diagnosis. The researcher decided to include age at diagnosis in the multiple regression analysis, and to excluded menopausal status. The rationale behind that decision was that menopausal status is a criterion to determine the use of AINs and gonadorelin analogues to treat BC, therefore, menopausal status could be predicted from these two treatments. Hence, age, as well as AINs and gonadorelin analogues use were included in the analysis, as the correlation between these two BC treatments and other variables in the models were below 0.7, and their tolerance

values were greater than 0.1, confirming that there were no collinearity issues with those variables.

Adjusted coefficient of determination (R^2) was used to evaluate the proportion of the variance in weight change explained by the models fitted. The adjusted R^2 and the p-value of the model were also used as criterion to evaluate the goodness of fit of the model and to choose the final model.

The final model will be presented with the unstandardised coefficients, 95% CI and their associated p-values. Included also will be the multiple Pearson's correlation coefficient (R), the ANOVA coefficients [regression' degrees of freedom (d.f.), the residuals' d.f. and the value of the F-statistics]. The statistical significance of the model will be also provided.

In the event that multiple linear regression showed that only one predictor was significant for a particular outcome, a simple linear regression was conducted provided that the outcome and the independent variable were linearly related. The assumptions of independence of observations, no outliers, homoscedasticity and normality of residuals were also assessed (Lund and Lund 2016).

A repeated analysis of the multiple regression was also conducted if the assumptions of the multiple regression analysis were not met (Appendix III). The repeated analysis indicated that the use of the multiple regression tests for the analysis presented in Chapter 4 was methodologically sound, except where stated.

3.7.1.2 Analysis of the frequency of weight change at 12, 24, 36 and 48 months post breast cancer diagnosis

The frequency of weight change was presented in absolute terms. Weight change was defined in three different ways, using different cut-offs. Firstly, weight change was defined as changes larger than 0.0 kg. Secondly, weight change was defined, as changes larger than 2.0 kg. Within this later classification, weight change was further sub-categorised as weight gain and as weight loss, and each sub-categories had

different groups (i.e. change of 2.1 to 5.0 kg, change of 5.1 to 10.0 kg and change larger than 10.1 kg). Finally, weight change was also explored using a relative cut-off: 0% of the weight at diagnosis. This was calculated as the percentage of weight change relative to weight at diagnosis:

> (Weight at a particular evaluation point – weight at diagnosis) x100 / weight at diagnosis

The relative weight change category was divided in three sub-categories: lost weight (more than 0.0% of their weight at diagnosis), gained weight (more than 0.0%) and maintained weight. Again, sub-groups were defined within the lost and gain weight sub-categories: weight change of from 0.1 to 5.0%, from 5.1 to 10.0%, from 10.1 to 15.0%, from 15.1 to 20.0% and more than 20.0%.

The decision of defining weight change in absolute and relative terms, as well as the use of those sub-categories (i.e. + 2 kg, or 5% weight change) were inspired by previous literature on weight change after BC (Rock et al. 1999; Lankester et al. 2002; Caan et al. 2006; Saquib et al. 2007; Caan et al. 2006, 2008; Han et al. 2009; Heideman et al. 2009; Caan et al. 2012b; Gu et al. 2010; Tredan et al. 2010; Chen et al. 2011; Nissen et al. 2011; Bradshaw et al. 2012; Sestak et al. 2012). The use of similar categories would facilitate the comparison of the results found in this study with previous literature. Furthermore, evidence showed that those figures could be clinically relevant (more details in Chapter 2).

3.7.2 Multilevel modelling for weight change after breast cancer diagnosis

The repeated weight measurements collected in this study were analysed using multilevel modelling. This is an advantageous technique to study weight change as it could answer two main types of questions: 1) how did each participant's weight change over time? And 2) is it possible to predict differences in weight change among participants with the variables collected in this study (i.e. BC treatment)?

Weight change multilevel modelling was conducted following the steps suggested by Singer and Willet (2003). It started with a descriptive exploratory analysis to get familiar with data, followed by the model fitting.

3.7.2.1 Multilevel model for change: descriptive analysis

Empirical weight growth plots were created to identify important data features, such as general patterns of weight change and functional forms, or to identify outlying participants whose weight values differ considerably from others (Singer and Willet 2003). Initially, the plots were summarised with a smooth trajectory, applying a non-parametric approach, and then using a parametric approach (ordinary least squares) regression (Singer and Willet 2003). The common functional form adopted for the exploratory analysis was a straight line, as this was the simplest functional form that could fit all data points. Nonetheless, because Heideman et al. (2009) modelled weight change after BC as a quadratic function, a set of polynomial shapes was also explored in this study to assess the quadratic effect of time (months from diagnosis) on weight change among participants. The visual inspection of the plots created suggested that weight change could be quadratic over time. Therefore, in order to simplify the analysis, the metric of the outcome (weight) and of time (months) were transformed aiming for linearity in the trajectory of weight change, but unfortunately, these techniques failed to achieve that goal. Consequently, data were initially modelled using a quadratic and a linear approach, as explained below.

3.7.2.2 Multilevel model for change: model fitting

The initial models fitted, explored weight change as a quadratic function. Participants with less than four recorded weights were excluded from the model in order to compare linear vs. quadratic weight change. Nonetheless, due to the complexity of the quadratic models fitted, a linear function was finally adopted to model weight change. This seemed adequate as previous research in the area also

analysed weight change post BC diagnosis as a linear trajectory (Camoriano et al. 1990; Rock et al. 1999; Demark-Wahnefried et al. 2001; Kroenke et al. 2005; Makari-Judson et al. 2007; Heideman et al. 2009; Gu et al. 2010; Vagenas et al. 2015).

The models fitted were divided into a level-1 sub-model, which described how each participant's weight changed after diagnosis, and a level-2 sub-model that described differences in weight change patterns across participants, as well as the association between predictors and the patterns of each person's weight change (Singer and Willet 2003). Each sub-model contained two parts: the structural part (fixed effects), which represented true scores and the stochastic part, which representes the measurement error (the residuals). The introduction of predictors (i.e. BC treatments) in the models aimed to reduce the magnitude of the residual variability left in the models fitted (Singer and Willet 2003).

Body weight was the outcome of level-1 sub-model, whereas the individual growth parameters (intercept and slopes) estimated in the level-1 sub-model were the outcomes of the level-2 sub-model. Consequently, the level-2 sub-model had two structural parts and two stochastic parts (one for each of its two outcomes). This feature allowed the level-1 parameters of one person to differ stochastically from those of others (Singer and Willet 2003).

The variables of interest or predictors were the 1) clinical factors (BC treatments and tumour characteristics); 2) biological and behavioural factors (menopausal status at diagnosis, transition to menopause, genetic profile, age, smoking status at diagnosis).

The parameters of the models were estimated using full maximum likelihood (ML) estimation. This is a popular approach for studies with large sample sizes because its estimates converge on the true values of the parameters of the population (asymptotically unbiased) and their sample distribution are approximately (asymptotically) normal with known variance. Furthermore, its SEs are smaller than those computed by other estimation methods (Singer and Willet 2003). The assumptions invoked for the ML estimation were: 1) the level-1 and level-2 residuals were normally distributed with mean zero, 2) the level-1 residuals were independent from level-2 residuals, and 3) all the residuals were independent of the predictors

included in the models (Singer and Willet 2003). An unstructured covariance matrix was specified for the model fitted.

The first two models created were the unconditional means model and the unconditional growth model. The unconditional growth model added the predictor 'months' into the level-1 sub-model, to account for the effect of time elapsed since diagnosis on body weight. These models were needed to investigate whether there was variation in weight that was worth exploring and to find out where the variation existed. Subsequent models adding and removing predictors were fitted to generate the potential final model that best fitted data. A predictor was removed from a model if the associated p-value provided by the software used to fit the models was consistent with a null hypothesis (i.e. the outcome for the average participant with the predictor tested was similar to the outcome for the average participant without the predictor) ($p>0.05$). An interpretation of the estimated fixed effects was carried in order to determine if the model predictors were conceptually sound.

In addition, pseudo-R^2 statistics were used to compare the proportion of variation in the level-1 or level-2 variances left unexplained between two models. Ideally, a reduction in the unexplained residual variation would be observed in the nested model compared to the full model it was nested from, which would indicate an improvement in the fit.

The Pseudo-R^2 statistics were computed as:

> Pseudo-R^2 statistics = (variance of model full model – variance of model nested model)/ variance of model full model

Deviance statistics were used as a criterion for hypothesis testing of the models. Deviance was preferred to single parameter tests of the fixed effects and variance components because (Singer and Willett 2003, pp.116):

- Had superior statistical properties.
- Permitted composite tests on several parameters simultaneously.
- Conserved the reservoir of type-one error.

Deviance statistics between two models were compared after ensuring that three conditions were met. First, the models were fitted using full ML. Second, the models were estimated using the same data. Third, one model (reduced model) was nested within the other (full model), in other words, every parameter of a nested (reduced) model appeared in the full model. The assumption hold by the difference in deviance statistics between the full and the nested model was that it is distributed asymptotically as a χ^2 distribution with d.f. equal to the number of contrains imposed (the difference in the number of parameters between the models compared). Therefore, the criteria followed to choose the final model were: 1) the value of its deviance statistics (these were similar or smaller than those in other models), and 2) because it was the simplest and conceptually sounded model that included significant predictors that seemed relevant to characterise individual weight post BC diagnosis based on data and the predictors collected in this study.

A correlation coefficient was used to explore the relationship between true initial status and true change, and was calculated using the following formula:

$$\text{Correlation} = \sigma_{01}/\sqrt{(\sigma_0^2\,\sigma_1^2)}$$

BC treatments were explored as time-invariant and as time-varying predictors. The main focus of the time-invariant models was the interaction between the BC treatment and time elapsed since diagnosis. Time-varying models assumed that any effect of the treatment started after the commencement date of the treatment and had a persistent effect. Therefore time-varying models provided additional information on whether the effects of the BC treatment on weight vary over time (i.e. the rate of weight change might speed up after the start of a treatment) and were useful to predict average differences in weight according to the predictors, at particular times. In both cases, the models fitted explored the main effects of the predictors, as well as the interaction between the BC treatments and months elapsed since diagnosis.

In the models with BC treatment used as time-varying predictors, the models looked at the main effects of the predictors as well as the interaction between the time-varying predictors and time passed since diagnosis (months), to allow the

trajectories' slopes to vary by the predictor values. The models only included the fixed effects of the treatments, as the random effects could not be fitted (as explained in the results Chapter). Therefore, the main effects of the time-varying predictors and their interaction with time elapsed since diagnosis was assumed to be constant across the population.

The first set of models fitted treated BC as time-invariant predictor. Therefore, each BC treatment had two mutually exclusive values: 1) users and 2) not-users. The models were split into sub-model 1 and sub-model 2. BC treatments were added as predictors in the between-person, level-2 sub-model. Hence, it was assumed that they were not going to be able to explain much of the within-person, level-1 sub-model's variance. The level-1 sub-model looked at individual weight change (within-person change) using months after BC diagnosis as a temporal predictor of the outcome (body weight) (Singer and Willet 2003):

$$Y_{ij} = {}_{0i} + {}_{1i}\,\text{Months}_{ij} + \varepsilon_{ij}$$

Where:

- Y_{ij} is woman i's weight value on occasion j.
- ${}_{0i}$ represents woman i's true initial status, the value of the outcome when Months_{ij} is zero.
- ${}_{1i}$ represents woman i's true rate of weight change during the period under study.
- ε_{ij} represents the portion of woman i's outcome that is not predicted by the model on occasion j.

The level-2 sub-model defined between-person differences in weight change. In this sub-model, the coefficients of the level-1 sub-model (true weight initial status ${}_{0i,}$ and true rate of weight change ${}_{1ij}$) became the sub-model outcomes, as follows (Singer and Willet 2003):

$${}_{0i} = \Upsilon_{00} + \Upsilon_{01}\,\text{PREDICTOR} + \zeta_{0i}$$

$${}_{1i} = \Upsilon_{10} + \Upsilon_{11}\,\text{PREDICTOR} + \zeta_{1i}$$

Where:

- Υ_{00} and Υ_{10} are the level-2 intercepts, and represent the population average initial weight (initial status, $_{0i}$) and the rate of weight change ($_{1i}$) respectively, when the predictors have a value of zero.
- $\Upsilon_{01}$and Υ_{11} are the level-2 slopes, and represent the effect of the predictor on the weight change trajectories, providing increments or decrements to initial weight ($_{0i}$) and rate of weight change ($_{1i}$) for the average woman with the predictor. If both parameters are zero, the average woman with the predictor weighs the same as the average women without the predictor, and their rate of weight change is also the same.
- ζ_{0i} and ζ_{1i} are the level-2 residuals, and indicate the portion of initial weight and of rate of weight change that are not explained at level-2.

The level-1 and level-2 sub-models could be condensed yielding a composite multilevel model for change (Singer and Willet 2003):

$$Y_{ij} = [\Upsilon_{00} + \Upsilon_{10}\text{Months}_{ij} + \Upsilon_{01}\,\text{PREDICTOR}_i + \Upsilon_{11}\text{PREDICTOR x (Months}_{ij})] + [\zeta_{0i} + \zeta_{1i}\text{Months}_{ij} + \varepsilon_{ij}]$$

Where:

- $\Upsilon_{00} + \Upsilon_{10}$ describe the population average of true initial status and true rate of change.
- $\Upsilon_{01} + \Upsilon_{11}$ describe between-person differences in initial status and rate of weight change, according to predictors.
- $[\zeta_{0i} + \zeta_{1i}\text{Months}_{ij} + \varepsilon_{ij}]$ describes the difference between the observed and the expected body weight for individual i on occasion j.

In this composite specification, body weight depended simultaneously on the number of months (a level-1 predictor), the predictor(s) at level-2 and the cross-level interaction between months and predictor(s) (Singer and Willet, 2003).

The next groups of models analysed BC treatments as time-varying predictors. The BC treatment values were: 1) using/had used (the treatment) and 2) have not used. Accordingly, any weight record measured before the commencement of the

treatment had a corresponding value of "have not used", whereas any weight record collected at or after the first day of the treatment was given a "using/had used" value. Therefore, these models assumed that any effect of the treatment would start after the commencement date of the treatment and have long lasting effects. As time-varying predictors, BC treatments were included as predictors in the level-1 sub-model, as follow:

$$Y_{ij} = {}_{0i} + {}_{1i}\,\text{Months}_{ij} + {}_{2i}\text{PREDICTOR} + \varepsilon_{ij}$$

On the other hand, the level-2 sub-model was defined as:

$${}_{0i} = \Upsilon_{00} + \Upsilon_{01} + \zeta_{0i}$$

$${}_{1i} = \Upsilon_{10} + \Upsilon_{11} + \zeta_{1i}$$

$${}_{2i} = \Upsilon_{20}$$

Where:

- ${}_{0i}$, the intercept, is the value of the outcome when months equals zero and no BC treatment (predictor) is used.
- ${}_{1i}$, the slope, is the rate of weight change, controlled for the effect of the predictor.
- ${}_{2i} = \Upsilon_{20}$

The composite multilevel model for change was represented as:

$$Y_{ij} = [\Upsilon_{00} + \Upsilon_{10}\text{Months}_{ij} + \Upsilon_{20}\text{PREDICTOR}_{ij} + \Upsilon_{30}\text{PREDICTOR}_{ij}\text{x Months}_{ij} + [\zeta_{0i} + \zeta_{1i}\text{Months}_{ij} + \varepsilon_{ij}]$$

Where:

- $\Upsilon_{00,}$ the intercept, represents the weight when months equals zero and predictor(s) have a value of zero (i.e. no BC treatment is used).
- Υ_{10} is the population average rate of weight change per month, controlling for the effects of the predictor(s).

- Υ_{20}, Υ_{30} and so on, represent the population average difference, over time, in weight values according to the predictor(s)' values (i.e. using/have used a BC treatment vs. non-users).

The properties of the residuals of the final models fitted were explored to assess whether the assumptions invoked in the models were met (Appendix IV). The following assumptions were made when fitting these models (Singer and Willet 2003).

- The shape of the individual weight change trajectory was linear. Similarly, the relationship of each individual growth parameters (intercept and slope) with the predictors included in the models was linear.
- The residuals were independently drawn from a univariate normal distribution at level-1 and a bivariate normal distribution at level-2.
- The residuals were not correlated with the predictors and were homoscedastic over all values of the predictors.
- The residuals could be autocorrelated and were heteroscedastic within-person.

Because information about the population from which the sample was drawn was lacking, it was not possible to ascertain completely the tenability of these assumptions (Singer and Willet 2003). Nonetheless, the observed properties of the residuals suggested that the models' assumptions were met (Appendix IV).

Results of the multilevel modelling are presented with the parameter value and its corresponding SE. For the final model fitted, 95% CI will be also included.

3.7.3 Adipose and metabolic parameters at the end of the follow up

The analysis of body adiposity parameters the metabolic status of participants and the factors associated with them followed the same data procedures as the analysis of weight change at the four evaluation points.

Nine participants known to have diabetes at the time of study entry were not included in the analysis of metabolic parameters. Laboratory data revealed that 32 of the included participants had an insulin level lower than 13.89 pmol/l, the lowest level the assay could detect. Creating insulin categories to analyse insulin levels as an ordinal variable would lose information. Therefore, it was decided that the variable would be analysed as numerical variable. Hence, three analyses were conducted. In the first one, all of these 32 participants were given an insulin level of 13.89 pmol/l, in the second one, a value of 7 pmol/l, and in the third one, a value of 1 pmol/l. Due to the fact the results were similar (Appendix V), it was decided that the main analysis would be conducted providing participants with an insulin value of 7 pmol/l.

Waist circumference and the metabolic parameters at the time of collection were not normally distributed (Appendix V). The value of their mean was comparable to their median (Appendix V). Therefore, for consistency with other outcomes presented in the study, the mean and SD will be used to summarise metabolic and adiposity parameters in Chapter 4. The sample sizes were larger than 15 (Frost 2015), and a sensitivity analysis conducted showed similar results when using parametric and non-parametric tests. Similarly, the presence of outliers did not modify the results of the parametric test (Appendix V). Therefore, parametric test were used to explore those outcomes, and outliers were included in the analyses presented in Chapter 4 (unless specified). Independent sample t-tests, correlation analysis and one-way ANOVA were used to explore factors associated to each body adiposity and metabolic parameters. Multiple linear regression analysis was performed to look at the combined effect of two or more variables that could predict those parameters, following the same steps conducted when exploring the magnitude of weight change at different evaluation points. As before, age at diagnosis was included in the analysis whereas the new variable created on menopausal status was excluded for the reasons explainer earlier on.

A repeated analysis of the multiple regressions was conducted if the assumptions of the multiple regression analysis were not met. This repeated analysis provided rationale for the use of this test, unless otherwise explained (Appendix V).

3.8 Ethical considerations

This study operates in accordance with Bournemouth University's ethic policies and procedures. The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust sponsored the study and since the target population involved NHS patients, the original study and the protocol ammendments obtained a favourable ethical opinioin and a site-specific assessment approval by the Dorset NHS Research Ethics Committee (REC reference 08/h0201/35). The study was also granted with the approval of the research governance from the NHS Research and development (R&D) department for the two NHS hospitals where participants were recruited and by the Research Governance Review Group at BU. The study was assessed as a basic science study type, as it involved procedures with human participants without affecting their clinical care that the participant would receive.As requested, the study commenced within 12 months of the date of the favourable ethical opinion and research governance approval. Annual progress reports were submitted to the Dorset NHS Research Ethics Committee, which also received the nofitication of the end of the study (June 2011), when data collection finished. A final report with a summary of the findings and arrangements for publication or dissemination will be submitted to the Dorset NHS Research Ethics Committee. All participants voluntarily gave written consent prior to taking part in the study and after they received accurate information about the study and its rights following local REC advice (Appendix VI).

A main ethical issue was that health professionals dealing directly with BC women were personally inviting them to take part in the study. That may have put some pressure on them to participate in the study, so in order to avoid that, women were reassured that the decision was voluntary and would not affect in any way the quality of treatment that they were receiving or about to receive. Due to the delicate condition of the target population, confidentiality was ensured. Participants knew what information was going to be collected from them. Data were collected and stored following Bournemouth University's Data Protection Policy and Guidelines and principles of The Data Protection Act of 1998.

Foreseen potential negative effects were both uncomfortable and distressing feelings when obtaining a blood sample and the minimal but potential risk of developing lymphoedema resulting from an infection in the venepuncture place (Clark et al. 2005). Therefore, in order to minimise the risks, qualified and experienced nurses took the blood samples. On the other hand, it is possible that participants felt comfort knowing that they were contributing to the improvement of future BC treatment. They knew they had access to the results of the test and study if they wished and that any adverse result identified during the study (i.e. unidentified diabetes) was going to be reported to the clinitians taking part in the study and the participant herself and that it was going to be effectively dealt with.

Accurate reports from this research project's findings will be shared with the scientific community, and a report with the main findings of this study will be available to the clinitians in the two hospitals where the study took place. They will be then able to share this findings with their patients and with the participants in this study, as they were told that they could have access to the findings of this study by contact their clinitians.

CHAPTER 4: RESULTS

4 Introduction

This chapter summarises data from the participants who were included in the database. The length of time that participants have been followed for varies across the cohort. Similarly, the numbers of participants with available outcome measures vary across the dataset, so sample sizes are provided for the analyses conducted.

The results presented here will follow the aims of the study as detailed in Chapter 2. It begins with a description of the participants included in the dataset. It continues by presenting the results related to the primary aim of the study (to explore the magnitude and frequency of weight change at 12, 24, 36 and 48 months post-diagnosis and the factors associated with the weight change), and then it shows the results of modelling weight change. The last section deals with the secondary aim of the study: exploring body adiposity and metabolic parameters at the end of the follow up, and the factors associated with them. A summary of the main findings is presented at the end of the chapter. Chapter 4 includes four appendixes. Appendix I collates the tables and figures of the main results described in this chapter, and appendixes III, IV and V present sensitivity analyses of the results presented here to validate the use of a parametric approach and to address the impact of outliers on the main findings.

4.1 Baseline characteristics of participants

4.1.1 General biological characteristics

This retrospective cohort consisted of a total of 239 participants newly diagnosed with BC between January 2003 and December 2009. Follow up began at the time of BC diagnosis and ended at the date of study entry, when the participants' final

weight record, metabolic and body adiposity parameters were collected by the researcher. Mean follow up time was 47.46 months (SD: 20.45), ranging from a minimum of 10.25 months to a maximum of 91.17 months post-diagnosis. This variability in the length of follow up is reflected in the decreasing number of participants followed as time post-diagnosis increases (Figure 1, Appendix I). For instance, 237 participants were followed at least 12 months post-diagnosis, whereas only 14 had 84 months of follow up.

Most participants (81.9%) were treated and recruited from Royal Bournemouth Hospital. On average, participants were diagnosed with BC when they were 57.5 years old (SD: 10.41). At that time, most of them were postmenopausal (72.8%), and reported to be non-smokers (89.2%) (Table 6, Appendix I).

Mean weight chosen as weight at diagnosis was 72.2 kg (SD: 13.6). This weight was recorded in the medical notes around 0.92 months (SD: 0.71) post-diagnosis. This was thought to represent a true baseline weight, despite the fact that 16 participants had just started their adjuvant treatment at the time of the record (four were receiving chemotherapy, three tamoxifen, eight were taking AINs and one was receiving gonadorelin analogues).

To determine the magnitude of the weight change effect size (small, medium, or large effect size in the popular Cohen's terminology), the SD of the weight at diagnosis (13.6) was multiplied by Cohen's standard d values that represent small, medium or large effect (d=0.2, 0.5 and 0.8 respectively) (Table 7, Appendix I) (Cohen 1992). The resulting values can be used as a guide to interpret the results of the statistical tests of weight change.

4.1.2 Breast cancer treatment received

Nearly all participants (96.2%) received adjuvant treatment (Table 8, Appendix I). Most participants received or were being treated with hormone therapy (86.1%) during or at the end of the follow up. Nearly 45% of them were treated with combined therapy, predominantly tamoxifen plus AINs (Table 9, Appendix I).

Tamoxifen treatment started a mean of 4.84 (SD: 5.31) months post-diagnosis. In total, 131 participants took tamoxifen for a mean of 23.65 (SD: 15.61) months. Eleven participants resumed tamoxifen treatment after initially having discontinued it. Anastrozole was the most common AIN used (n=106), and was used for the longest duration (mean 30.73, SD: 18.34 months). Anastrozole therapy started a mean of 9.94 (SD: 10.76) months post-diagnosis. Three participants resumed therapy for a further 22.78 (SD: 10.18) months. Letrozole treatment (n=18) started 20.29 (SD: 20.34) months post-diagnosis, and was used for a mean of 15.78 (SD: 11.89) months. Exemestane (n=48) commenced a mean of 27.49 (SD: 13.70) months after being diagnosed with BC, and was taken for a mean of 18.05 (SD: 13.25) months. Finally, gonadorelin use (n=21) started closer to diagnosis (4.98 months post-diagnosis, SD: 8.99), and was continued for a mean of 24.81 (SD: 14.67) months.

Chemotherapy was administered to over half of the sample (56.1%), whereas biological therapy with trastuzumab was rarely prescribed (5.5%) (Table 10, Appendix I). Chemotherapy was administered from a mean of 2.16 (SD: 1.31) months post-diagnosis to 6.47 (SD: 1.65) months post-diagnosis. Most participants treated with chemotherapy had anthracycline-containing regimens (95.5%) and 52.3% of them also received taxanes.

4.2 Number of participants with available weight data

This section details the number of participants included in the analyses of the weight change at 12 (± 4), 24 (± 4), 36 (± 4) and 48 (± 4) months post-diagnosis. A ± 4 months window period was chosen for those particular evaluation points because it produced larger sample size than smaller window periods (Appendix II).

The numbers of participants that were expected to be followed at 12, 24, 36 and 48 months differ from those represented in Figure 1 (Appendix I) (n=237, 207, 161 and 112, respectively) (Appendix I). The reasons for this discrepancy is that the window period for each evaluation point was widened from ± 0.5 to ± 4 months so that more participants could be included (Figure 1, Appendix I). Consequently, the total

number of participants with a minimum follow up of 12 (± 4) months was 239 (Table 11, Appendix I). A minimum follow up of 24 (± 4) months was achieved for 224 participants and 178 and 128 participants were followed for at least 36 (± 4) months or 48 (± 4) months respectively.

Participants were expected to have weight records available at diagnosis as well as at 12, 24, 36 and 48 months post-diagnosis. However, at the time of diagnosis weight records were only available for 92.5% of the sample population. The percentage of participants with recorded weight measurements at 12 months post-diagnosis dropped to 42.3% and continued at a similar level at 24 and 36 months post-diagnosis (46.4% and 43.8%, respectively). Weight at 48 months post-diagnosis was available for 68 out of the 128 (53%) participants who were expected to have a weight recorded at that evaluation point.

Participants with missing weight records either at diagnosis or at the month under exploration were excluded from the relevant analyses. Accordingly, only 41.4% (n=99) of the eligible participants were included in the analysis of weight change at 12 months post-diagnosis, 44.6% (n=100) at 24 months, 41.6% (n=74) at 36 months, and finally, 48% (n=62) were analysed for weight change at 48 months post-diagnosis.

There were no statistical differences in weight at diagnosis between participants included in the analyses presented in this chapter and those excluded due to missing values (details in Appendix II). Consequently, a complete-case analysis approach was taken for data analysis of weight change at 12, 24, 36 and 48 months post-diagnosis.

4.3 Magnitude and frequency of weight change after breast cancer diagnosis

One participant had two weights recorded on the same date. Because the weight values were different (77 and 76 kg respectively), the mean weight for that participant on that date was calculated. There were no weights measured at equidistant time from the desired evaluation point.

During the first 12 months post-diagnosis, mean weight change across all participants was + 1.30 kg (95% CI: 0.33 to 2.32, p=0.01), the equivalent of a mean of + 2.2% (95% CI: 0.91 to 3.41, p=0.01) over their baseline weight (Table 12 and Figure 2, Appendix I). The largest magnitude of mean weight change across the sample was observed at 36 months post-diagnosis: + 1.59 kg (95% CI: 0.39 to 2.79, p=0.01), the equivalent of a 2.67% increase relative to weight at diagnosis (95% CI: 1.05 to 4.29, p<0.01). The smallest magnitude of mean weight change occurred a year later (mean change + 0.42 kg, 95% CI: -0.99 to 1.84), which represented a non-statistically significant increase of 0.88% over the diagnosis weight (95% CI: -0.97 to 2.73, p=0.34).

The frequency of weight change varied according to the cut-off used. When looking at changes larger than 0.0 kg, results showed that most women experienced weight changes in the first two years of diagnosis (92.0% and 98% respectively). In the last two evaluation points explored, the totality of the sample100% had weight changes (Table 13, Appendix I). When weight change was defined as changes larger than 2 kg, data revealed that between 32.3 and 38% of the sample maintained their weight. Among those who experience weight changes larger than 2.0 kg, the percentage of women who gained weight at the four evaluation points ranged from 38.7% to 48.6%, and was larger than the percentage of women who lost weight at those points (ranging from 17.6% to 29.0%) (Table 14, Appendix I).

Seven participants had no weight change (more than 0.0 kg) at 12 months (Table 13, Appendix I). Nonetheless, records showed that five of them had lost weight by the next evaluation points, and one gained weight. The last one had missing data on weight beyond 12 months period.

Available data from 35 of the 60 participants who gained weight (at least + 0.1kg) during the first 12 months post-diagnosis revealed that eight of them had a net weight loss by the end of the follow up period: four of them lost between 0-5% of their baseline weight at diagnosis, three lost up to 10% of their baseline weight and one participant lost more than 10% of her weight at diagnosis. The remaining 27 participants gained weight at the end of the follow up in relation to their baseline weight.

On the other hand, data from 27 of the 32 participants who lost weight by the end of the first year post-diagnosis showed that 67% of them continued to lose weight by the end of the follow up period, whereas 33% had a net weight gain despite their initial weight loss.

In relative terms, weight changes larger than 0.0% of the weight at diagnosis were seen in nearly all participants. Half of the sample experienced weight changes larger than 5% of their baseline weight in the first year of diagnosis, and weight change over 10% relative to baseline weight were seen in around 12% (Table 15, Appendix I). In the following years, the frequency of weight change larger than 10% increased to 14% at 24 months post-diagnosis, to 16.2% at 36 months post-diagnosis and to 12.8% at 48 months post-diagnosis (Table 15, Appendix I). At all periods explored, the frequency of weight gain was superior to the frequency of weight loss. For instance, in the first year of diagnosis, 35.4% of participants gained more than 5% of their baseline weight and 8.1% have more than 10% weight increases. Conversely, 15.1% of the sample experienced weight loses of 5% of baseline weight, and 4% of participants had a weight loss of more than 10% of their weight at diagnosis (Table 15, Appendix I).

The effect size of the weight changes at 12, 24, 36 and 48 months-post-diagnosis was small according to Cohen's convention (Table 7, Appendix I).

4.4 Factors associated with weight change post-diagnosis

4.4.1 Univariable analysis of weight change

4.4.1.1 Breast cancer treatment

Tamoxifen and anastrozole were the only two treatments statistically associated with weight change (Table 16, Appendix I).

In all four evaluation points explored, the magnitude of the mean weight change was always larger and positive, among participants who had taken or were taking

tamoxifen (mean weight change ranting from +1.52 kg to +2.87 kg) compared to those who had never used it, who experienced an average negative weight change at 24 and 48 months post-diagnosis (mean weight change -0.81 kg and -1.01 kg respectively), and an average positive weight change in the remaining periods (mean weight change +0.67 and + 0.23 kg). Nonetheless, the differences in mean weight change between tamoxifen users and non-users reached statistical significance only at 24 and 36 months post-diagnosis (mean difference 3.68 kg, 95% CI: 1.62 to 5.75, $p<0.01$, and 2.38 kg, 95% CI: 0.01 to 4.75, $p=0.05$, respectively). Conversely, the magnitude of mean weight change was inferior among the group of participants that used anastrozole compared to non-users. The differences between the groups reached statistically significance at 48 months post-diagnosis, when anastrozole users' body weight decreased by an average of -0.97 kg, whereas non-users had a mean weight change of + 2.16 kg (mean difference between the groups: -3.13 kg, 95% CI: -5.92 to -0.34, $p=0.03$).

No other treatment was statistically associated with weight change post-diagnosis (Table 16, Appendix I). The magnitude of all the differences in mean weight change found was small considering Cohen's criteria (Table 7, Appendix I).

4.4.1.2 Menopausal status

On average, participants diagnosed before the menopause had a positive weight change in all of the four evaluation points explored, which ranged from + 1.90 kg to + 3.90 kg. The magnitude of their average weight change was larger than the mean weight change observed among postmenopausal participants, whose weight change was more erratic: It was positive in the first three years post-diagnosis (ranging from + 0.37 kg to + 1.23 kg), and negative at 48 months post-diagnosis (-0.69 kg). Differences in mean weight change between the groups were statistically significant at 48 months post-diagnosis only (mean difference 4.59 kg, 95% CI: 1.47 to 7.70, $p<0.01$) (Table 17, Appendix I).

After diagnosis, fewer than 30% of the pre and perimenopausal participants become peri or postmenopausal (Table 18, Appendix I), and on average, there was a larger weight change in this group of participants (mean weight change ranging from +2.01 kg to 3.91 kg) compared to participants who did not experience a change in menopausal status (mean weight change ranging from -0.35 kg to +1.33 kg). The magnitude of the differences in weight change between the two groups was small and only statistically significant at 12 and 48 months post-diagnosis when the mean differences were 2.29 kg (95% CI: 0.20 to 4.37, p=0.03) and 4.27 kg (95% CI: 1.08 to 7.45, p=0.01), respectively (Table 17, Appendix I).

4.4.1.3 Genetic profile

Participants carrying the A-allele of the FTO gene that has been associated with increased body weight had similar body weight at the time of BC diagnosis to non-carriers (mean difference -1.31 kg, 95% CI: -6.88 to 4.25, p=0.64). Both groups experienced an average positive weight change at the four evaluation points explored ranging from + 0.97 kg to + 1.30 kg in the A-allele carriers group, and from + 0.32 kg to 3.30 kg in the non-carriers group. Findings on the association of weight change post-diagnosis with FTO profile are inconsistent. Larger average weight changes were seen in the carriers group at 24 and 48 months post-diagnosis, whereas the opposite was seen at 12 and 36 months post-diagnosis. Nonetheless, the differences in mean weight change between the groups were not statistically significant (Table 19, Appendix I).

Similarly, there was no statistically significant differences in weight at diagnosis between carriers of the allele of the Mc4R gene associated with higher body weight (C allele) and non-carriers (mean difference 0.12 kg, 95% CI: -5.11 to 5.35, p=0.96). Unexpectedly, on average, carriers of the risk allele C experienced negative weight change at 24 and 48 months post-diagnosis (mean weight change -0.16 kg and -1.61 kg respectively). Conversely the non-carriers group had an average positive mean weight change at all the four evaluation points explored, and the magnitude of overall weight change was always larger compared to the non-carriers group (mean

weight differences between the groups ranged from -0.86 kg to -3.19 kg) (Table 19, Appendix I). Nonetheless, the magnitude of the differences was small and not statistically significant.

4.4.1.4 Other covariates of interest

Mean weight change was negatively correlated with lower body weight at diagnosis (at 36 months post-diagnosis: r=-0.32, p<0.01), and with age at the time of diagnosis (at 48 months post-diagnosis: r=-0.29, p=0.03 (Table 20, Appendix I).

Participants who smoke at the time of BC diagnosis experienced an average positive weight change post-diagnosis at the four evaluation points (ranging from + 0.66 kg to + 4.55 kg). The magnitude of their mean weight change was larger compared to the one observed among non-smokers, who experienced mean weight changes ranging from - 0.16 kg to + 1.38 kg. The differences between the groups were statistically significant at 12 and at 48 months post-diagnosis (mean difference + 3.49 kg, 95% CI: -0.18 to 6.80, p=0.04, and + 4.00 kg, 95% CI: 0.08 to 7.91, p=0.04 respectively) (Table 20, Appendix I).

4.4.2 Multivariable analysis of weight change

4.4.2.1 Weight change at 12 months after breast cancer diagnosis

Multiple regression analysis confirmed that weight at diagnosis and smoking status at diagnosis were statistically significantly associated with weight change in the first year of diagnosis (Table 21, Appendix I). The model that included these two variables was able to predict 19.5% of the variability in weight change observed at 12 months post-diagnosis (as determined by adjusted $R^{2,}$ p<0.01).

When all the other predictors were constant, the model predicted that at 12 months post-diagnosis:

- Each kg increase in body weight at diagnosis reduced body weight by 0.12 kg.
- Additionally, smokers had a weight change value that was + 5.44 kg (on average) higher than non-smokers.

4.4.2.2 Weight change at 24 months after breast cancer diagnosis

The model that explained the largest amount of the variance (18.9%) of weight change at 24 months included two variables: tamoxifen use and weight at diagnosis (both $p<0.01$) (Table 22, Appendix I).

In this model, when weight at diagnosis was similar, the model predicted that:

- Tamoxifen users had a weight change value that was + 4.05 kg (on average) higher than that of non-users.
- Each kg of body weight at diagnosis was associated with a reduction of 0.08 kg in the weight at 24 months post-diagnosis.

The analysis was repeated excluding an outlying participant who gained 15.92 kg and had a large studentized deleted residual (3.33). Both analyses showed similar results (Table 53, Appendix III).

4.4.2.3 Weight change at 36 months after breast cancer diagnosis

Weight at diagnosis and tamoxifen use were able to predict 12.5% of the variability in weight change at 36 months post-diagnosis ($p<0.01$, Table 23, Appendix I).

The model indicated that each kg at BC diagnosis was associated with a - 0.12 kg weight change at 36 months post-diagnosis ($p<0.01$), suggesting that heavier women experienced less weight change. Tamoxifen users had a weight change value that was 2.23 kg (on average) higher than that of non-users ($p=0.05$).

Data from one outlying participant with large weight change (+ 20.1 kg) and studentized deleted residual value (4.33) were included, as her inclusion in the analysis did not alter the results (Table 54, Appendix III).

4.4.2.4 Weight change at 48 months after breast cancer diagnosis

The model exploring weight change at 48 months excluded one outlier participant whose weight change was -18.5 kg, as sensitivity analysis showed that the exclusion of this participant from the analysis reduced R^2 (from 0.15 to 0.09) and changed the statistical significance of the predictors included in the model (Tables 55 and 56 in Appendix III).

Sensitivity analysis showed that after excluding this outlier participant, the only variable that remained associated with weight change at 48 months post-diagnosis was age at diagnosis.

A visual inspection of a scatter/dot plot of weight change at 48 months post-diagnosis against age at diagnosis (figure not shown) indicated a linear relationship between these two variables. All the assumptions were met to conduct a linear regression analysis: independence of observations, no outliers, homoscedasticity and normality of residuals.

Average age at diagnosis statistically significantly predicted weight change at 48 months post-diagnosis [F (1, 54) =5.31, p=0.02] and accounted for 7.3% of the variation in weight change at that point, a small size effect. In this linear model, each year of age at diagnosis reduced weight change at 48 months post-diagnosis by 0.12 kg (p=0.02, 95% CI: 1.41 to 13.86).

Overall, although the difference in weight change related to the predictors in these models was statistically significant, its magnitude was small. Nonetheless, the estimates of models fitted had medium to large effect sizes (R value) (Cohen 1992).

4.5 Multilevel model for weight change after breast cancer diagnosis

The multilevel models fitted describe individual's weight change after BC diagnosis and explore whether the individual trajectories of body weight differ according to the clinical (i.e. BC treatments), behavioural (i.e. smoking) and biological (i.e. genetic profile) variables analysed earlier. The outcomes shown in Tables 24-27 (Appendix I) represent part of the journey to find the best model to fit data.

4.5.1 Trajectory of weight change after breast cancer diagnosis

An exploratory assessment of data using empirical weight growth plots suggested some degree of heterogeneity in the pattern of weight change post-diagnosis. Some participants seemed to have a linear trend in weight change, whereas others' weight trajectories seemed to be quadratic (data not shown). Consequently, the first models were fitted to evaluate whether a higher order trajectory would fit data better than a linear one. The final number of participants included was 165. The first quadratic model seemed to provide a better fit than the linear change model, as evidenced by its smaller deviance statistic value compared to the linear model (difference 386), a value that exceeded the 0.05 probability threshold of a χ^2 distribution with four d.f. (value of 9.49). Unfortunately, the next quadratic models fitted to explore the effect of the variables (predictors) of interest became very complex, i.e. the coefficient for the quadratic term was statistically significant in the models with five or more predictors (data not shown), and there were convergence problems when exploring the random effects. Therefore, considering this and the fact that the dataset was severely unbalanced, it was deemed inappropriate to continue using quadratic models to explore weight change post BC-diagnosis. A cubic model was also fitted (data not shown) with those participants that had five or more weights recorded (n=138), however, there were convergence problems. Consequently, weight change was explored as a linear function.

In the first linear models created, BC treatments were included as time-invariant predictors (i.e. their values did not change over time). In the next section, BC

treatments were treated as time-varying predictors. Their values for any participant changed during the follow up.

4.5.2 Linear models with breast cancer treatment as time-invariant predictors

The models in Table 24 (Appendix I) explored the fixed effects of the BC treatments analysed as time-invariant predictors.

Model A, the unconditional means model (Table 24, Appendix I), was the first model fitted. The estimated within-person variance (8.95, SE: 0.03) and the estimated between-person variance (173.48, SE: 16.10) in this model were both statistically significant, suggesting that the average woman's body weight varies over time and that women's weight differ from each other. Because these variance components were statistically significant, their values could be reduced with the addition of predictors.

The intra-class correlation coefficient of 0.95 indicated that most of the variance was attributable to differences between participants. It also showed that for each person, the average correlation between any pair of residuals (i.e. between occasions 1 and 2, or 2 and 3) was 0.95. Hence, the composite residuals were auto-correlated.

Model B, the unconditional growth model (presented in Tables 24 and 26), accounted for the effect of time elapsed since diagnosis on body weight. The fixed effects showed that weight of the average woman diagnosed with BC increases steadily at a rate of 0.03 kg per month. The sub-level-1 within-person variance of Model B (σ_ε^2: 5.79, SE: 0.20) was smaller than the within-person variance in Model A (σ_ε^2: 8.95, SE: 0.29), which indicated that some of the within-person variation in weight could be attributable to months passed since diagnosis. The pseudo-R2 statistic computed from the differences in within-person variances in models A and B confirmed that finding: 35% of the within-person variation in weight could be explained by time elapsed since diagnosis in a linear trajectory. Therefore, Model B was better at predicting weight than Model A. Nonetheless, the within-person variance was still statistically significant, hence, there was room for other level-1

time-varying predictors that could further explain within-person variation in body weight. The variance components at level-2 showed that there was statistically significant variation in true individual initial status (σ_0^2**:** 171.61, SE:16.05) and, more importantly, in true rate of weight change (σ_1^2**:** 0.01, SE: 0.0014), which implied that there are differences among women in rate of weight change post-diagnosis that could be explained by predictors at level-2.

The population covariance of the level-2 residuals σ_{01} transformed into a correlation coefficient (value 0.10) showed that the relationship between the residual body weight at diagnosis and the residual true rate of weight change was negative and weak. Moreover, it was not statistically significant suggesting that weight at diagnosis was not statistically associated with rate of weight change.

The models C, D, E and F included BC treatments explored as time-invariant predictors (Table 24, Appendix I). Models C and D (and others not shown) guided decision-making leading to the final model (F). Model E included months, tamoxifen use and exemestane use as predictors of weight post-BC diagnosis. These two BC treatments were the only two that made a statistically significant contribution to the outcomes in previous models. The difference in deviance statistics between Model E and Model B was 13.47, a value that exceeded the 0.05 probability threshold of a χ^2 distribution with four d.f. (value of 9.49). Therefore, Model E with time-invariant predictors (tamoxifen and exemestane use) provided a better fit than Model B, which only accounted for the effects of months passed since diagnosis. The fixed effects shown in Table 24 (Appendix I) revealed that in Model E the interaction between exemestane use and months elapsed was not statistically significant. Hence, exemestane was not included in the next model fitted (F). Deviance statistics in Model F were statistically similar to the previous model E (Table 24, Appendix I), as the value of the difference (4.36) was inferior to the 0.05 probability threshold of a χ^2 distribution with two d.f. (value of 5.99). Because of the similarity of the deviance statistics and because Model F was more parsimonious than Model E, the former was considered the final model and is described as follows:

Model F included tamoxifen use as a predictor of initial status and of rate of weight change. The fixed effects in the final model F suggested that:

- The estimated initial weight for a women diagnosed with BC who received no treatment was 72.9 kg (SE: 2.64, $p<0.01$).
- The estimated rate of weight change for an average woman diagnosed with BC who was not taking tamoxifen was 0.0 kg per month (SE: 0.02, $p<0.01$).
- The estimated differential in the rate of weight change between tamoxifen users and non-users was 0.05 kg per month (SE: 0.01, $p<0.01$).

The level-1 residual (σ_ε^2 within-person) variance in model F was nearly similar to that in the unconditional growth model B (5.7900 vs. 5.7915). This was expected because time-invariant predictors cannot explain much of the within-person variance.

Nonetheless, this variance was still statistically significant, therefore it could be possible to reduce it by introducing more time-varying predictors. The only time-varying predictor in this study was menopausal status. However, because change in menopausal status correlated with age at diagnosis (r=0.74), age at diagnosis was chosen to be tested in multilevel modelling, as it is a more reliable measure than menopausal status. Age at diagnosis was also correlated with gonadorelin analogues use (r=0.61), a value that allowed both variables to be tested in the analysis. Nonetheless, neither age at diagnosis nor gonadorelin analogues use contributed significantly to the final model. The pseudo-R^2 statistic computed from the differences in between-person variances in rate of change in models B and models F showed that just 4.62% of the between-person variation in true rate of change was explained by tamoxifen use. As both level-2 variances were significant (individual initial status σ_0^2**:** 171.75, SE: 16.07, rate of weight change σ_1^2**:** 0.01, SE: 0.0014) additional predictors at level-2 could further reduce them. However, no other variable explored in this study (i.e. genes, smoking status at diagnosis, invasive tumour size or cancer stage) contributed statistically to Model F. Therefore, using the composite multilevel model for change, Model F was described as:

$$Weight_{ij} = [72.9 + 0.10Months_{ij} - 0.46tamoxifen_i - 0.05(tamoxifen_i \times Months_{ij})] + [\zeta_{0i} + \zeta_{1i}Months_{ij} + \varepsilon_{ij}]$$

4.5.3 Linear models with breast cancer treatments treated as time-varying predictors

The first two models fitted were the unconditional means model A and the unconditional growth model B (Table 24, Appendix I). Their features and characteristics were described earlier and will not be repeated in here. Nonetheless, the parameters estimated in unconditional growth model B were presented again in Table 26 (Appendix I) as they were used for comparison with later models to assess whether they fit data better.

Models in Table 26 (Appendix I) (and others not included in that table) present part of the journey of model fitting leading to the final model (4). Model 1 included all the BC treatments and models 2 and 3 were fitted after removing BC treatments that did not contribute statistically to the previous model fitted (Table 26, Appendix I).

The final model 4 included tamoxifen, anastrozole and letrozole. The value of the differences in deviance statistics between Model 4 and the unconditional growth model B was 118.84, a value that exceeded the 0.05 probability threshold of a χ^2 distribution with four d.f. (value of 9.49). Therefore, in accounting for the effects of these three BC treatments, Model 4 provided a better fit than the unconditional growth model B which only accounted for the effects of months passed since diagnosis.

The fixed effects in the final model 4 indicated that:

- The estimated weight for a women diagnosed with BC, at the time of diagnosis and who was not receiving tamoxifen, anastrozole or letrozole was 70.7 kg (SE: 1.59, $p<0.01$).
- The estimated population average rate of weight change was -0.06 kg/month (SE: 0.02, $p<0.01$), after controlling for the effects of these three treatments.
- The estimated population average difference, in weight values between those who used (or were using) tamoxifen and non-users was 1.09 kg (SE: 0.27, $p<0.01$).

- The estimated population average difference, in weight values between those who used (or were using) anastrozole and non-users was 0.85 kg (SE: 0.32, $p<0.01$).
- The estimated population average difference, in weight values between those who used (or were using) letrozole and non-users was 2.60 kg (SE: 0.54, $p<0.01$).
- The coefficient for interaction of anastrozole with months was statistically significant (0.05, $p<0.01$), indicating that the effect of anastrozole on weight varies over time.

The level-1 residual (σ_ε^2 within-person) variance in Model 4 was lower than the variance component in the unconditional growth model B (5.64 vs. 5.79). The variance was statistically significant; therefore the addition of other time-varying predictors might have reduced its value. However, no other predictor in this study improved the variance components of Model 4. Level-2 variance was also lower in Model 4 compared to Model B, nonetheless, changes in level-2 variances might not be meaningful (Singer and Willet, 2003) and therefore will not be mentioned.

Using the composite multilevel model for change, Model 4 was described as:

$$\text{Weight}_{ij} = [70.7 + 0.06\text{Months}_{ij} - 1.09\text{tamoxifen}_{ij} - 0.85\text{anastrozole}_{ij} + 2.60\text{letrozole}_{ij} + 0.05\text{anastrozole}_{ij}\text{x Months}_{ij} + [\zeta_{0i} + \zeta_{1i}\text{Months}_{ij} + \varepsilon_{ij}]$$

The parameters for the main effects of tamoxifen and anastrozole were negative, implying that treatment users had higher weight compared to non-users. Conversely, the parameters for the main effect of letrozole were positive, therefore, the estimated weight of letrozole users was lower than that of non-users. The estimated weights for each year of diagnosis as predicted by Model 4 are presented in Table 28 (Appendix I).

A final question that was explored was whether the main effects of tamoxifen, anastrozole, or letrozole could vary across the population. Unfortunately, due to convergence problems it was not possible to fit a model exploring the random effects of the main effects of these three BC treatments to data. Nonetheless, a new model looking at the random effects of the interaction of anastrozole with months passed

since diagnosis was fitted (Model 5 in Table 27, Appendix I). However, allowing the interaction of anastrozole with months passed since diagnosis to vary across the participants, and not allowing the main effect of the treatment to vary would not be wise. For that reason, Model 4 was chosen as the final model.

4.6 Adiposity parameters at the end of the follow up

On average, participants had a mean of 37.8% (SD: 6.24) body fat, a mean of 28.48 kg (SD: 9.43) of FM, a mean of 45.05 kg (SD: 5.65) of FFM and a mean FM/FFM ratio of 0.62 (SD: 0.16). The mean waist circumference was 92.3 cm (SD: 11.82).

4.6.1 Univariable analysis of body adiposity parameters at the end of the follow up

4.6.1.1 Breast cancer treatment

Chemotherapy users had healthier body adiposity parameters than non-users, with lower fat levels and lower waist circumference values, although the differences between users and non-users were not statistically significant (Table 29, Appendix I). Taxane-containing regimens were not statistically associated with the body adiposity parameters measured (data not shown). Anastrozole was the only hormonal treatment associated with body adiposity outcomes. Participants who had taken or were taking anastrozole had significantly higher body fat % (mean difference 2.04%, 95% CI: 0.39 to 3.69, p=0.02) and higher FM/FFM ratio (mean difference 0.05, 95% CI: 0.00 to 0.09, p=0.03) compared to non-users. Waist circumference was also higher among anastrozole users, but the differences were not statistically significant (Table 29, Appendix I).

4.6.1.2 Menopausal status

Participants diagnosed with BC after the menopause had significantly higher levels of body fat percentage (mean difference 2.42%, 95% CI: -4.25 to -0.58, p=0.01), FM/FFM ratio (mean difference 0.05, 95% CI: -0.10 to -0.00, p=0.03) and waist circumference (mean difference 4.45 cm, 95% CI: -8.52 to -0.38, p=0.03) than participants who were pre or perimenopausal at the time of BC diagnosis (Table 30, Appendix I).

Change in menopausal status post-diagnosis was also statistically related to body adiposity parameters. Fewer than 30% of participants experienced a change in menopausal status during the follow up period. Those who did had less body fat and a lower waist circumference than those whose menopausal status did not change after BC diagnosis (mean difference of body fat percentage: 2.42%, 95% CI: -4.31 to -0.53, p=0.02, difference of FM/FFM ratio: 0.05, 95% CI: -0.10 to -0.00, p=0.04, and difference of waist circumference: 3.65cm, 95% CI: -7.85 to -0.55, p=0.05) (Table 30, Appendix I).

4.6.1.3 Genetic profile

Participants with the risk A and C alleles of the FTO and Mc4R genes had a non-significantly lower body fat percentage and smaller waist circumferences than non-carriers (Table 31, Appendix I).

4.6.1.4 Other covariates of interest

Older age was associated with larger body adiposity parameters (Pearson's r coefficient ranged from 0.15 to 0.21, p-values from <0.01 to 0.03) (Table 32, Appendix I).

4.6.2 Multivariable analysis of body adiposity parameters at the end of the follow up

4.6.2.1 Percentage of body fat

Percentage of body fat were predicted by a model that included weight and age at diagnosis (adjusted R^2 =0.53, p<0.01) (Table 33, Appendix I). When these variables were accounted for, anastrozole was no longer associated with fat %.

The model indicated that when all the other predictors were similar:

- Body fat percentage increased by 0.35 per one kg of body weight at diagnosis.
- Each one year of age increased body fat by 0.11%.

The results of the model did not vary after excluding one outlier participant with a studentized deleted residual value of - 3.70, and 27.7% of body fat (Table 69, Appendix V).

4.6.2.2 Fat mass/fat free mass ratio

The combination of weight at diagnosis and age at diagnosis was able to explain 54.4% of the variance of FM/FFM ratio (p<0.01) (Table 34, Appendix I).

According to the model:

- Each kg of weight at diagnosis increased the FM/FFM ratio by 0.01.
- Each year of age added 0.002 to the FM/FFM ratio value.

The magnitudes of the coefficients and of the p-values were very similar to those conducted excluding two participants with studentized deleted residual values of -3.60, and 3.28 respectively, and with FM/FFM ratio values of 0.38 and 1.04, respectively (Table 70, Appendix V).

4.6.2.3 Waist circumference

After considering all the variables explored, waist circumference was best predicted by the combination of weight at diagnosis and age at diagnosis (all $p<0.01$, adjusted R^2: 0.56) (Table 35, Appendix I).

According to the model:

- Each one year of age predicted an increase of 0.17 cm to the waist circumference, when weight at diagnosis was kept constant.
- Each kg of body weight at BC diagnosis increased waist circumference by 0.66 cm.

A repeated analysis excluding one participant with a large studentized deleted residual value (-3.71) and a waist circumference of 86 cm produced similar coefficients and p-values (Table 71, Appendix V). Hence, her data were included in the analysis presented in Table 35 (Appendix I).

4.7 Metabolic parameters at the end of the follow up

Nine participants known to have diabetes at the time of study entry were excluded from the analysis of metabolic parameters. Data from the remaining participants with available records showed that average fasting glucose was 5.14 milimol per litre (mmol/l) (SD: 0.77), ranging from 3.10 to 10.80 mmol/l (n=167), and their average fasting insulin level was 39.31 pmol/l (SD: 2.63), ranging from 7.00 to 184.74 pmol/l (n=147). HOMA IS and HOMA IR values were 128.92 (SD: 59.19) and 0.99 (SD: 0.56) respectively.

Eight participants (4.8%) had fasting glucose levels above the normal level (more than 6.0 mmol/l). Three of them had values corresponding to pre-diabetes (6.1 to 6.9 mmol/l) and five participants had glucose levels that fall within the diabetic range (more than 6.9 mmol/l) (Diabetes.co.uk 2016).

4.7.1 Univariable analysis of metabolic parameters at the end of the follow up

Chemotherapy and exemestane use were statistically significantly associated with insulin levels. The mean difference between chemotherapy users and non-users was -10.43 pmol/l (95% CI: -20.74 to -12, p=0.04), and mean difference between exemestane users and non-users was - 6.00 pmol/l (95% CI: -24.82 to -1.10, p=0.03). Conversely, letrozole users had higher insulin levels compared to non-users (mean difference 24.03 pmol/l, 95% CI: 4.56 to 43.49, p=0.02) (Table 36, Appendix I).

Menopausal status was not associated with metabolic parameters (Table 37, Appendix I).

Carriers of the risk alleles for higher body weight of both FTO and Mc4R genes had statistically non-significantly lower glucose levels and higher insulin levels than non-carriers (Table 38, Appendix I).

Insulin levels were negatively related to time elapsed between BC diagnosis and measurement of metabolic parameters (r=-0.21, p=0.01), but not with weight change post-diagnosis (r=-0.07, p=0.44) (Table 39, Appendix I).

4.7.2 Multivariable analysis of metabolic parameters at the end of the follow up

Initially, the model predicting glucose levels that best fitted data included weight at diagnosis and letrozole use. However, sensitivity analysis showed that after excluding two outlier participants with the highest glucose levels (10.8 and 8.80 mmol/l, studentized deleted residual values: -7.90 and 4.43, and Cook's distance: 1.80), letrozole use no longer added any statistical value to the model's prediction of glucose levels (Tables 72 and 73, Appendix V), and R^2 decreased from 0.13 to 0.09. Therefore, Multiple linear regression analysis was repeated without those participants and the final model included weight at diagnosis and FTO genetic profile (adjusted R^2: 0.10, p=0.02) (Table 40, Appendix I). The model predicted that when the other predictors were held constant:

- Each kg of body weight at the time of diagnosis was associated with an increase in glucose levels by 0.01 pmol/l ($p<0.01$).
- Carriers of the risk FTO A-allele had 0.28 pmol/l higher glucose levels compared to non-carriers.

The resulting model met all the assumptions of a multiple regression analysis.

Similarly, fasting insulin level was best predicted by a model that included weight at diagnosis and FTO genetic profile (R^2: 0.37%, $p<0.01$) (Table 41, Appendix I). When the other predictors were held constant, the model predicted that:

- Each kg of body weight at the time of diagnosis was associated with an increase of insulin levels by 1.17 pmol/l ($p<0.01$).
- Carriers of the risk FTO A-allele had 15.20 pmol/l higher insulin levels than those of non-carriers.

This model included a participant with an outlying large studentized deleted residual value (3.5) and fasting insulin level of 142.48 pmol/l as inclusion of her data did not alter the findings (Table 74, Appendix V).

The addition of other predictors to the model did not add any significant predictive value to the models fitted.

4.8 Summary of the findings

A mean positive weight change was found from diagnosis to the four evaluation points explored. Weight change across the sample was statistically significant, although it magnitude was small. Most participants experienced weight change (defined as changes larger than 0.0kg or 0.0% of baseline weight). At each of the four evaluation points explored, the number of participants that experienced weight gain was larger than the number of participants that experience weight loss. For instance, among those whose weight changed, relative weight gains over 5% were seen in 24.1% to 35.5% of participants, whereas a 5% or more decrease of weight was seen in 13.5% to 17.7% of participants.

Multilevel modelling of weight change also indicated that weight varied over time and that most of the variance in body weight was found between-person rather than within-person. It also suggested that weight change was heterogenic across the sample, and for most participants, it followed a quadratic trajectory rather than a linear trajectory. In line with that, weight change was observed in both directions, and a small number of participants who gained weight in the first 12 months post-diagnosis lost it later on, and some of those who lost weight in the first year gained weight by the time of study entry.

The factors associated with weight changes from diagnosis to 12, 24, 36 and 48 months post-diagnosis were body weight, smoking status and age at diagnosis, as well as tamoxifen use post-diagnosis. Multilevel modelling predicted that the rate of weight change varied according to tamoxifen use and that body weight at any time post-diagnosis could be predicted by tamoxifen, anastrozole and letrozole use.

Multiple regression analysis showed that body adiposity parameters at study entry were associated with weight and age at the time of diagnosis. Together with FTO genetic profile, weight at diagnosis was also a predictor of insulin and glucose levels at study entry. No other factor explored in this study was associated with participants' adiposity and metabolic parameters.

CHAPTER 5: DISCUSSION AND CONCLUSSIONS

5 Introduction

This retrospective cohort study of 239 participants diagnosed with early BC aimed to explore weight changes from diagnosis to the end of the follow up period, on average, 48 months post-diagnosis, and to look at the association of BC treatments, menopausal status and genes with weight change post-diagnosis. A further aim was to test the association of those parameters with body adiposity and metabolic parameters collected at the end of the follow up period.

This chapter discusses the results presented in the previous chapter, demonstrating how they coincide with other studies, and how they add new evidence which might open lines of investigation for a better understanding of changes in weight, metabolic and adiposity parameters after BC diagnosis. The implications for practice will be detailed just before the conclusions of the discussion.

5.1 The sample

It was expected that at least 500 women treated with early BC would be seen in the Oncology and Surgical follow up clinics. Nonetheless, it was not possible to know whether they all would meet the inclusion criteria of this study, and therefore, the rate of participation in the study is unknown. Moreover, since the researcher was not the person directly inviting BC patients to take part in this study, it is not possible to know the exact number of BC patients approached. However, it is known that non-participation existed, as some BC patients filled out the optional decline slip to inform the research team that she did not want to take part in the study and the reasons why. It is also unknown whether those who declined to take part in this study were different to those who participated.

It is possible that self-selection bias are present if those who gained a lot of weight and felt embarrassed by and did not take part in the study. Similarly, it is possible

that those who lost weight or those who felt their weight was not altered after diagnosis thought that this study was not applicable to them. This risk of selection bias was addressed by explaining to the potential participants the importance of taking part in the study, regardless of whether they experienced weight changes or either direction of weight change.

Arguably, the participants in this study are fairly representative of the UK population of BC patients. The participants' characteristics presented in Chapter 4 seem to reflect UK national figures. Nearly 50% of study participants were 60 years of age or older, a proportion that is representative of BC women over 60 years diagnosed in the UK each year (46%) (Cancer Research UK 2014a). In addition, the prevalence of ER and HER-2 tumours in the sample is comparable to cases in the UK (Cancer Research UK 2014b). Furthermore, the percentages of different non-white ethnic groups mirror the English national figures: 1.5% Asian cases, 1% black cases, or 0.2% Chinese cases (National Cancer Intelligence Network 2010).

Interestingly, findings showed that in this study, 117 participants (around 60% of the sample) were overweight or obese at the time of diagnosis. Eighty nine of them were postmenopausal and 28 were pre or peri menopausal (data not shown). These figures are consistent with the fact that obesity is a risk factor for post-menopausal BC (Neuhouser et al. 2015). Nonetheless, the prevalence of overweight or obesity in this study is slightly higher than the figures reported in other cohort studies like the WHEL (57%) (Saquib et al. 2007), or the LACE and WHEL combined (50.85%) (Caan et al. 2006). The higher prevalence of overweight/obese participants in this study might have led to an underestimation of the true association between predictors and weight change, as findings showed that weight at the time of diagnosis was negatively correlated with weight change in the first three years post-diagnosis, which suggests that heavier participants tend to experience less weight change than leaner participants. Arguably, this finding could be the result of the regression to the mean, a common statistical artefact in repeated datasets with non-systematic (random) errors and random fluctuation, where initial extreme values far from the true mean (i.e. low or large body weight) would be likely to be followed by less extreme values nearer the subject's true mean (Barnett et al. 2005). Nonetheless, it is a finding that has been reported by large cohort studies (i.e. Kroenke et al. 2005;

Caan et al. 2006; Saquib et al. 2007; Chen et al. 2011), Consequently, it is possible that weight changes of higher magnitude could have been observed if the percentage of overweight or obese participants would have been lower.

Most participants were recruited from Royal Bournemouth Hospital because recruitment in Poole Hospital started ten months later. It is possible that patients attending Royal Bournemouth Hospital might be different from those attending Poole Hospital. Nonetheless, no statistically significant differences were found between the two groups in the frequency of baseline characteristics or outcomes values (i.e. age, education level, percentage of smokers at diagnosis, rate of pre/perimenopausal women/postmenopausal status at diagnosis, treatment and tumour characteristics, weight at diagnosis, months from diagnosis to the end of the follow up, weight at study entry, adiposity parameters, insulin and glucose levels) (data not shown). Therefore, the fact that most participants were recruited from Royal Bournemouth Hospital might not have implications affecting the findings in this study, and hence, both groups of participants might benefit equally from the recommendations derived from the results of this study detailed in the next paragraphs.

5.2 Weight change after breast cancer diagnosis

5.2.1 Magnitude and frequency of weight change after breast cancer diagnosis

The analysis of the magnitude and the frequency of weight change revealed that weight changed at 12, 24, 36 and 48 months post-diagnosis. On average, the largest magnitude of weight change was observed 36 months post-diagnosis (mean change of + 2.67% of diagnosis weight, representing a weight change of + 1.59 kg). The smallest change in weight was found a year later (mean + 0.88%, a + 0.42 kg weight change). This finding is consistent with previous studies in the field which indicated that for 10% of BC survivors, the weight gained post-diagnosis reverses during follow up (Saquib et al. 2007). In this study nearly 23% of participants who gained weight 12 months post-diagnosis lost all the weight gained by the end of follow up.

Interestingly, more than 33% of the participants who initially lost weight gained weight later on.

The true weight at diagnosis in the population from which this sample was drawn is unknown. Therefore, it is difficult to ascertain whether the weight change found is a real weight change, or an expected finding resulting from the regression to the mean. Nonetheless, the regression to the mean effect was addressed in the multivariable analysis, which controls for the effect of weight at diagnosis.

The percentage of participants that experienced weight change larger than 0.0 kg was over 92% in the first year and nearly 100% in the years after that. Weight changes over 5% and 10% were seen in both directions. At each of the four period explored, around 8% of the sample gained more than 10% of their diagnosis weight and around 5% of the sample lost that amount. And the percentage of participants that experienced 5% weight gain, or 5% weight loss, ranged from 24.1 to 35.4% and from 13.5 to 17.7% respectively. These findings are comparable to previous small and large cohort studies, including the ABCPP, that reported that 10% to 60% of BC survivors gained more than 5.0% of initial body weight, and 15% to 27% lost weight, over an average of two to seven years post-diagnosis (Rock et al. 1999; Caan et al. 2006, 2008; Han et al. 2009; Tredan et al. 2010; Nissen et al. 2011; Bradshaw et al. 2012; Caan et al. 2012b; Sedjo et al. 2014).

In this study, weight from diagnosis to 12 months remained unchanged in seven participants (0.0% weight change), although available data indicated that their weight changed after that. Several studies reported that some women diagnosed with BC maintained their weight post-diagnosis (Lankester et al. 2002; Han et al. 2009; Gu et al. 2010; Chen et al. 2011; Bradshaw et al. 2012). Nonetheless, it is worth mentioning that the definition of weight maintenance, in other words, no weight change, varies across the studies, and in most cases, it involved some degree of weight change. Therefore, findings in this study did not contradict previous literature. Most commonly used definition of weight maintenance in the field of weight change post BC included within 2 kg of baseline (Lankester et al. 2002; Gu et al. 2010; Chen et al. 2011), or within 5% of body weight (Rock et al. 1999; Han et al. 2009; Nissen et al. 2011; Bradshaw et al. 2012; Caan et al. 2012b).

Exploring the magnitude as well as the frequency of weight change allowed researcher to indentify different groups of participants: those who experienced small magnitude of weight change, those whose weight suffered large variations, as well as those whose weight did not change (at least in the first year of diagnosis). The clinical implications for these subgroups of participants seem to be different in the light of knowledge derived from survival studies, as it is explained below.

5.2.1.1 Is the magnitude of weight change different from the healthy population?

In the general population, weight change is common among women as they age. The National Health and Nutrition Examination Survey found that in a 10-year period, women aged 25 to 34 gained 3.45 kg (0.35 kg/year), and those 45 to 55 years gained 0.79 kg (0.08 kg/yr), whereas women 55 and older lost weight (Williamson et al. 1990). Results from the IBSI-1 of postmenopausal women with a high risk of BC found weight gains of 1.0 kg (SD: 1.5) after 12 months follow up, and a gain of 1.3 kg (SD: 5.7) at 60 months (Sestak et al. 2012). Although the findings from those studies cannot be compared directly with the results in the present investigation, their findings and this study's findings suggest that on average, women diagnosed with BC experienced larger weight changes (which on average were positive) than women not diagnosed with BC. Supporting that, at least three studies have provided evidence that BC patients gain more weight than age-matched controls (Sestak et al. 2012; Gross et al. 2015; Vagenas et al. 2015). BC survivors gained significantly more weight [β = + 3.06 pounds (lb), 95% CI: 0.94 to 5.17] than cancer-free women in Gross'et al. (2015) study. Vagenas and colleagues (2015) found that 56% of BC patients gained weight (mean + 5.3 kg) between six and 72 months post- BC surgery, and nearly 80% of them experienced greater gains than the average weight gain seen in sex- and age-matched controls.

5.2.1.2 Potential clinical implications of the weight change found

There is evidence that large weight changes (in both directions) post-diagnosis could have and undesirable effect on BC prognosis and on women's health.

Nichols et al. (2009) found that each 5 kg gained during the first six years post-diagnosis was associated with a 12% increase in all-cause mortality, a 13% increase in BC specific mortality, and a 19% increase in mortality from cardiovascular disease. Nonetheless, in the ABCPP, the risk of death was only associated with substantial weight gain (more than 10% of diagnosis weight, mean + 10.5 kg) during the first two years of diagnosis (Caan et al. 2012b). The Nurses Health Study also showed an adverse prognosis among non-smoking women who gained a mean of 6.6 lb (around 3 kg) within the first three years post-diagnosis (RR all cause mortality: 1.59, 95% CI: 1.07 to 2.51 and RR of BC recurrence: 1.53, 95% CI: 1.04 to 2.24) (Kroenke et al. 2005). Weight gains of 5% after BC diagnosis have also been associated with worse physical functioning (Imayama et al. 2013). Furthermore, BC survivors who gained more than 10% of their baseline weight by two years post-diagnosis had an increased risk of developing type-two diabetes (OR: 1.38, 95% CI: 1.06 to 4.76, p=0.04) (Erickson et al. 2012), and were more likely to report hot-flushes (Caan et al. 2012a).

On the other hand, evidence has shown that weight loses of more than 10% of the baseline weight in the first two years of diagnosis increased the risk of all cause mortality in subgroups of populations: for instance, in women who smoked (HR: 1.58, 95% CI: 1.20 to 2.09), in underweight women with no comorbidities (HR: 2.16, 95% CI: 1.25 to 3.75), and in overweight/obese women with comorbidities (HR: 1.89, 95% CI: 1.25 to 3.75) (Caan et al. 2012b). Nevertheless, normo-weight women might also have an increased risk of mortality following BC if they experience moderate weight losses of 5% to 10% (mean: - 4.9 kg) (Caan et al. 2012b). Similarly, in a previous study, BC survivors who lost more than 1 kg by 18 months post-diagnosis had higher risk of all cause mortality (HR: 2.41, 95% CI: 1.62 to 3.58) and BC recurrence (HR: 1.60, 95% CI: 1.03 to 2.48) (Chen et al. 2010). Nonetheless, the current evidence on the association between weight loss and BC prognosis should be taken with cautious as it might be the result of unintentional

weight loss that might hide other issues (i.e. more advanced stages of BC, or comorbidities) (Jackson et al. 2017).

In the present study, although the mean weight change post-diagnosis was statistically significant, its magnitude was small according to Cohen's criteria (Cohen 1992) ranging from + 1.44% to + 2.67% of diagnosis weight (a mean of + 0.85 kg to + 1.59 kg). In the light of those survival studies, the magnitude of weight change experienced by most participants in this study is unlikely to be clinically relevant. Nonetheless, the study uncovered that at least 20% of participants gained 5 kg or more in the first year of diagnosis, and when exploring weight change in relative terms, between 8 and 10% of participants gained more than 10% of their diagnosis weight. Furthermore, findings revealed that 4% to 6% of the sample lost large amount of weight (more than 10% of their diagnosis weight) in the first four years post-diagnosis. Based on the results of the survival studies above cited, attention should be given to these subgroups of participants whose magnitude of weight change (either weight gain or weight lost) poses a threat to their health and BC prognosis.

Attention should be also paid to the 60% of participants who were already overweight/obese at the time of diagnosis. Obesity at diagnosis is a negative prognostic factor (Protani et al. 2010; Druesne-Pecollo et al. 2012; Niraula et al. 2012; Jain et al. 2013; Azrad and Demark-Wahnefried 2014; Chan et al. 2014). In a study conducted by Nichols et al. (2009), women categorized as obese (BMI more than 30 kg/m^2) before a BC diagnosis had a 52% increase in all-cause mortality risk (95% CI: 1.17 to 1.98), a risk of death from BC 2.28 times higher (95% CI: 1.43 to 3.64) and a cardiovascular disease mortality rate 1.65 times higher (95% CI: 0.97 to 2.83) than that of women with a normo-weight (BMI of 18.5 to 24.9 kg/m^2). Azra and Demark-Wahnefried (2014) conducted a review of the literature and concluded that obesity contributed to negative clinical outcomes in BC patients. The association appeared to be strongest in women with ER positive tumours regardless of menopausal status, whereas there was no evidence to suggest that obesity was associated with outcomes in women with triple negative disease.

To date, the association between obesity and BC outcomes is poorly understood and many hypotheses have been suggested, including that BC occurs at more advanced stages in obese women, or less aggressive treatment might be given to those patients (Goodwin 2015a). It is also possible that BC treatments are less effective in obese women. Results from two clinical trials, the ATAC trial (Howell et al. 2005) and the Australian Breast and Colorectal Cancer Study Group trial 6 (ABCSG-6) (Schmid et al. 2003) suggest that obesity at the time of BC diagnosis might alter the efficacy of AINs. Furthermore, the physiological changes arising from a metabolically active adipose tissue (i.e. changes in insulin, oestrogens and other inflammatory substances) could also explain the association between adiposity and BC (Goodwin 2015b), as detailed later on in this chapter.

The findings reported in this study confirm that weight changes occur post-diagnosis and that the magnitude of weight change varies across the sample. This highlights the need to investigate the causes leading to weight changes post BC diagnosis. In order to contribute to that knowledge, the second objective of this study was to explore whether weight change was associated with BC treatment, biological and behavioural factors. Because of the retrospective nature of the study, the findings reported here only indicate a relationship and not a causal association (Hill 1965).

5.2.2 Factors associated with weight change post-diagnosis

5.2.2.1 Chemotherapy

In this study, a non-statistically significant mean weight increase post-diagnosis was seen among participants treated with chemotherapy and among participants who did not receive chemotherapy. The differences in the magnitude of weight change between the two groups were erratic and both multiple linear regression analysis and multilevel weight change modelling confirmed that chemotherapy was not associated with weight change. This finding correlates with several studies that did not find a relationship between chemotherapy and weight gain post-diagnosis (Kutynec et al. 1999; Freedman et al. 2004; Gu et al. 2010; Sestak et al. 2012; Reddy et al. 2013;

Arpino et al. 2015), but contradicts many others showing a statistically significant association between the two (Goodwin et al. 1999; Rock et al. 1999; Demark-Wahnefried et al. 2001; Caan et al. 2006, 2008; Chen et al. 2011). One possible explanation for the inconsistent association between chemotherapy and positive weight change post BC diagnosis is the difference in chemotherapy agents used. Although at least two studies found that all chemotherapy regimens resulted in some weight gain (Goodwin et al. 1999; Irwin et al. 2005), emerging literature suggests that anthracycline-containing regimens are associated with less weight gain than other regimens (Rock et al. 1999; Saquib et al. 2007; Reddy et al. 2013; Makari-Judson et al. 2014). For example, one study found that the highest weight gain (+ 2.9 kg) was recorded in a group of women receiving non-anthracycline agents (i.e. cyclophosphamide, methotrexate and fluorouracil), compared to + 0.9 kg weight change observed in the anthracycline-based group (Liu et al. 2014). In the current study, most chemotherapy users (95.5%) were treated with anthracycline-containing regimens either alone or in combination with taxanes. Hence, this could explain the lack of association with weight change. Due to the small number of chemotherapy users who did not receive anthracyclines in this study, it was not possible to compare weight changes based on anthracycline use. However, comparing taxane-based chemotherapy with other chemotherapy regimens found similar weight changes. Because of the evolving nature of BC treatment, it is important to continue investigating the role that different chemotherapy regimens play in body weight changes, as the variations in pharmacokinetics that could provide a plausible explanation for the differences in weight change, if they exist.

5.2.2.2 Hormone therapy

Univariable analysis showed that weight change was larger (and overall positive), among tamoxifen and gonadorelin analogue users at all four evaluation points explored, compared to the average weight change seen among non-users, who on average, had a negative weight change.

On the other hand, both letrozole users and non-users, and exemestane users and non-users have a mean positive weight change post-diagnosis. Interestingly, the magnitude of weight change was larger among letrozole and exemestane non-users at 12 and 24 months post-diagnosis, whereas in the next two periods explored, users had larger weight changes than non-users. Like chemotherapy, anastrozole use was associated with erratic patterns of weight change, with positive and negative mean weight change post-diagnosis.

Multilevel weight change modelling support the role of these hormone therapy agents in weight change post-diagnosis, an association that is biologically plausible. Hormone therapy aims to reduce the availability of endogenous oestrogen to tumour cells (Goel et al. 2009), and it is known that oestrogens can reduce food intake and affect body weight (Roesch 2006). Studies conducted in animals have provided evidence that AINs can produce weight gain (Kubatka et al. 2008a; Kubatka et al. 2008b; Sadlonova et al. 2009). In multiple linear regression analysis, tamoxifen use was associated with positive weight change at 24 months post-diagnosis, with a quite low significance level (p-value <0.01). Moreover, weight change modelling showed that the rate of weight change was higher among tamoxifen users and the estimated body weight at any time post-BC diagnosis was higher among tamoxifen and as well as among anastrozole users compared to non-users. Conversely, the estimated body weight was lower among letrozole users. Nonetheless, findings about the apparently contradictory effect of anastrozole and letrozole on weight use should be taken with caution as they might the result of the reduced sample size of letrozole users.

In the BC research field, the association of tamoxifen use with weight gain is not new. Some studies have shown higher weight gain in BC survivors using tamoxifen compared with a control group of women not receiving hormone therapy (Hoskin et al. 1992). In the Nurses Health Study, women treated with chemotherapy or tamoxifen tended to experience the largest weight gains (Kroenke et al. 2005). Sedjo et al. (2014) reported for the first time lower weight gain among AINs users as compared with tamoxifen users. However, the literature reporting the role of hormone therapy in weight gain is controversial, as there is also evidence of greater increases among non-users. Data from the WHEL study revealed that tamoxifen use was not associated with significant weight gain and did not modify the effect of

chemotherapy use on weight gain (Saquib et al. 2007). WHEL's findings are in line with other studies (Camoriano et al. 1990; Kumar et al. 1997; Lankester et al. 2002; Freedman et al. 2004; Irwin et al. 2005; Genton et al. 2006; Makari-Judson et al. 2007; Gu et al. 2010; Chen et al. 2011; Sestak et al. 2012). However, a light into the controversy comes from Nissen et al. (2011), who found that although tamoxifen use did not affect weight change, it increased body fat. These findings are compatible with those reported by Nguyen et al. (2001), although they contradict many others (Freedman et al. 2004; Irwin et al. 2005; Francini et al. 2006).

5.2.2.3 Menopausal status and change in menopausal status

In this, and in several studies (Camoriano et al. 1990; McInnes and Knobf 2001; Freedman et al. 2004; Caan et al. 2006, 2008; Heideman et al. 2009; Basaran et al. 2011; Chen et al. 2011; Caan et al. 2012b) the magnitude of mean weight change following BC diagnosis was positive in the premenopausal/perimenopausal group and was larger than the magnitude of weight change found among postmenopausal women.

Average weight changes were also positive among those who became peri or postmenopausal post-diagnosis. The magnitude of the weight change observed in this group was larger compared to that seen among those whose menopausal status did not change (postmenopausal women and a few premenopausal women) and was statistically significant at 12 ($p=0.03$) and 48 months ($p=0.01$) post-diagnosis. Although it is possible that the significant associations found are a type-one error inflation, the associations are not surprising. In the general population, the transition to menopause is commonly associated with weight gain and with a redistribution of body fat (Toth et al. 2000; Boonyaratanakornkit and Pateetin 2015). A limited number of studies have also reported an association between transition to menopause and weight gain in BC survivors. In one of them the effect vanished after other variables were taken into account (Reddy et al. 2013), whereas in another it remained a significant predictor together with chemotherapy use (Goodwin et al. 1999). On the other hand, Irwin et al. (2005) found that in both adjusted and non-

adjusted analysis, postmenopausal women gained more weight compared to premenopausal women and women who became postmenopausal after diagnosis.

In the current study, the association between menopausal status change and weight change mirrored the association seen between chemotherapy and weight change. Weight change was positive among chemotherapy users and non-users. Nonetheless, the magnitude of the change was larger among chemotherapy users at 12 and 48 months post-diagnosis, whereas at 24 and 36 months post-diagnosis, the magnitude of weight change was inferior among chemotherapy users compared to non-users. Age and menopausal status at diagnosis were highly correlated, and like others (Sedjo et al. 2014), this study used the variable age in the multiple linear regression analysis and in the multilevel weight change modelling. Both showed that neither age nor chemotherapy use were predictors of weight change after accounting for other variables. Hence, it is likely that other factors (i.e. tamoxifen use) might be more relevant to explain weight change post-diagnosis. However, it is possible that smaller sample size of this study compared with others studies and the difficulties in defining menopausal status after one year post-diagnosis (as discussed by Reddy and colleagues, 2013) could explain the opposite results found by other researchers (Goodwin et al. 1999; Irwin et al. 2005; Reddy et al. 2013). In addition, it is possible that the timing of recording baseline weight could have masked the impact of treatment-related amenorrhoea on body weight change (i.e. if adjuvant treatment had already commenced). Therefore, the question of whether the observed weight changes can be attributed to menopausal status change, ageing, or BC treatment remains to be answered.

5.2.2.4 Genes

Growing research is pointing to the importance of FTO and Mc4R in determining body weight.

The prevalence of the homozygous risk allele A at the rs9939609 FTO variant in this study was 8.3%, a bit lower than the prevalence reported in the general population

(Frayling et al. 2007; Lourenço et al. 2014). This finding is not consistent with results from one study showing that the presence of risk allele A at the rs9939609 SNP increases the risk of BC (additive effect OR: 3.719, 95% CI: 1.43 to 9.68) (Kaklamani et al. 2011). Nonetheless, evidence on the relationship between FTO genotype and BC risk is not firm and another study found no differences in the frequencies of the FTO gene A allele and the Mc4R gene C allele between BC patients and controls (Kusinska et al. 2012). Consistent with this, the prevalence of homozygosity (also 8.3%) for the C-allele of the Mc4R gene rs17782313 polymorphism among participants in this study is comparable with that found by Liu et al. (2011) among the general population, although other studies have found lower prevalence of the risky alleles (Arrizabalaga et al. 2014). Therefore, the reason for the prevalence of the risky alleles among participants is not understood.

To the researcher knowledge, this is the second study looking at the effect of FTO on weight changes post BC diagnosis and the first one exploring the role of Mc4R. Findings revealed that all categories of FTO and Mc4R genotypes experienced a positive weight change. The pattern of weight change associated with FTO SNP rs9939609 was not consistent, a finding not supported by those reported by Reddy and colleagues (2013), who conducted the first study published looking at FTO in the field. In their study, weight change post-diagnosis was best predicted by a model that incorporated age and BMI and diagnosis, as well as data on all 14 SNPs explored from FTO and adiponectin genes. The model had a high discriminatory power to identify those at risk of weight gain post-diagnosis (Reddy et al. 2013).

On the other hand, findings from this study showed a non-significantly lower weight change at all 4 evaluation points explored among those with the obesity-increasing C allele (high-risk allele) of the Mc4R gene compared to those without the high-risk allele. This finding contradicts the literature showing that the high-risk allele predisposes people to obesity (OR=1.18, 95% CI: 1.15 to 1.21, $p<0.001$) (Xi et al. 2012a).

This study's findings should be taken with caution. Genetic studies usually require larger sample sizes, hence, the small sample size of this study might only detect larger effects (Reinhart 2015). Additionally, in this study insufficient funding left 96

samples un-analysed. In addition, the large SD found in this study suggests that the mean weight change would be likely to vary should other samples of participants be analysed (Reinhart 2015).

Although FTO and Mc4R have been linked to common forms of obesity, the molecular bases of the relationship is not clear (Olszewski et al. 2009, Xi et al. 2012a; Arrizabalaga et al. 2014). These two genes seem to be highly expressed in the central nervous system which regulates the energy metabolism (Xi et al. 2012a). It is possible that the effect of the environment or other changes associated with the diagnosis of BC could interact with the genes, leading to weight changes post-diagnosis. Therefore, further studies with bigger sample sizes are warranted, as they could provide evidence on whether these two genes could be used to understand weight change post-diagnosis and to identify women at risk of gaining weight post-diagnosis.

5.2.2.5 Other variables of interest

Multiple linear regression analysis showed that weight at diagnosis was significantly and negatively correlated with weight change. Arguably, this could be a type-one error due to the use of un-adjusted significance testing. Nonetheless, it is worth mentioning that that association found could have met more strict significance levels, as the p-value found for the association was lower than 0.01. Furthermore, the finding agrees with most of the literature in the field showing larger weight gain among leaner women (Rock et al. 1999; Saquib et al. 2007; Gu et al. 2010; Nissen et al. 2011; Caan et al. 2012b; Reddy et al. 2013; Sedjo et al. 2014). However, the multilevel modelling conducted with all weight records collected from participants showed that weight at the time of diagnosis was not a predictor of rate of weight change. Therefore, it is possible that the statistical artefact of regression to the mean could be behind the association between weight at diagnosis and subsequent weight change.

Multiple linear regression analysis revealed that smoking status at diagnosis was also a significant predictor of the mean positive weight change found at 12 months post-diagnosis. The nine participants who were smokers at the time of BC diagnosis tended to have a higher magnitude of weight change, which was positive, compared to non-smokers, which is consistent with other studies (Chen et al. 2011; Sedjo et al. 2014), although Sestak et al. (2012) did not find significant differences in the risk of gaining weight post-diagnosis between smokers and non-smokers at the time of BC diagnosis. The reason for the association found in this study between being smoker at the time of BC diagnosis and an average positive weight change post-diagnosis is unclear. Findings could be the result of a small sample size, as in this study, like in that of Chen's et al. (2011) the number of smokers was small compared to the number of non-smokers. However, the most parsimonious explanation for the association found might be related to changes in smoking status post-diagnosis. Numerous studies have shown that smokers have typically lower body weight than non-smokers (Albanes et al. 1987; Himokata et al. 1998; Molarius et al. 1997). There is also abundant evidence that quitting smoking is associated with weight gain (Travier et al. 2012). For instance, results from a meta-analysis of 62 studies showed that smoking cessation is associated with a mean weight gain of 4.5 kg during the first 12 months of abstinence (Aubin et al. 2012). Although the mechanisms leading to post-cessation weight gain remain unclear, it is possible that the metabolic effects of the nicotine (i.e. increases in metabolic rate and decreases in appetite) play a role (Chiolero et al. 2008). In this study, five out of the nine participants that were smokers at BC diagnosis and were included in the analysis of weight change at 12 months post-diagnosis quitted smoking after diagnosis. In addition, another participant reduced the number of cigarettes per day and the remaining three participants said they did not change their smoking habits. These changes in smoking habits post-BC diagnosis among participants in this study could explain the larger weight change found among smokers. In fact, Sedjo et al. (2014) claimed that was the reasons for the positive association found in their study between smoking and weight gain post BC diagnosis. Unfortunately, participants were not able to specify the exact time at which they quit smoking. Therefore this study did not assess the impact of smoking cessation on weight gain post BC diagnosis. This is something

that might be worth taking into account in other studies looking at weight changes among BC patients.

5.3 Multilevel models for weight change

In addition to the findings from the multilevel weight change modelling already discussed in the previous paragraphs, the weight change models fitted also showed that the pattern of weight change post BC diagnosis seemed to follow a quadratic trajectory. However, due to the peculiarities of data, weight change could only be modelled using a linear function, an approach that added to previous research in the field using linear regression analysis (Camoriano et al. 1990; Rock et al. 1999; Demark-Wahnefried et al. 2001; Kroenke et al. 2005; Makari-Judson et al. 2007; Heideman et al. 2009; Gu et al. 2010; Vagenas et al. 2015).

The magnitude of the rate of weight change estimated in the models was small but statistically significant. Findings indicate that women differ in their rate of change, and that the only predictor explaining those differences was tamoxifen use. Furthermore, the time-varying models fitted allowed the effects of BC treatment to vary over time, adding further support for the role of hormone therapy in weight change post-diagnosis as mentioned earlier. The models showed that estimated weight following BC diagnosis depends on tamoxifen, anastrozole and letrozole use.

5.4 Body adiposity parameters

Participants had a mean of 28.5 kg (SD: 9.43) of body fat, a fat percent of 37.8% (SD: 6.24) of body weight and an FM/FFM ratio of 0.62 (SD: 0.16). On average, their waist circumference measured a mean of 92.3 cm (SD: 11.82). These body adiposity parameters are comparable to those reported in the literature of BC (Kutynec et al. 1999; Demark-Wahnefried et al. 2001; McTiernan et al. 2003; Freedman et al. 2004; Harvie et al. 2004; Guinan et al. 2014; Alacacioglu et al. 2016).

5.4.1 Are these adiposity parameters different from the healthy population?

Although findings cannot be directly compared, the body fat and waist circumference values found in this study, as well as those reported in other samples with BC women seem to be higher than in the average population of women in the UK (Kutynec et al. 1999; Demark-Wahnefried et al. 2001; McTiernan et al. 2003; Freedman et al. 2004; Genton et al. 2006; Harvie et al. 2004; Guinan et al. 2014; Alacacioglu et al. 2016). Premenopausal women in the UK were found to have an average body FM of 24.4 kg (SD: 9.6), body fat percentage of 33.8% (SD: 7.0) and a waist circumference of 80.6 cm (SD: 11.3), whereas postmenopausal women had higher levels of body fat mass (25.8 kg, SD: 8.8), body fat percentage (36.2%, SD: 6.4) and waist circumference (83.2 cm, SD: 11.2) (Guo et al. 2015).

Typical weight gain is often associated with an increase in FM and FFM (Forbes et al. 1986). In this study, FM, FFM and waist circumference were only measured once at the end of the follow up (mean 48 months post-diagnosis), therefore, changes in those parameters following BC diagnosis were not assessed. Nonetheless, previous studies in the field have shown deteriorations in adiposity parameters at different points after BC diagnosis (Arpino et al. 2015 and Sheean et al. 2012 for a review of previous studies). The magnitude of the increment in fat percentage levels reported by some studies varied from 0.72% to 2.5% during chemotherapy (Aslani et al. 1999, Freedman et al. 2004), and from 1.37% to 6.2% one year after diagnosis and the final fat percent reported reached values ranging from 35.2% to 46.9% (Kutynec et al. 1999; Demark-Wahnefried et al. 2001; McTiernan et al. 2003; Freedman et al. 2004; Harvie et al. 2004; Thomson et al. 2009; Guinan et al. 2014; Alacacioglu et al. 2016). Similarly, increases in waist circumference from 0.9 cm to 5.1cm have been observed around one or two years after diagnosis, which resulted in final levels of 82.4 to 89.9 cm (Goodwin et al. 1999; Freedman et al. 2004; Harvie et al. 2004; Arpino et al. 2015). Differences in the magnitude of change found in previous studies could be attributed to the different instruments used to measure body fat (i.e. BIA analysis, skinfold thickness, dual energy x-ray absorptiometry, or CT scan) as discussed by Freedman and colleagues (2004).

5.4.2 Potential clinical implications of the excess body fat and waist circumference observed

The adipose tissue is an important endocrine organ (Kershaw and Flier 2004) that participates dynamically in regulation of energy (Ruan and Lodish 2003) and body weight (Su et al. 2012) through different ways: 1) its effects on other hormones also implicated in the regulation of energy, like insulin, 2) through active interaction with other organs (Morton and Schwartz 2011), and 3) the substances that it secretes, including oestrogen and leptin (Galic et al. 2010).

In this study, the participants' mean body fat percentage was above the healthy range (36% for women 60 years and older, 34% for 40- to 59-year-old women) (Tanita Corporation 2014), a worrying finding since it has been suggested that excessive adipose tissue might change the cellular composition of the adipose tissue, leading to alterations in the number, phenotype and location of cells, affecting the secretory activity of the adipose tissue (Ouchi et al. 2011), and ultimately contributing to cellular transformation and carcinogenesis (Ozbay and Nahta 2008). Adipose tissue is found in the mammary gland, around breast tumours and in the tumours themselves (Dirat et al. 2010). Consequently, it could potentially exert its effects on BC through endocrine, paracrine and autocrine mechanisms (Vona-Davis and Rose 2007). In fact, Wang et al. (2012) suggests that the effects of the adipose tissue located in the breast might be increased in obese women, which could explain in part the adverse BC prognosis associated with obesity found in many investigations (Kumar et al. 2000; Rose et al. 2002; de Azambuja et al. 2010; Azra and Demark-Wahnefried 2014; Sun et al. 2015). Also worrying is the fact that a study conducted with Asian women indicated that high levels of abdominal fat were correlated with greater chemotherapy toxicity, a finding that, as the author concluded, adds to the emerging evidence suggesting that body composition has a role in chemotherapy dose determination (Wong et al. 2014). And as mentioned earlier on, it is believed that obesity influences the response and resistance of BC tumours to AINs (Schmid et al. 2003; Howell et al. 2005; Pfeiler et al. 2012; Schech et al. 2015).

Waist circumference (a marker of abdominal adiposity) and general obesity are also associated with cardiovascular disease risk, type-two diabetes, overall mortality, as

well as colorectal cancer and postmenopausal BC (World Health Organization 2008). According to the International Diabetes Federation, a waist circumference more than 80 cm increases the risk of metabolic complications in women from different ethnic groups (Alberti et al. 2009). In the current study, participants' mean waist circumference of 92.3 cm exceeded that cut-off point, which highlights the potential risk that this group of women are exposed to.

Finally, the greater content of body fat among BC patients, if accompanied by lower FFM, could lead to sarcopenic obesity, and can have several adverse effects. Sarcopenia has been associated with higher risk of developing dose-limiting toxicity in BC treatment (Prado et al. 2009), and with poorer prognosis, independently of body adiposity (Villasenor et al. 2012). It also leads to reduced physical performance in the general population (Lang et al. 2010).

5.4.3 Factors associated with body adiposity parameters at the end of the follow up

5.4.3.1 Chemotherapy

In this study, chemotherapy users had non-significantly lower body fat and waist circumference levels at the end of the follow up. The lack of association between body adiposity parameters and chemotherapy is consistent with some studies that report no changes in body fat associated with BC treatment at two years post-diagnosis (Irwin et al. 2005), or three years post-diagnosis (Guinan et al. 2014). In contrast, numerous studies have reported increased levels of fat content during chemotherapy or during the first year of diagnosis (Cheney et al. 1997; Aslani et al. 1999; Kutynec et al. 1999; Demark-Wahnefried et al. 2001; Del Rio et al. 2002; Harvie et al. 2004; Campbell et al. 2007a; Gordon et al. 2011; Porciúncula Frenzel et al. 2013). Altogether, these findings suggest that the effect, if any, of chemotherapy on body fat might not be sustained over time, which is a hypothesis that deserves further exploration.

5.4.3.2 Hormone therapy

Anastrozole was statistically significantly associated with higher waist circumference levels, and with fat percentage in univariable analysis, but not in the multiple linear regressions conducted. Results also showed a non-significant trend for higher body fat and waist circumference levels among letrozole users. Tamoxifen users had non-significant larger weight circumference levels, which could explain their common verbalised complaint that the drug is causing them to put on weight. On the other hand, exemestane users tended to have lower body fat and waist circumference levels than non-users, a finding in line with that reported by Francini and colleagues (2006), who found that switching obese patients from tamoxifen to exemestane reduced FM.

In general, these findings add to the increasing literature in the field that insinuates a role for hormone therapy, or tamoxifen in particular, in body adiposity levels (Nguyen et al. 2001; Knobf et al. 2008; Nissen et al. 2011). However, the fact that not all the literature shows an association between adiposity and BC treatment or tamoxifen use (McTiernan et al. 2003; Irwin et al. 2005) indicates that it is worthwhile to continue exploring the role of hormone therapy on post-diagnosis changes in adiposity, using studies that have long follow up and adequate control groups. This is important since the association is biologically plausible. It is known that a reduction of oestrogens, which is one of the effects of AINs (Burstein 2011), is associated with changes in body fat (Davis et al. 2012). Letrozole is a potent AIN (Jain et al. 2013). Suppression of oestrogens is greater with letrozole compared with anastrozole across the full range of BMIs (Folkerd et al. 2012). The small number of participants who took letrozole in this study (less than 44) might not have provided enough power to find a statistical association between letrozole and body adiposity parameters when other variables were accounted for.

5.4.3.3 Menopausal status and change in menopausal status

Worth noting is the fact that an increasing number of studies in the field are reporting a distinctive pattern of weight change consistent with sarcopenia (Cheney et al. 1997; Kutynec et al. 1999; Demark-Wahnefried et al. 2001; Freedman et al.

2004; Gordon et al. 2011; Nissen et al. 2011; Hojan et al. 2013). Interestingly, Nissen and colleagues (2011) found that this peculiar pattern of weight gain was only seen in normo-weight women, rather than in overweight or obese women. Currently it is unclear whether sarcopenia is caused by BC treatment, or is the consequence of ageing and entry into the menopause (Sheean et al. 2012). A preliminary study by Gordon and colleagues (2011) showed that women who suffered chemotherapy-induced ovarian failure tended to gain FM (+ 1.8 kg) and lose FFM (- 0.6 kg), whereas those who retained their ovarian function during chemotherapy treatment also gained FM (+ 0.9 kg) but did not have significant changes in FFM. In the present study, the opposite was found: those pre and perimenopausal participants who changed their menopausal status post-diagnosis (nearly 92%) had lower body adiposity parameters than those who did not. Arguably, the finding could be the consequence of the analytical approach. The number of participants who remained pre or perimenopausal was small (n=5), therefore, their data were combined with data from postmenopausal women (73% of the sample), whose body adiposity parameters were significantly higher than those of pre and perimenopausal women. The higher adiposity parameters seen among postmenopausal participants might have masked the effect of the transition to the menopause on body fat in pre and perimenopausal participants. In fact, fat percentage and FM/FFM showed an upward trend associated with gonadorelin analogue use, a finding consistent with Gordon's study. This was not a surprise as goserelin, the type of gonadorelin analogue used in this study to produce chemical ovarian ablation, can suppress circulating oestrogens to postmenopausal levels (Cancer Research UK 2014). And, as stated previously, it is known that the change in the hormonal milieu during the transition into the menopause is associated with an increase in abdominal and total body fat (Davis et al. 2012).

Nonetheless, in this study, multiple linear regression analysis indicated that menopausal status was not a statistically significant predictor of body adiposity parameters. Hence, other factors might be more relevant to explain body adiposity parameters after BC.

5.4.3.4 Genes

The FTO and the Mc4R have been associated with common forms of obesity. In this study, the presence of the FTO SNP rs9939609 risk A-allele and the risk C-allele at SNP rs17782313 of the Mc4R gene were both non-significantly associated with healthier adiposity parameters, a finding which is in line with their association with weight change, but that contradicts findings from previous studies with small (Arrizabalaga et al. 2014) and large samples (Frayling et al. 2007; Liu et al. 2010; Hertel et al. 2011; Xi et al. 2012a and 2012b; Kvaløy et al. 2013) that supported the effects of those risk alleles on increased adiposity. In view of the results of these investigations, this study might have not had enough power to identify an association between genotype and adiposity levels post-diagnosis. Nonetheless, it is important to uncover the factors that could explain changes in adiposity, since higher levels of body fat have been linked with poorer prognosis (Sun et al. 2015), with greater chemotherapy toxicity (Wong et al. 2014) and with the response and resistance of BC tumours to AINs (Schech et al. 2015).

5.4.3.5 Other variables of interest

Participants' age at the time of BC diagnosis was positively correlated with menopausal status. Accordingly, postmenopausal participants had statistically significant higher body adiposity parameters than premenopausal women. This is an expected finding as substantial evidence has demonstrated that the transition to the menopause favours increases in FM, particularly abdominal fat (Davis et al. 2012), and waist circumference is known to increase with age (World Health Organization 2008). Multiple linear regression analysis showed that when other factors were accounted for, the only predictors of body adiposity parameters in this study were age at diagnosis and weight at diagnosis (both $p<0.01$). Arguably, these associations could be the result of lack of p-level adjustment. Nonetheless, the associations could have meet more strict significance levels than the used ($p<0.05$), and they are present in the general population (World Health Organization 2008).

In this study, adiposity parameters were cross-sectional only and therefore, a cause-effect relationship cannot be concluded (Hill 1965). Nonetheless, a temporal feature exists as age and weight were recorded at diagnosis, the starting point of the follow up period, whereas body fat and waist circumference were measured at the end of follow up. It is also worth mentioning that although multiple linear regression analysis was conducted, there could be other factors contributing to the magnitude of the adiposity parameters found among participants that were not explored (i.e. diet or physical activity). In line with this, it is acknowledged that this study might not have enough power to detect statistically significant effects of other variables (i.e. genes).

5.5 Metabolic parameters at the end of the follow up

The literature on metabolic status after BC diagnosis is limited. In this study, the average fasting glucose level was 5.14 mmol/l (SD: 0.77), ranging from 3.10 to 10.80 mmol/l. Average fasting insulin level was 39.31 pmol/l (SD: 2.63) ranging from 7.00 to 184.74 pmol/l. HOMA IS and HOMA IR values were 128.92 (SD: 59.19) and 0.99 (SD: 0.56) respectively. Glucose levels measured at the end of follow up are similar in magnitude to figures reported in another BC population (Goodwin et al. 2012). And Thompson et al. (2009) found glucose levels of 98.7 mg/dl (SD: 12.9) (equivalent to 5.48 mmol/l) when exploring metabolic syndrome in a sample of BC patients who were 6 to 72 months from surgery or completion of radiation or chemotherapy. Conversely, in this study, participants' insulin mean and range values were higher than those reported by Alacacioglu and colleages (2016) six months post-chemotherapy (30 pmol/l, SD: 77.9), but lower than those reported by other authors who found insulin levels ranging from 44.6 pmol/l (SD: 31.1) to 92.9 pmol/l (SD: 77.9) pre-adjuvant therapy (Goodwin et al. 2002; Borugian et al. 2004), or a mean fasting insulin value of 66.9 pmol/ml, measured two-and-a-half years post-diagnosis (Duggan et al. 2011).

After excluding nine participants with known diabetes at study entry, blood samples taken a mean of 48 months post-diagnosis revealed that 1.8% of the participants with available fasting glucose levels had values corresponding to pre-diabetes (6.1 to 6.9 mmol/l) and nearly 3% had glucose levels that fell within the diabetic range (more than 6.9 mmol/l) (Diabetes.co.uk 2016). This finding, together with the high prevalence of overweight/obesity found among participants at the time of their BC diagnosis is consistent with those from the WHEL study which showed that higher BMI was statistically significantly associated with incidence of diabetes post-diagnosis (Erickson et al.2012). Therefore, findings in this study highlight the need of exploring metabolic parameters among BC survivors. Furthermore,

5.5.1 Potential clinical implications of the high glucose and insulin levels observed

Elevated fasting glucose has been associated with chemo-resistance in multiple tumour types (Song et al. 2016) and with poor prognosis after BC diagnosis (Monzavi-Karbassi et al. 2016). Similarly, high insulin levels have been associated with several diseases, including heart disease and type-two diabetes (Reaven 1988). Insulin's role in cancer is well documented, and currently, insulin offers a potential mechanism to explain the postulated causal relationship between obesity and BC (Vona-Davis and Rose 2009). Insulin can activate insulin, IGF and oestrogen signalling pathways related to carcinogenesis, tumour progression, invasion and metastasis (Goodwin 2015b). In line with that, insulin has been implicated in tumour-related angiogenesis (Vona-Davis and Rose 2009). Studies have also demonstrated that higher levels of insulin and IR both are associated with adverse BC prognosis, even after adjusting for BMI and other prognosis factors (Goodwin et al. 2002; Borugian et al. 2004; Duggan et al. 2011), particularly during the first five years of diagnosis (Goodwin et al. 2012). Hence, IR has been described as one of the most significant metabolic disturbances in cancer (Hursting et al. 2007). In the context of this study examining insulin and glucose levels acquires additional relevance as Chapter 2 detailed, they can also take part in the regulation of body weight, body fat , appetite and leptin levels (Trayhurn and Beattie 2001; Dulloo

2005; Pliquett et al. 2006; Su et al. 2012). It has been suggested that a reduction in the response to the effects of insulin in areas in the brain relevant to eating behaviours could lead to the association between IR and weight gain (Anthony et al. 2006), an hypothesis that may be worth exploring further to understand reasons for weight gain post BC diagnosis.

5.5.2 Factors associated with metabolic parameters at the end of the follow up

5.5.2.1 Chemotherapy

In this study, insulin levels were statistically significantly lower among participants who received chemotherapy ($p<0.04$). This finding adds to the limited and inconsistent literature on the effect of chemotherapy on insulin levels. Two studies found an increase of insulin levels during chemotherapy treatment (Makari-Judson et al. 2009; Alacacioglu et al. 2016), whereas another found a decrease (Chala et al. 2006). Furthermore, a local study found a statistically significant increase in blood glucose levels in the last cycles of chemotherapy prescribed with dexamethasone, and 14 out of the 39 participants in that study developed degrees of glucose intolerance (Hickish et al. 2009). Finally, a population-based study showed that BC survivors had an increased risk of diabetes (HR for chemotherapy users: 1.24, 95% CI 1.12 to 1.38 and HR for non-chemotherapy users: 1.11, 95% CI 1.05 to 1.16) (Lipscombe et al. 2013). Differences between insulin levels observed in the current and published studies may be attributed to the longer follow up of this study, although effects of the characteristics of participants in the study, or a type-I error due to the use of multiple hypothesis testing (the level of significance was 4%) cannot be ruled out. In fact, chemotherapy was not a statistically significant predictor of metabolic parameters in multiple regression analysis.

It has been suggested that chemotherapy drugs affect the liver and the muscle, leading to disruptions of glucose metabolism and IR (Makari-Judson et al. 2014). In addition, the elevated insulin levels post-chemotherapy in BC patients might result from the chemotherapy regimen used (Alacacioglu et al. 2016), as it is known that

the chemotherapy agent docetaxel can activate the extracellular signal-regulated kinases (ERK1/2) pathway in ovarian carcinomas (Seidman et al. 2001), a pathway that has been shown to play a part in glucose-stimulated insulin secretion in a subgroup of pancreatic mouse cells (Niu et al. 2016). More research is needed to firstly, explore a possible association between chemotherapy and insulin and glucose levels, secondly, to understand the mechanisms underlying that possible association, and thirdly, to identify the prognostic consequences of altered metabolic parameters.

The negative correlation between time elapsed from BC diagnosis to the end of follow up (the time when insulin levels were taken) suggests that insulin levels decrease during follow up. Unfortunately, this investigation did not explore changes in insulin levels post BC-diagnosis. Nonetheless, a study which explored glucose metabolism in the long term (before adjuvant BC therapy, during treatment and two years after BC diagnosis) found no statistically significant changes in glucose and insulin levels over time (Arpino et al. 2015). Given this evidence, more research is needed to understand whether insulin and glucose levels fluctuate or their trajectory is linear after BC diagnosis.

5.5.2.2 Hormone therapy

In this study, exemestane and tamoxifen users had higher glucose levels and lower insulin levels than non-users. Conversely, anastrozole and letrozole treatments were associated with higher levels of serum insulin. It should be noted that only the associations with letrozole and exemestane reached statistical significance ($p=0.02$ and $p=0.03$). Similarly to the association found between chemotherapy and insulin, these significant findings should be taken with caution as they could also be type-one errors due to the use of multiple hypothesis testing, as they were not significant predictors of insulin in multiple regression analysis. Nevertheless, findings are in line with evidence from animal models, which suggests that tamoxifen use could interfere with overall hepatic function (Marek et al. 2011). A previous study, tamoxifen-treated rats had statistically higher insulin levels after tamoxifen treatment than control rats (Hozumi et al. 2005). Nonetheless, two recent studies showed

mixed results. In one of them tamoxifen administration induced mitogen-activated protein kinase kinase kinase kinase four (MAP4K4) deletion in mice, causing a decrease of fasting glucose levels and improvements in insulin signalling in the adipose tissue and liver (Danai et al. 2015). In the second study tamoxifen significantly increased glycated haemoglobin (HbA1c), a measure that reflects previous glucose levels (Hesselbarth et al. 2015).

Interestingly, the findings related to insulin, chemotherapy and hormone therapy are in line with those found when exploring the association between waist circumference and BC treatments. Therefore, in view of the suggestive findings in this study, and considering evidence from investigations conducted on animals, it is concluded that the effects of hormone therapy (of tamoxifen in particular) deserve further exploration.

5.5.2.3 Other variables of interest

Multiple linear regression analysis showed that only weight at diagnosis and FTO genotype remained significant predictors of glucose and insulin levels measured at the end of the follow up, after accounting for the effect of BC treatment. These findings are the result of unadjusted p-value analysis, nonetheless, the significance level was quite small ($p<0.01$ and $p=0.01$), which suggest that the association might be true. The positive correlation found between body weight at the time of diagnosis with insulin levels at the end of the follow up is consistent with the knowledge that obese individuals have higher basal insulin levels than lean individuals (Frank et al. 2014). Unfortunately, the findings in relation to FTO genotype cannot be supported with previous literature as at the point of writing this discussion, no published study has reported the effect of weight at diagnosis and FTO genotype on insulin levels in the BC population. Nonetheless, among the general population, the association between FTO and insulin and glucose metabolism is not new: non-significantly higher levels of insulin and IR have been found in carriers of the high-risk A-allele of the FTO gene SNP rs9939609 in obese and overweight participants taking part in a weight loss intervention (Zheng et al. 2015). A study with an Indian population

found a significant positive association between the FTO gene SNP rs9939609 and fasting insulin, insulin resistant traits and HOMA-index (Prakash et al. 2016). In addition, the AA genotype has been related to higher glucose levels in postmenopausal Brazilian (Ramos et al. 2011), Pakistani (Shahid et al. 2016) and Tunisian women (Elouej et al. 2016). These findings, in combination with those reporting that specific FTO genotypes are associated with increased risk of diabetes in different populations (Frayling et al. 2007; Ortega-Azorín et al. 2012; Xi et al. 2012a; Vasan et al. 2014) suggest that the A-allele of the SNP rs9939609 might alter glucose metabolism (Prakash et al. 2016). However, other studies have found no association between the A-allele and measures of glucose homeostasis in African-Americans, Hispanic-Americans or non-Hispanics white Americans (Wing et al. 2011).

As Chapter 2 explains, insulin is involved in body weight regulation (Dullo 2005; Szablewsksi 2011). Unfortunately, insulin levels in this study were cross-sectional data collected at the end of the follow up, and therefore, it was not possible to explore the role of changes of insulin levels on body weight post BC diagnosis. On the other hand, an increase of body weight could affect metabolic parameters (Vagenas et al. 2015). The correlation test conducted in this study indicated that weight change post-diagnosis was not associated with insulin levels at the end of the follow up. Arguably, the fact that in this study weight change was bidirectional (weight gain and weight loss were observed) could have masked a plausible association between those variables.

5.6 Contribution to the knowledge. Implications for clinical practice and research

Findings in this study showed that participants' adiposity levels were high and that weight changed following BC diagnosis. Although on average the magnitude of weight change across the sample was small and probably not clinically relevant, there were a few women for whom larger weight changes (either gains or losses) could pose a health risk, as suggested by survival studies that have shown the

negative effects body change and adiposity on BC prognosis and other aspects of health. Therefore, this study joins many others in the conclusion that there is a need for professionals to understand which factors are associated with the weight changes observed after BC diagnosis.

Although BC usually has a good prognosis, there is a growing interest in understanding the changes in body adiposity and metabolic parameters that this clinical population may experience, as they might have a negative impact in women's health. Acknowledging the factors that contribute to those adverse changes, either clinical (i.e. BC treatment), biological (i.e. ageing, genes), behavioural (diet or physical activity), or a combination of all, and putting in place best available care based on current knowledge may result in a prevention of unhealthy body weight and unhealthy metabolic changes, and possibly could improve BC outcomes and overall health. This study has addressed that need. Its findings expand on the current and controversial knowledge of the effects of chemotherapy and hormone therapy, and present novel evidence on the possible association of factors such as obesity-related genes with the weight changes observed.

The information derived from the use of multilevel modelling to characterise weight change following BC diagnosis contributes significantly to the knowledge of the topic. Multilevel modelling is a very appealing approach, but it has been relatively under-utilised in this field, perhaps because it requires several waves of data, something that this study's dataset has. Findings from the weight change modelling expand the knowledge of weight change as it not only looked at differences between participants, but also described how weight varied within each individual, suggesting that the weight change trajectory might be quadratic rather than linear, and that the association of the BC treatments with body weight might vary over time.

This study reports on the metabolic status of women diagnosed with BC in the long term (average 48 months post-diagnosis), which expands on findings from previous literature that have focused on insulin and/or glucose levels at periods closer to diagnosis. This information contributes to the knowledge of the phenomenon of changes in body weight, adiposity post BC diagnosis and, as emerging literature is starting to show, alterations in metabolic parameters. The repercussions of these

changes on BC outcomes and other aspects of women's health have been highlighted earlier in the discussion.

There are different ways in which knowledge gained from this study might be applied to improve care of BC patients: 1) it advocates the need for implementing a protocol to assess not just weight, but also body adiposity parameters and metabolic status post-diagnosis; and 2) it provides evidence suggesting that newly diagnosed BC patients might benefit from tailored care plans to manage those outcomes.

5.6.1 Assessment of body weight, body adiposity and metabolic parameters

Some participants in this study gained a significant amount of weight post-diagnosis, had elevated levels of body fat, and some also presented with high insulin and glucose levels.

Considering the above-mentioned potential adverse consequences that both obesity at the time of BC diagnosis and subsequent weight changes post-diagnosis seem to pose for women diagnosed with BC, findings in this study imply that is imperative to integrate the regular assessment of body weight in the BC care plan. This recommendation could be particularly useful for the Surgery team in the follow up clinics at the hospitals where participants were recruited, as at the time of data collection, the measurement of patient's body weight was not part of the routine care. Moreover, the presence of missing body weight data in the medical notes highlight 1) the need to inform clinicians the impact that changes in body weight can have on patient's health, and 2) the need to ensure that the body weight measures are registered in the medical notes.

Findings from this study also suggest that it is necessary to measure not just body weight, but also adiposity parameters because firstly, changes in FM or FFM cannot be captured by measuring changes in weight or BMI alone, and secondly, because imaging studies in cancer populations have shown that body adiposity varies across the BMI spectrum (Sheean et al. 2012). Excessive adipose tissue could have metabolic effects including IR, elevated glucose levels, or dyslipidemia (Kershaw

and Flier 2004). Nonetheless, the risk of IR is likely to be higher in individuals with increased visceral adipose tissue as compared with increased subcutaneous fat (Laharrague and Castiella 2010). In addition, visceral adiposity has been associated with higher levels of glucose (Kershaw and Flier 2004), which is one of the components of the metabolic syndrome (Alberti et al. 2009).

This study has identified a small percentage of participants whose glucose levels corresponded to pre-diabetes or fall within the diabetic range. This finding and those related to high body fat levels provide an opportunity to advocate the assessment and management of metabolic status during BC treatment. This also invites further research into metabolic changes post-diagnosis and BC-related factors associated with unhealthy glucose and insulin changes. This would add to the emerging literature revealing that BC chemotherapy could affect insulin levels (Makari-Judson et al. 2009; Alacacioglu et al. 2016), and that higher levels of glucose might produce chemo-resistance (Song et al. 2016).

Highlighting the clinical implications of the assessment of body weight, body adiposity and metabolic parameters, to both patients and clinical staff, might promote the implementation of these recommendations in the clinical setting. Benefits from an accurate assessment would also extend to researchers, who will be able to continue investigating the effects of body weight, adiposity and insulin on BC prognosis, as well as other aspects such as psychological and functional well-being post BC diagnosis.

Weight and FM and FFM can be easily and quickly measured through BIA using a Tanita machine. However, the measurement of waist circumference might be subject to error as there are different places to locate the tape when measuring it. The World Health Organization (2008) recommends measuring at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, although the method of measuring waist circumference does not substantially affect the association between its value and health outcomes (i.e. risk of diabetes) (Ross et al. 2008).

5.6.2 Care plan to improve control of weight, adiposity and metabolic parameters

The findings from this study have identified some factors which are statistically significantly associated with weight change and larger body fat and insulin levels (i.e. smoking status, body weight at diagnosis, hormone therapy). These are valuable findings which suggest that some subgroups of patients might be at higher risk of experiencing potentially harmful changes in body weight and body fat post-diagnosis. In addition, the study identified a large number of participants that were overweight or obese at the time of BC diagnosis.

Considering the apparently negative impact of both obesity and post-diagnosis weight changes on BC prognosis (Nichols et al. 2009; Protani et al. 2010; Druesne-Pecollo et al. 2012; Niraula et al. 2012; Jain et al. 2013; Azrad and Demark-Wahnefried 2014; Chan et al. 2014), findings from this study propose that newly diagnosed overweight/obese women, as well as those with an increased risk of weight change (i.e. to those who smoke, those who are leaner or younger and those who will be treated with hormone therapy) might benefit from weight control/management interventions as compared with other subgroups of patients. This recommendation is supported by the American Cancer Society, which advises patients to maintain a healthy body weight during and after cancer treatment (Rock et al. 2012).

Additionally, in view of the emerging literature on the impact of chemotherapy on glucose levels, metabolic monitoring might be beneficial for women receiving chemotherapy. Luckily, numerous observational and interventional studies have demonstrated that body weight and the body adiposity and metabolic outcomes explored in this study can be modified to reach optimal levels. Vanegas et al. (2015) found that those BC patients meeting national physical activity guidelines had lower body weights than those who were insufficiently active or sedentary. In addition, behavioural interventions and counselling on exercise and diet have been shown to contribute to prevention of body fat gains (for a review Sheean et al. 2012), to favourable changes in body weight and body adiposity levels (Campbell et al. 2007b; Irwin et al. 2009; Friedenreich et al. 2011; Playdon et al. 2013), to improvements in

insulin levels and other biomarkers (Campbell et al. 2007b; Ligibel et al. 2008; Harrigan et al. 2016) and to better psychological and functional well-being among BC survivors (Knobf et al. 2014).

Weight loss interventions published have taken different approaches (in-person, telephone, individual or group counselling). In general, these interventions seem to be feasible for BC survivors, although there is a need to promote activities to increase compliance with the interventions (Paydon et al. 2013). As explained in Chapter 1, weight loss have been associated with poorer BC prognosis, nonetheless, it is likely that that weight loss was unintentional rather than intentional (Jackson et al. 2017). Among the general population, intentional weight loss leads to changes in biomarkers that are plausibly linked with cancer-related outcomes (Jackson et al. 2017). Therefore, it is likely that weight loss interventions in BC patients could be beneficial. In fact, several (intentional) weight loss interventions among BC survivors have improved symptoms and side effects of BC treatments, showing the potential benefits of weight loss interventions among BC survivors (Jackson et al. 2017). Moreover, the fact that obesity is associated with poorer prognosis clearly underline the importance of interventions aiming to reduce overweight and obesity prevalence among BC survivors (Druesne-Pecollo et al. 2012). Hence, an emphasis should be put in integrating behavioural interventions into a BC care plan to promote better health outcomes (Knobf and Coviello 2011).

Research has shown that weight-loss multi-component interventions (i.e. diet and exercise) are more successful than single-component interventions (Playdon et al. 2013), nonetheless, more research is needed to ascertain details of these interventions, such as the most effective intervention for each specific outcome or the optimal timing of the intervention (Vagenas et al. 2015). Also further research is needed on the prevention of potentially harmful unintentional weight changes following BC diagnosis, and on the repercussions that intentional weight loss and improvements in metabolic and adiposity parameters might have on BC outcomes (Playdon et al. 2013; Goodwin 2015a). This would enable professionals to provide timely interventions to prevent increases in body fat and body weight, or to achieve an optimal body weight for those participants with initial weight concerns.

5.7 Limitations of the study

Several limitations of this study warrant discussion.

5.7.1 Representativeness of the sample

Because of the lack of random sampling, it is difficult to ensure that the sample is truly representative of the BC population in the UK. This might limit the generalisability of the findings. Nonetheless, as discussed earlier, the characteristics of the participants seem equivalent to those of the BC population in the UK. Moreover, many findings are comparable to other studies with BC patients, suggesting that the study has good external validity.

5.7.2 Validity of data collected

Most data were retrospective. The validity of the weights collected from the medical notes from the time of diagnosis to the end of the follow up was dependent on the reliability and validity of the scales measuring it and on the precision and accuracy of the clinical staff recording it. This might have lead to measurement errors, data entry errors, or missing data, which can affect the internal validity of the findings (De Vaus 2001). Multilevel modelling showed that the intra-class correlation, a test which can be used to assess the magnitude of measurement error (Peat et al. 2002), was 0.95, which indicates that the repeated weight measures were highly correlated. Waist circumference measurements can be expected to have an intra-measurement error of 1.31 cm and inter-measurer error of 1.56 cm (World Health Organization 2008).

In order to reduce errors when measuring weight and body adiposity parameters, measurements were taken by trained nurses following the same data collection protocol. Furthermore, an attempt was made to ensure uniform measurement conditions across participants. For instance, measurements were taken early in the

morning and participants were asked to fast, avoid physical activity as much as possible, and to empty their bladder before the measurement, as it is known that BIA is susceptible to changes in the quantity and distribution of body water (Tanita Corportation 2014). Finally, data entry was double-checked in order to minimise errors (Web Center for Social Research Methods 2006). Measurement error may have made the results less precise. Hence, when possible, it was reported whether the magnitude found falls within the range of results from previous studies.

The issue of missing data is a common problem in retrospective studies and was explored in depth in Chapter 3 and in Appendix II, with the aim of identifying sources of missing data and the mechanisms leading to missing data. This exhaustive analysis led to the conclusion that a complete case analysis was the best approach to deal with missing data. In addition, the multilevel modelling conducted in this study was able to handle missing data (Singer and Willet 2003).

A final potential source of error in this study is that participants reported the values of some of the variables collected (i.e. change in menopausal status, smoking status at diagnosis). Recall and/or social desirability bias might have affected the validity of those variables. When possible, their answers were validated with data from their medical notes to demonstrate veracity.

5.7.3 Lack of control group

The lack of a healthy control group makes it difficult to ascertain whether the weight changes found in women with BC differ from the usual weight change of healthy women of a comparable age. Unfortunately, the number of participants receiving local therapy alone (surgery and radiotherapy) was small so they could not serve as a control arm. However, this reflects current clinical practice in the UK (most women with BC receive adjuvant therapy) and this study was designed to overcome these limitations by comparing weight change in participants with and without the factor explored.

5.7.4 Lack of statistical power

The total number of participants is slightly lower than the target sample size planned from power calculations conducted prior to enrolling the study. This might have resulted in a lack of statistical power to detect statistically significant results when exploring the associations tested. With a target sample size of 250 participants, the study was expected to be able to detect minimum effect sizes of 0.35 when looking at differences in weight change between groups of treatment use. Fortunately, the effect sizes for the difference in weight change between treatment groups are reassuring (data not shown). The magnitudes of the effect sizes found when looking at the effect of tamoxifen use ranged from 0.31 to 0.72, figures that were mainly larger than 0.35. Furthermore, the effect size of the difference in weight change at 36 months post-diagnosis depending on chemotherapy use was 0.36, which is also larger than 0.35. These figures are reassuring and indicate that despite the smaller sample size, the study was powered to detect differences when looking at weight changes associated with some independent variables.

Missing data and the characteristics of the sample itself (i.e. small number of participants with or without a factor) might also have reduced statistical power when conducting some analyses. Nonetheless, many of the findings agreed with previous studies. When discrepancies were found, an attempt was made to find a plausible explanation for the results, or alternatively, the findings were taken as preliminary, and for hypothesis generation.

5.7.5 Unbalanced data

Weight values were collected retrospectively from the medical notes. Therefore it was not possible to control the number of observations recorded nor the timing of the measures, which led to a severely unbalanced dataset. This reduced the sample size of the analyses conducted and complicated the planned multilevel analysis, as some models (i.e. quadratic models) could not be fitted due to convergence problems which did not allow the estimation of one or more variance components.

Furthermore, the large range of months of follow up (from 10.25 to 91.17 months), and the fact that body adiposity and metabolic assessments were not completed at a standardised time post-diagnosis led to considerable sample heterogeneity.

5.7.6 Causality

Because of the study's retrospective nature, it was not possible to prove a causal relationship between factors that were statistically significantly associated with weight change and the weight change itself, a limitation that extends to the cross-sectional data collected on adiposity and metabolic parameters. Nonetheless, other features of the research add support for a causal relationship for the associations found in this study, including the magnitudes of the effect sizes of the associations found between independent variables and the outcomes, the agreement with results from other studies, and the fact that current knowledge and findings from experimental studies provide evidence of the biological plausibility of the associations found (Hill 1965).

It is worth acknowledging that the weight change observed as well as the levels of adiposity and metabolic status might be influenced by other factors that due to the retrospective nature of the study were not able to be explored (i.e. energy intake and expenditure, psychological status, sleeping patterns).

5.7.7 Data analysis

Weight change has been analysed looking at mean changes from diagnosis to several evaluation points (i.e. 12, 24, 36 and 48 months post-diagnosis) and, as in the analysis of the adiposity and metabolic parameters, using a large number of single inference parametric and non-parametric significance tests to check their association with the independent variables explored (i.e. BC treatment, genes, menopausal status, etc.). As stated earlier, the use of multiple hypotheses tests in the same group of participants could have increased the probability of finding a false statistically

significant association between weight change and some of the factors, when there is not a true association (phenomena known as type-one error) (Bland and altman 1995). This limitation could have been overcome using one of the several methods to control for the probability of making a type-one error when performing multiple hypotheses tests (i.e. the Bonferroni correction, the Holm–Bonferroni method and the Šidák correction) (Miller 1966; Frane 2015). Nonetheless, although these results should be taken with caution as the multiplicity was not adjusted for, it is worth mentioning several points. First, the independent variables analysed in this study were chosen based on previous studies in the field of this research, after considering the fact those variables could provide a plausible biological justification for the association tested. Secondly, the findings in this study have been discussed in relation to existing literature in the field, offering explanation for the associations found. Thirdly, a number of significant associations found in the study would have met stricter significance levels (e.g. 1% or 0.1%), as discussed earlier on. Finally, the use of multilevel modelling, an analytical approach that address the problem of multiple hypothesis testing (Gelman et al. 2012), have confirmed some of the statistical significant associations found in univariable and multiple regression analysis (i.e. the effect of tamoxifen).

The study analysed weight change and factors associated with weight change measured as a continuous variable. Consistent with most literature in the field, findings revealed that on average, weight tended to increase post-diagnosis. Nonetheless, it should be noted that at an individual level, some participants gained weight, some participants lost weight and others remained the same. This is relevant as both large weight gain and weight loss post-diagnosis could have clinical implications. By looking at the frequency of weight change, the study have identified a small group of participants with large weight changes (i.e. more than 10% of their weight at diagnosis), which according to results from survival studies among BC cancer survivors, might put them at risk of poorer BC outcomes and other health issue. Investigating the characteristics of these subgroups of participants with large weight gains or large weight loses on a larger sample size would be an area of further research, but was out of the scope of this study. Furthermore, the number of participants who experienced large weight changes at those time points was small

(five to eight participants gained more than 10% and three to six participants lost more than 10%). Therefore, the study might not have been powered to explore the clinical and biological characteristics associated with different categories of weight change, nor to observe differences between them. Aige et al. (2014) observed that findings comparing factors associated with weight change can vary depending on how weight change is modelled and defined. For instance, the found that education level was not associated with mean weight change (continuous variable). However, when weight change was modelled as a categorical variable, they found a statistically significant association between the two variables. Consequently, further research could look at weight change and factors related to it, using both continuous and categorical weight change. This will avoid losing directional information on weight change and would increase comparability with other studies in the field, which as Table 1 (Appendix I) showed, was explored in different ways.

A final limitation comes from the fact that the number of weight records held by participants decreased considerably after the first year of diagnosis, which may have influenced the estimation of the weight change function (Singer & Willett 2003).

5.8 Strengths of the study

Some of the strengths of this study come from the methods of conducting the study.

5.8.1 Data collection

Body fat and FFM levels as well as metabolic data were collected with validated measurement tools (i.e. Tanita machine) following recommended protocols as stated by the manufacturer or the recognised authorities (i.e. the World Health Organization).

Weight change was measured from diagnosis, an advantage over other studies that explored weight change from later points, as it is possible that some women might

have already experienced changes in body weight after diagnosis but before enrolment into those studies.

5.8.2 Repeated measures and long follow up

This study is one of the few that has examined weight changes post-BC diagnosis beyond three years, which allowed the researcher to explore the effects of chemotherapy in the long term and allowed the effects of hormone therapy (if any) on weight change to emerge.

Longitudinal data such as the one found in this study are required for exploring change (Singer and Willet 2003). The repeated weight measures from diagnosis to end of follow up offered the potential to explore different aspects of the outcome, such as number of participants that experienced weight change, weight changes at key evaluation points from diagnosis and patterns of occurrence. This is an improvement compared with many studies in the field that were restricted to weights measures at two time points.

5.8.3 Data analysis

The meticulous data analysis also should be noted**.** An intense assessment of the data collected was conducted with the aim of assessing the validity of the results, determining the adequacy of using a parametric approach for data analysis, identifying the source and impact of missing data, and the presence and effects of outliers.

This study explores an array of factors potentially responsible for the weight changes and the values of adiposity and insulin levels observed among participants. These factors were chosen based on literature review and were explored in detail, looking at subcategories of hormone therapy (tamoxifen, anastrozole, letrozole, etc.). In addition, the study was designed to look at the impact of FTO and Mc4R genotypes

on the outcomes, as there was no published evidence looking at those genes in the context of this study.

Multiple linear regression analysis was used to improve the validity of the study by taking into account the combined effect of these known and novel factors which might be related to weight gain post-diagnosis.

Final strengths of this study are two important elements exploring weight change: 1) many participants had more than three weight records and 2) a sensible metric for clocking time. These allowed the researcher to conduct a multilevel analysis to characterise each person's pattern of weight change over time and also to identify differences in weight and rate of weight change among women, as well as factors which could predict those differences (Singer and Willet 2003).

5.9 Conclusions

This study showed changes in weight post BC diagnosis. The magnitude of body weight, fat and waist circumference seemed to be higher than those found in the general population, which could negatively affect the quality of life of BC survivors, and may put them at risk of metabolic disorders. Worryingly, a small percentage of the sample lost large amounts of weight and one quarter of participants gained more than 5% of their body weight at diagnosis, magnitudes that have been associated with worse BC prognosis and increase risk of obesity-related diseases such as diabetes. In fact, this study identified a small percentage of participants whose glucose levels corresponded to pre-diabetes or fell within the diabetic range.

Understanding the characteristics associated with changes or levels of those outcomes can help health professionals identify patients at risk. Findings in this study suggests that hormone therapy might contribute to differences in weight and rate of weight change post-diagnosis, and that weight at the time of BC diagnosis could predict body adiposity parameters and metabolic status post-diagnosis. Nonetheless, the effects of other variables (i.e. smoking status, genetic profile and other variables not included in this study) cannot be ruled out.

Since higher levels of body weight, fat and insulin are associated with poorer prognosis and other health risks, it is imperative to assess those parameters following BC diagnosis and to implement care plans to control them. This may help newly diagnosed BC patients to achieve optimal weight, body adiposity parameters, and a healthy metabolic profile. Given that the tendency to change weight seems to continue in the long term, these strategies should continue during the adjuvant chemotherapy and hormone therapy.

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APPENDIX I: TABLES AND FIGURES

Appendix I Introduction

This appendix contains all the tables mentioned in the Chapters 1 to 5.

Table 1 Appendix I - Summary of results from previous studies on weight change after breast cancer diagnosis and factors associated with weight change

Authors	Follow up for weight recordings	Sample Size	Frequency of weight gain and weight lost	Magnitude of changes in weight and body adiposity parameters[a]	Factors associated with weight change and body adiposity[a] changes
Aslani et al. 1999	Prospective. During chemotherapy.	25	Not given.	Weight: + 2.35 kg[b] FM: + 1.49 kg	Weight gain not associated with menopausal status or age within each menopausal status.
Basaran et al. 2011	Retrospective. From chemotherapy, to one year later.	176	At completion of chemotherapy: 67% gained weight. 22% lost weight. One year follow up: 72% gained weight. 18% lost weight.	At completion: Weight: + 1.7 kg (2.4% of baseline weight). One year follow up: Weight: + 3 kg (4.2% of baseline weight).	Weight gain associated with younger age, premenopausal status, multiparity and comorbidity.
Bradshaw et al. 2012	Prospective. One year prior diagnosis to five years post-diagnosis.	1,436	One year post-diagnosis: 22% gained (>5% of initial weight). 23% lost >5% At five years follow up: 35% gained weight. 21% lost weight.	Overall mean weight change not reported.	Not measured.

Caan et al. 2006	Prospective. From one year prior diagnosis to mean of 22.9 months post-diagnosis.	3,215	39.59% gained weight (>5% of initial body weight). 14.96% lost weight (>5%).	Mean weight gain: Chemotherapy: Yes: + 4.6% No: + 2.5% Tamoxifen: Never: + 4.3% Past: + 5.5% Current: + 3.4% Premenopausal: + 5.6% Postmenopausal: + 3.0%	Weight gain associated with chemotherapy, height, and inversely associated with pre-diagnosis BMI. Younger and premenopausal women were more likely to have greater weight gain than older or postmenopausal women.
Caan et al. 2008	Prospective. From one year prior diagnosis to mean of 22.7 months post-diagnosis.	1,692	25.9% gained (within5% of initial weight). 27.1% lost weight.	Weight: + 1.7 kg	Weight gain associated to chemotherapy and cancer stage. Younger and premenopausal women were more likely to gain more weight than older or postmenopausal women.
Caan et al. 2012b	Prospective. From one year prior diagnosis to mean of 2.1 years post-diagnosis.	12,915	United States site: 33.7% gained >5% of initial weight. 15.0% loss weight. Shanghai site: 36.6% gained weight. 13.9% loss weight.	Weight: + 1.6 kg	Weight gain was more common in normo-weight women and in premenopausal women.
Camoriano et al. 1990	Retrospective. During chemotherapy.	545	88.95% gained weight.	Median weight change: Treated premenopausal: + 5.9 kg Treated postmenopausal: + 3.6 kg Non-treated postmenopausal: + 1.8 kg	Weight gain associated with premenopausal status and lower baseline weight (in premenopausal women). Weight gain was not associated with: age, developing amenhorrea, or tamoxifen.

Campbell et al. 2007	Prospective. During chemotherapy.	10	7 gained weight. 3 lost weight.	No significant weight change. FM: + 2.3 kg No changes in muscle mass or resting energy expenditure (REE)[c]	Body weight, REE[c] and body composition were not associated with treatment. Premenopausal women tended to gain more weight compared with postmenopausal women.
Chen et al. 2011	Retrospective. One year pre-diagnosis to 18 months post-diagnosis.	4,561	51% gained >2 kg 27% lost >2 kg 14% maintained their weight (± 2 kg).	Weight: + 1.73 kg	Weight gain associated with chemotherapy and radiotherapy use, younger age, premenopausal status, high caloric intake, advanced disease stage and smoking. Weight gain not associated to treatment, exercise, menopausal symptoms.
Costa et al. 2002	Retrospective. During chemotherapy.	74	81.1% gained weight.	Weight: + 0.91% per month.	Weight gain was not associated with age, menopausal status or BMI at diagnosis.
Del Rio et al. 2002	Prospective. During chemotherapy and six months later.	30	100% gained weight.	Weight: + 2.8 kg FFM: + 1.6 kg FM: + 1.2 kg	Weight gain not associated to changes in REE[c] . Chemotherapy was not associated with changes in REE[c]. Increased REE[c] likely due to the increase in FFM.
Demark-Wahnefried et al. 1997	Prospective. From chemotherapy to one year later.	20	Not reported.	At the end of chemotherapy: No changes in weight or FM during chemotherapy. Trend towards losing FFM (- 0.4 kg). At one year follow up Weight: + 3.8 kg	Lack of weight change not associated with changes in caloric intake, physical activity or resting metabolic rate.

Demark-Wahnefried et al. 2001	Prospective. 12 months from starting treatment.	53	Not reported.	Chemotherapy user: Weight: + 2.1 kg FM: + 2.3 kg FFM: - 0.4 kg Surgery ± radiotherapy group: Weight: + 1 kg FM: + 0.1 kg FFM: + 0.8 kg	Weight gain not associated with caloric intake, or REE[c], but might be associated to a decrease of physical activity.
Francini et al. 2006	Prospective. 12 months post randomisation.	60	Not provided.	Exemestane group: weight - 1.99 kg, FM and FFM/FM ratio: decreased. Tamoxifen group: weight: - 0.58 kg. There was no change in FM and FFM/FM ratio.	Significant differences in FM and FFM/FM ratio between the groups.
Freedman et al. 2004	Prospective. During and six months after chemotherapy.	20	During Chemotherapy: Treated group: 12 gained, and 8 lost weight. After Chemotherapy: 12 gained and 7 lost weight.	Chemotherapy users: % FM, minimal waist and hip circumference increased and FFM decreased. At the end of follow up: Chemotherapy group: weight + 0.1 g/day. Control group: weight + 2.1 g/day.	Weight gain associated to premenopausal status. Weight change was not associated with chemotherapy, physical activity, or appetite. No differences in weight change between the groups.
Genton et al. 2006	Prospective. During and six weeks post radiotherapy.	37	81.08% gained. 6 weeks post radiotherapy.	From diagnosis to radiotherapy: Weight: + 2.2 kg Six weeks post-radiotherapy: Weight: + 3.3 kg FFM: + 0.8 kg	Weight gain not associated with treatment, physical activity or appetite.

Goodwin et al. 1999	Prospective. 12 months post-diagnosis.	445	84% gained weight.	Mean weight gain: Chemotherapy group: + 2.50 kg Tamoxifen group: weight + 1.26 kg No treatment group: weight + 0.63 kg Waist: + 1cm Hip: + 1.4 cm	Weight gain associated with chemotherapy and change of menopause status. Weight gain not associated with energy intake or physical activity.
Gu et al. 2010	Prospective. Diagnosis to 36 months post-diagnosis.	5,014	At 36 months: 46% gained > 2 kg And 17% lost > 2 kg	Median weight change from diagnosis to: 6 months: + 1.0 kg 18 months: + 2.0 kg 36 months: + 1.0 kg	Weight gain associated with younger age, lower BMI at diagnosis, less comorbidty and lower disease stage. Weight gain not associated with treatment 36 months post-diagnosis.
Guinan et al. 2014	Retrospective. From surgery to mean 3.28 years post surgery.	61	No significant weight gain.	Weight: + 0.91 kg % FM: + 1.37% FFM: - 0.09 kg	Adjuvant treatment was not associated with weight or body adiposity levels changes.
Han et al. 2009	Prospective. Baseline to 24 months of adjuvant treatment.	260	10.4% gained > 5% of baseline body weight at 1 year.	Weight: At 3 months: + 0.30 kg At 6 months: + 0.16 kg At 12 months: - 0.34 kg At 24 months: - 0.40 kg	Weight gain not associated with any clinical variable explored.

Harvie et al. 2004	Prospective. During and six months post chemotherapy.	17	Not reported.	Weight: + 5.0 kg FM: + 7.15 kg %FM: + 6.2% FFM: - 1.7 kg Waist circumference: +5.1cm	Not reported.
Heideman et al. 2009	Retrospective. Diagnosis to five years post-diagnosis.	271	At 1 year: 26% gained >5 kg	Weight change at one year: Radiotherapy only: + 1.5 kg Chemotherapy only: + 2.2 kg Hormone therapy only: + 1.4 kg Chemo + Hormone therapy: + 2.6 kg At end of follow up: + 2.4 kg	Greatest weight gain at end of follow up associated with combination of chemotherapy and hormone therapy. Radiotherapy or hormone therapy alone not associated with weight gain.
Irwin et al. 2005	Prospective. From within the first year of diagnosis to two years later.	514	68% gained weight. 32% lost weight.	Weight: + 1.7 kg FM: + 2.1% Chemotherapy ± tamoxifen: + 3.0 kg Tamoxifen ± chemotherapy + 1.7 kg Surgery only: + 1.5 kg Premenopausal: + 0.3 kg Pre to post: + 2.0 kg Postmenopausal: + 3.3 kg	Weight gain associated with greater disease stage, younger age, postmenopausal status and reduced physical activity, and with chemotherapy in a subgroup of patients. It was not associated with tamoxifen. Change in body fat was associated with decrease of physical activity, but not with chemotherapy, tamoxifen or menopausal status.
Kroenke et al. 2005	Prospective. From two years before to three years post-diagnosis.	5,204	46.3% gained (>0.5 kg/m^2 of their baseline BMI). 22.69% lost BMI	Weight: + 3.8 lb	Women with the largest gains in weight tended to be more likely to have received chemotherapy or tamoxifen, and to have later stage tumours.

Kumar et al. 2004	Prospective. During and six months post chemotherapy.	198	22% gained > 5 lb	Weight: + 3.1 kg	Weight gain associated to fatigue and sex hormone serum globulin levels. Weight gain not associated to physical activity, or caloric intake.
Kutynec et al. 1999	Prospective. During 12 weeks of treatment.	18	After 66 weeks: Chemotherapy group: 4 of 7 participants gained > 1 kg After 103 weeks: Radiotherapy group: 4 of 6 participants gained > 1 kg	Chemotherapy group: % FM: + 1.4% FFM: - 1.0 kg Radiotherapy group: % FM: + 1.0% FFM: - 0.6 kg	No differences in weight, FM, FFM changes between the two treatment groups.
Lankester et al. 2002	Prospective. During chemotherapy.	100	64% gained > 2 kg 31% maintained weight. 5% lost > 2 kg	Weight: + 3.68 kg	Weight gain was not associated with disease stage, different chemotherapy regimens, tamoxifen, or menopausal status.
Makari-Judson et al. 2007	Retrospective. Diagnosis to three years post-diagnosis.	185	71% gained weight at year 1. 29% lost weight at year 1.	From diagnosis to: Year 1: + 1.5 kg Year 2: + 2.7 kg Year 3: + 2.8 kg	Weight gain at year 1 and 2 was associated with chemotherapy, younger age and BMI. No factor associated to weight gain from year 2 to year 3. Hormone therapy, chemotherapy regimens, duration of chemotherapy, or dexamethasone not associated with weight gain.

Makari-Judson et al. 2009	Prospective. Before adjuvant therapy to 12 months later.	95	Not available.	Not changes in waist or waist to hip ratio. At 6 months: weight : + 0.4 kg At 12 months: weight: + 0.9 kg	Chemotherapy associated with significant changes in homeostasis model assessment of insulin resistance (HOMA-IR), glucose/insulin and waist to hip ratio at 12 months. Hormone therapy or exercise not associated with significant changes in outcomes.
McInnes and Knobf 2001	Retrospective. Three years from diagnosis.	44	> 65% gained >5 lb at years one, two and three.	Weight gain from diagnosis to: 1 year: + 9.0 lb 2 years: + 8.93 lb 3 years: + 10.85 lb	Weight gain was associated with premenopausal status. It was not associated with tamoxifen.
Nissen et al. 2011	Prospective. Start of chemotherapy to 12 months later.	49	13 out of 49 gained <5% of initial body weight.	Weight change: Normo-weight women: + 4.3 lb Overweight women: - 3.0 lb Obese women: - 4.1 lb Normo-weight women gained FM and FFM. Neither obese or overweight women gained FM, but they lost FFM.	Weight gain was associated with decreased physical activity and BMI at diagnosis. It was not associated with chemotherapy regimens, tamoxifen, AINs, or with caloric intake.
Reddy et al. 2013	Retrospective. Diagnosis, to 18 months post-diagnosis.	459	At six months: 13% gained >5 kg/m^2. At 18 months: 36% gained >5 kg/m^2.	Weight change: At 6 months: + 0.3 kg At 12 months: + 1.0.kg At 18 months: + 1.9.kg	Weight gain was inversely associated with age and BMI at diagnosis, and positively with absence of chemotherapy. There was support for weight gain with tamoxifen, although results were not significant across all points.

Rock et al. 1999	Retrospective. From one year before diagnosis to the end of the follow up (mean 23.7 months).	1,116	60% gained >5% of initial body weight. 26% lost weight.	Weight change: No chemotherapy group: + 1.5 kg Chemotherapy groups[b]: AC: +1.8 kg CFA: + 3.3 kg CMF: + 4.4 kg Other regimen: + 3.4 kg	Weight gain positively associated with chemotherapy, time since diagnosis and caloric intake, and negatively associated with BMI at diagnosis and physical activity. Hormone therapy or radiotherapy not associated with weight gain.
Saquib et al. 2007	Retrospective. From one year before diagnosis to a mean 23.7 months.	3,045	Not reported.	OR for weight gain (>5% or more) in women receiving: Chemotherapy only: 1.65 Tamoxifen only: 1.03 Both treatments: 1.69	Weight gain associated with chemotherapy, but not with tamoxifen or with chemotherapy regimens. Younger age and women with lower pre-cancer BMI tend to gain more weight.
Sestak et al. 2012	Retrospective. Zero to 60 months post-randomisation.	6,186	At 12 months: 39.7% gained > 2 kg 14.8% lost weight. At 60 months: 40% gained weight. 29% lost weight.	Weight change: 12 months: + 1.4 kg 60 months: - 0.35 kg	Weight gain at 12 months associated with being younger than 60 years old, smoking at entry and having had a mastectomy, and not associated with treatment. Radiotherapy was associated with less weight gain.
Tredan et al. 2010	Prospective. During to 15 months later.	272	At nine months: 52.1%gained weight. 29.8% lost weight. At 15 months: 59.7% gained weight. 29.2% lost weight.	Weight gain: At 9 months: + 0.6 kg (1%of baseline weight) At 15 months: + 1.5kg (+2.3%).	Weight gain not associated with age, baseline BMI, chemotherapy, or menopausal status.

Vagenas et al. 2015	Prospective. From sx to 72 months post-surgery.	287	From 6-18 months: 24% gained >1 unit BMI. 15% lost >1 unit BMI. From 6-72 months: 39% gained >1 unit BMI. 24% lost >1 unit BMI.	Weight change 6-18 months: Chemotherapy group: + 0.2 kg No chemotherapy: + 0.1 kg Hormone therapy: - 0.2 kg No hormone therapy: + 0.5 kg	Weight gain associated with radiotherapy.
Wayne et al. 2004	Prospective. Diagnosis to two years post-diagnosis.	260	23% gained >3kg. 7.69% lost weight.	Weight: + 1.5 kg	Weight gain not associated with caloric intake.

[a] Body adiposity parameters: FM: fat mass, FFM: fat free mass, WHR: waist to hip ratio.

[b] Abbreviations used: kg: kilograms, REE: resting energy expenditure.BMI: body mass index, HOMA-IR: homeostasis model assessment of insulin resistance, AINs: aromatase inhibitors and chemotherapy regimens: AC = doxorubicin + cyclophosphamide, CFA: cyclophosphamide + 5-fluorouacil + doxorubicin, CMF = cyclophosphamide + methotrexate + 5-fluorouacil.

Table 2 Appendix I - Standardised effect sizes of weight change after breast cancer diagnosis found in previous studies in the field

Authors	Sample size and follow up	Standardised effect size (Cohen's *d*) for weight change according to treatment	Standardised effect size for weight change according to menopausal staus, body mas index, weight at diagnosis and age
Caan et al. 2006	3,215 From one year pre diagnosis to almost two years post-diagnosis.	Chemotherapy vs. no chemotherapy: 0.214 Past users of tamoxifen vs. never users: 0.118 Current tamoxifen users and never users: -0.091	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.271
Chen et al. 2011	4,561 18 months post-diagnosis.	Chemotherapy vs. no chemotherapy: 0.395 Tamoxifen vs. no tamoxifen: 0.246	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.465 Comparing younger age (40-49 years) vs. older age (50-59 years) (Cohen's *d*): 0.291
Goodwin et al. 1999	445 12 months post-diagnosis.	Not enough information to calculate it.	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.219 Pearson's r values for weight gain and**:** Age: -0.09 (p=0.05) Baseline weight: 0.08 (p=0.11)
Heideman et al. 2009	271 12 months post-diagnosis.	<u>At year one:</u> Chemotherapy vs. radiotherapy: 0.174 Chemotherapy vs. hormone therapy: 0.191 Chemotherapy + hormone therapy vs. radiotherapy: 0.212 <u>At year five:</u> Chemotherapy vs. radiotherapy: 0.183 Chemotherapy vs. hormone therapy: 0.161 Chemo + hormone therapy vs. radiotherapy: 0.700	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): at year one: 0.123 at year five: 0.522

Irwin et al. 2005	514 From a mean of six months from diagnosis to two years later.	Chemotherapy vs. surgery only: 0.308 Tamoxifen vs. no tamoxifen: -0.021	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.025 Comparing change of menopausal status vs. remaining premenopausal (Cohen's *d*): 0.313
Makari-Judson et al. 2007	185 From diagnosis to year one, two and three.	Chemotherapy vs. no chemotherapy: 0.969 Hormone therapy vs. no hormone therapy: -0.259	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.417 Pearson's r values for weight gain and: Age: -0.030 (p=0.0001) Body mass index diagnosis: -0.2 (p=0.026)
Nissen et al. 2011	49 From start of chemotherapy and 12 months later.	Tamoxifen users vs. never users: 0.5336	Not enough information to calculate it.
Rock et al. 1999	1,116 From one year before diagnosis to maximum four years post-diagnosis.	Chemotherapy vs. no chemotherapy: 0.040 Hormone therapy past used vs. never used: 0.128	Cohen's *d* when comparing: *:* Young post vs. premenopausal: 0.27 Old post vs. premenopausal: 0.06 Linear regression for weight gain R^2 value =0.13
Saquib et al. 2007	3,045 From one year before diagnosis to a mean of two years.	OR (gain >5% or more of baseline weight) Chemotherapy only : 1.65 Tamoxifen only : 1.03 Tamoxifen + chemotherapy: 1.69	Not enough information to calculate it.
Tredan et al. 2010	272 During to 15 months later.	n/a	Comparing premenopausal vs. postmenopausal women (Cohen's *d*): 0.177

Table 3 Appendix I - Number of weight records hold by participants from breast cancer diagnosis to the end of the follow up

	All follow up (from diagnosis to the end of the follow up)	**During the 1st year (from diagnosis to 12.50 months)**	**During the 2nd year (from 12.51 to 24.50 months)**	**During the 3rd year (from 24.51 to 36.50 months)**	**During the 4th year (from 36.51 to 48.50 months)**	**During the 5th year (from 48.51 to 60.50 months)**	**During the 6th year (from 60.51 to 72.50 months)**	**During the 7th year (from 72.51 to 84.50 months)**
Number of weight records	2,172[a]	1,290	266	219	173	111	63	38
Mean number of weight records (Standard deviation)	9.09 (7.34)	5.42 (4.91)	1.13 (1.51)	1.09 (1.32)	1.08 (1.33)	1.02 (1.35)	0.98 (0.92)	1.05 (0.94)
Number of weight records	**Number of participants**							
None	0	14	105	89	56	37	23	9
1	8	62	56	52	63	54	26	23
2	39	36	45	35	30	11	11	2
3	27	17	14	15	8	2	5	1
4	27	8	11	7	2	2	0	2
5	15	4	4	2	0	1	0	0
6	9	6	0	1	1	1	0	0
7	9	6	0	0	0	0	0	0

8	1	6	0	1	0	0	0	0
9	3	17	0	0	0	0	0	0
10	7	11	0	0	0	1	0	0
11	4	20	0	0	0	0	0	0
12	9	12	0	0	1	0	0	0
13	9	6	0	0	0	0	0	0
14	15	5	1	0	0	0	0	0
15	7	2	0	0	0	0	0	0
16	11	2	0	0	0	0	0	0
17	7	2	0	0	0	0	0	0
18	4	1	0	0	0	0	0	0
19	5	2	0	0	0	0	0	0
20	2	0	0	0	0	0	0	0
21	3	0	0	0	0	0	0	0
22	5	0	0	0	0	0	0	0
23	5	0	0	0	0	0	0	0
25	2	0	0	0	0	0	0	0
26	3	0	0	0	0	0	0	0
27	1	0	0	0	0	0	0	0
28	1	0	0	0	0	0	0	0
43	1	0	0	0	0	0	0	0
Number of participants	239	239	236	202	161	109	65	37

[a] This number includes one woman with missing information on how many months her only weight recorded was taken after diagnosis. This number also includes eleven women, each one having 1 weight recorded more than 88 months post-diagnosis.

Table 4 Appendix I - Number of pre, peri and postmenopausal participants at breast cancer diagnosis and at 12, 24, 36 and 48 months post-diagnosis

	At diagnosis n=239	**At 12 months post-diagnosis[a]**	**At 24 months post-diagnosis[a]**	**At 36 months post-diagnosis[a]**	**At 48 months post-diagnosis[a]**
Premenopausal n (%)	52 (21.8%)	4 (4.0%)	4 (4.5%)	2 (3.0%)	1 (1.9%)
Perimenopausal n (%)	13 (5.4%)	15 (15.2%)	5 (5.7%)	4 (6.1%)	2 (3.7%)
Postmenopausal n (%)	174 (72.8%)	80 (80.8%)	79 (89.8%)	60 (90.9%)	51 (94.4%)

[a] Participants included in each period explored were those that were included in the analysis of weight change at 12, 24, 36 and 48 months post-diagnosis, and whose menopausal status was available (n=99, n=88, n=66 and n=54, respectively).

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Table 5 Appendix I - Number of participants grouped according to genetic profile included in the analysis of weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	Weight change from diagnosis to:			
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**
FTO (n):				
AA	8	7	4	4
AT	37	38	23	22
TT	24	21	13	7
McR4 (n):				
CC	5	6	2	2
TC	22	19	14	9
TT	42	41	24	22

Table 6 Appendix I - Participants' biological characteristics at breast cancer diagnosis

Participants' biological characteristics	Mean (SD)[a]
Weight (kg[b])	72.2 (13.6)
Missing, n	18
Height (m[c])	1.6 (0.1)
Missing, n	39
BMI[d] range (kg/m^2):	26.9 ± 5.0
<18.5 n(%)	1 (0.5%)
18.5-24.9	68 (36.6%)
25-30	77 (41.4%)
>30, obesity	40 (21.5%)
Missing	53
Age (years):	57.5 ±10.4
<40, n (%)	14 (6.2%)
40-49	34 (15.1%)
50-59	67 (29.8%)
60-69	86 (38.2%)
≥ 70	24 (10.7%)
Missing	14
Genetic profile, (n%):	
FTO:	
AA	10 (8.3%)
AT	72 (60.0%)
TT	38 (31.7%)
Missing	119
Mc4R:	69 (57.4%)
TT	41 (34.7%)
TC	10 (8.3%)
CC	
Missing	119
Menopausal status, n (%):	
Premenopausal	52 (21.8%)
Perimenopausal	13 (5.4%)
Postmenopausal	174 (72.8%)
Education level, n (%):	
	41 (17.2%)
No qualification	58 (24.4%)
Other qualification	70 (29.4%)
GCSE grades	69 (28.9%)
Higher education	
Missing	1
Number of cigarettes, n (%):	
None	214 (89.9%)
1-5	3 (1.3%)
6-10	9 (3.8%)
11-15	7(2.9%)
16-20	3(1.3%)
>21	2(0.8%)
Missing	1
Ethnic background, n (%):	
White British	231 (96.6%)
White Irish	2 (0.8%)
Indian	2 (0.8%)
Chinese	1 (0.4%)
Black Caribbean	1 (0.4%)
Black African	1 (0.4%)

[a] Unless otherwise indicated.
[b] Kg stands for kilograms. First weight available from two months prior diagnosis to up to 4 months post-diagnosis recorded a mean of 0.92 (SD: 0.71) months post-diagnosis.
[c] M stands for metres.
[d] BMI: body mass index using participants' height (in meters, m) and weight (in kilograms, kg) at diagnosis.

Table 7 Appendix I - Magnitude of the weight change post-diagnosis calculated in this study, corresponding to the conventional small, medium and large Cohen's conventional criteria

Cohen's *d*	**Weight change (kilograms)[a]**	**Cohen's conventional criteria**
0.2 small	2.72	Small effect size
0.5 medium	6.79	Medium effect size
0.8 large	10.86	Large effect size

[a] Calculated as Cohen's *d* value multiplied by the sample's standard deviation of weight at diagnosis.

Table 8 Appendix I - Participants' clinical characteristics

Participants' clinical characteristics	n (%)
Tumour stage [a]:	
I	105 (45.1%)
II	88 (37.7%)
III	40 (17.2%)
Missing	6
Nodal status:	
Positive	85 (36.0%)
Negative	151 (64.0%)
Missing	3
ER status:	
Positive	206 (86.6%)
Negative	32 (13.4%)
Missing	1
Her-2 R status:	
Positive	23 (15.9%)
Negative	121 (83.1%)
Missing	94
Treatment type:	
Surgery ± radiotherapy only	9 (3.8%)
Adjuvant	197 (82.8%)
Neo-adjuvant	32 (13.4%)
Missing	1
Surgery:	
Mastectomy	79 (33.2%)
Conservation	159 (66.8%)
Missing	1
Chemotherapy:	
Yes	134 (56.1%)
No	105 (43.9%)
Hormone therapy:	
No	33 (13.8%)
Yes	205 (86.1%)
Missing	1
Radiotherapy:	
Yes	180 (75.6%)
No	58 (24.4%)
Missing	1
Biological therapy:	
Yes	13 (5.5%)
No	225 (94.5%)
Missing	1

[a] Cancer stage: including participants treated with neo-adjuvant treatment.

Table 9 Appendix I - Details of the hormone therapy used by the 205 participants treated with hormone therapy after breast cancer diagnosis

Hormone therapy characteristics	n (%)
Hormone therapy, n (%)[a]:	
No	33 (13.8%)
Yes	205 (86. 1%)
Tamoxifen[a]	131 (55.0%)
Aromatase inhibitors (AINs)[a]	152 (63.9%)
Gonadorelin analogues [a]	21 (8.82%)
Missing	1
Hormone therapy modality n (%)[b]:	
Single therapy[b]:	113 (55.1%)
Tamoxifen only[c]	43 (38. 1%)
AINs only[c]	69 (61%)
Gonadorelin analogues only[c]	1(0.9%)
Combined therapy[b]:	92 (44.8%)
Tamoxifen + AINs[d]	72 (78.3%)
Tamoxifen + Gonadorelin analogues[d]	9 (9.8%)
AINs +Gonadorelin analogues[d]	4 (4.3%)
Tamoxifen + AINs + Gonadorelin analogues[d]	7 (7.6%)

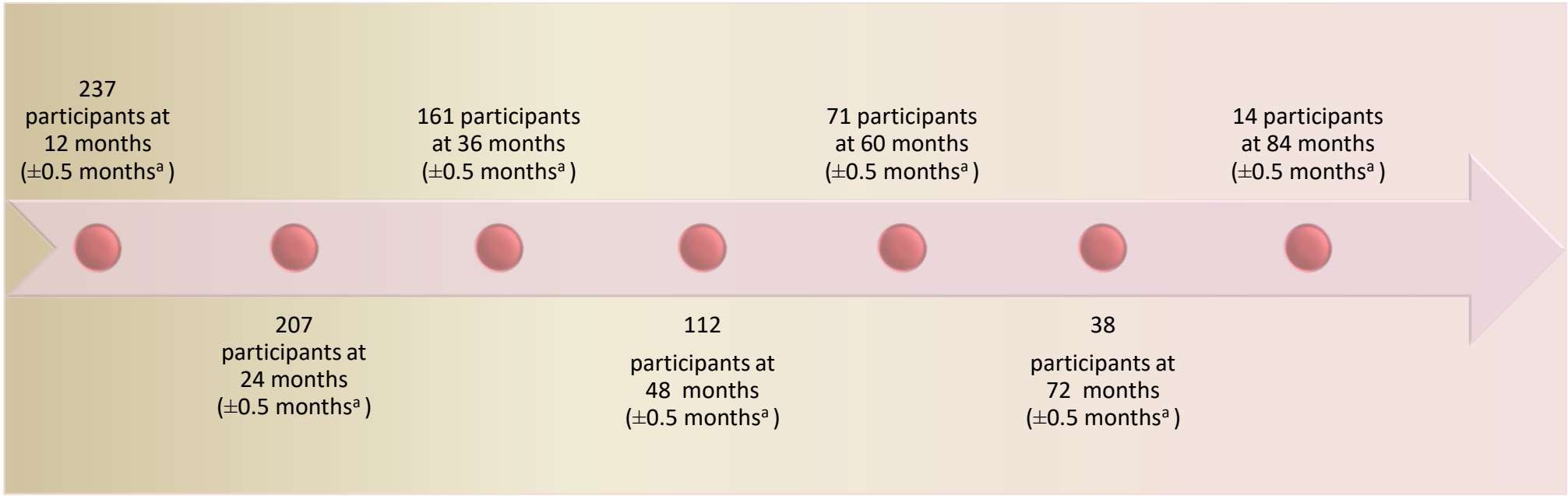
[a] Percentage calculated over the total number of participants in this cohort (excluding 1 with missing information on treatment received) (n=238).
[b] Percentage calculated over the total number of participants who received hormone therapy (n=205).
[c] Percentage calculated over the total number of participants who received single hormone therapy (n=113).
[d] Percentage calculated over the total number of participants who received combined hormone therapy (n=92).

Table 10 Appendix I - Details of chemotherapy regimens used by the 134 participants treated with chemotherapy after breast cancer diagnosis

Chemotherapy regimens	n (%)
Anthracycline containing regimens:	
Yes[a]:	126 (95.5%)
Epirubicin[b]	126 (100.0%)
Doxorubicin[b]	2 (1.6%)
No[a]	6 (4.5%)
Missing	2
Taxane containing regimens:	
Yes[a]:	69 (52.3%)
Paclitaxel[c]	44 (63.8%)
Docetaxel[c]	26(37.7%)
No[a]	63 (47.7%)
Missing	2
Regimens[a&d]:	
CMF	2 (1.5%)
FEC	59 (44.7%)
FEC + docetaxel	19(14.3%)
FEC + paclitaxel	1 (0.8%)
FEC +docetaxel + paclitaxel	1 (0.8%)
FEC +docetaxel + gemitabine + cisplatin	1 (0.8%)
EC + paclitaxel	19 (14.3%)
EC + paclitaxel + gemitabine	21 (15.9%)
EC + docetaxel	5 (3.8%)
paclitaxel + gemitabine	1 (0.8%)
paclitaxel + gemitabine + cyclophosphamide	1 (0.8%)
Methotrexate + mitozantrone	2 (1.5%)
Missing	2

[a] Percentage calculated over the total number of participants treated with chemotherapy, excluding 2 participants with missing information (n=132).
[b] Percentage calculated over the total number of participants treated with anthracycline agent (n=126).
[c] Percentage calculated over the total number of participants treated with taxanes (n=69).
[d] CMF (cyclophosphamide, methotrexate and fluorouracil), FEC (fluorouracil, cyclophosphamide and epirubicin), EC (cyclophosphamide and epirubicin).

Figure 1 Appendix I - Number of participants followed at 12, 24, 36, 48, 60, 72 and 84 months post-diagnosis



[a] A ± 0.5 months window period was chosen for consistency with data provided in Chapter 3.

Table 11 Appendix I - Number of participants with available and missing weight records at diagnosis and at 12, 24, 36 and 48 months after breast cancer diagnosis

	At diagnosis[a]	**At 12 months[b]**	**At 24 months[b]**	**At 36 months[b]**	**At 48 months[b]**
Total number of participants	239	239	224	178	128
Participants with available weight records (%)[c]	221 (92.5%)	101 (42.3%)	104 (46.4%)	78 (43.8%)	68 (53.1%)
Participants with missing weight records (%)[c]	18 (7.5%)	138 (57.7%)	120 (53.6%)	100 (56.2%)	60 (46.9%)
Participants with weight available at both diagnosis and each month explored (%)[c]	n/a	99 (41.4%)	100 (44.6%)	74 (41.6%)	62 (48.4%)

[a] From minus 2 months to up to four months of diagnosis.
[b] ± 4 months.
[c] Percentage based on total number of participants within each evaluation point (at 12 months: 239; at 24 months: 224; at 36 months: 178 and at 48 months: 128).

Table 12 Appendix I - Participants' body weight at, and body weight change from, breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	Diagnosis[d]	**12 months**	**Diagnosis[d]**	**24 months**	**Diagnosis[d]**	**36 months**	**Diagnosis[d]**	**48 months**
Number of participants[a] (%)[b]	99 (41.4%)		100 (44.6%)		74 (41.6%)		62 (48.4%)	
Mean weight(kg[c]) (SD[c])	70.8 (12.9)	72.1 (12.5)	72.6 (14.2)	73.5 (14.2)	70.3 (12.9)	71.9 (12.3)	73.4 (15.7)	73.8 (15.7)
Mean weight change: Kg, (95% CI[c]), p-value	1.30 (0.33 to 2.27) p=0.01[e]		0.85 (-2.24 to 1.93) p=0.12[e]		1.59 (0.39 to 2.79) p=0.01[e]		0.42 (-0.99 to 1.84) p=0.55[e]	
% change[d] p-value	2.20 (0.91 to 3.49) p=0.01		1.44 (0.05 to 2.84) p=0.04		2.67 (1.05 to 4.29) p<0.01		0.88 (-0.97 to 2.73) p=0.34	

[a] Number of participants with available weight records for both at diagnosis and the explored evaluation point.
[b] Percentage based on the total number of participants available within each follow up period (at 12 months: 239, at 24 months: 224, at 36 months: 178 and at 48 months:128).
[c] Kg stands for kilograms, SD stands for standard deviation, CI stands for confidence interval.
[d] Diagnosis date from minus 2 months prior diagnosis to 4 months post-diagnosis.
[d] Paired t-test p-value. Percentage of weight change relative to weight at diagnosis.

Figure 2 Appendix I - Weight change from diagnosis to 12, 24, 36 and 48 months post BC diagnosis

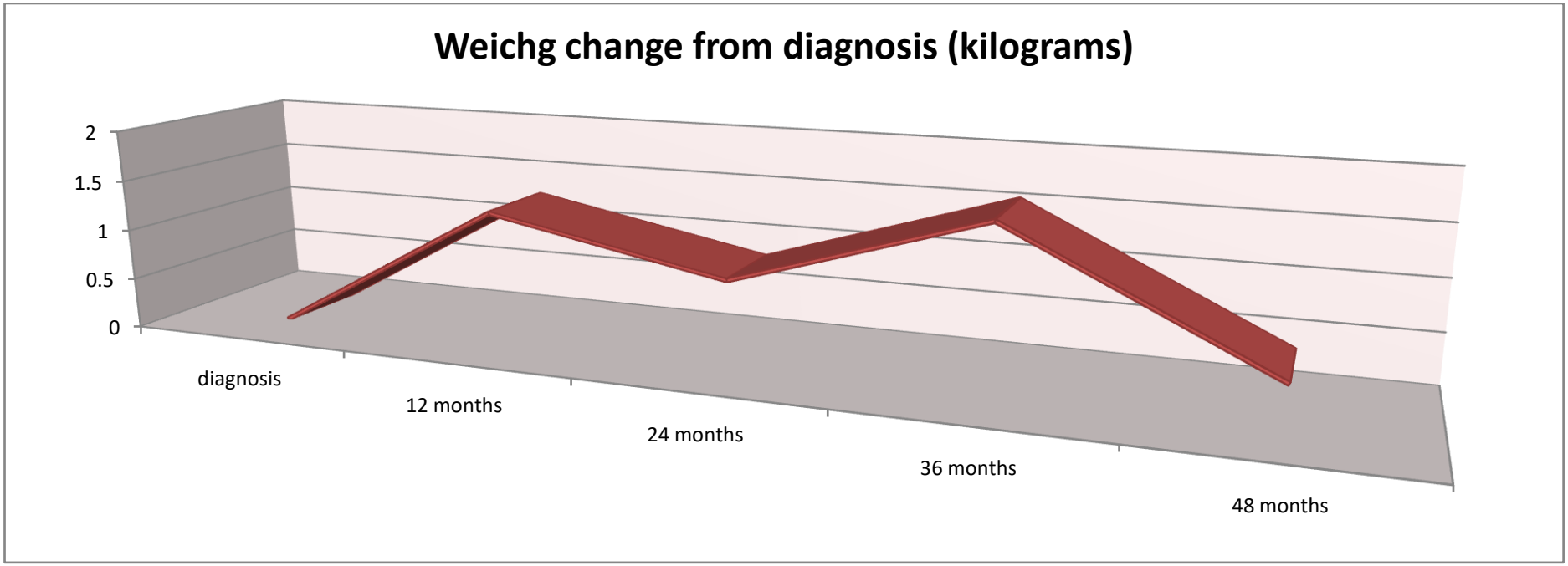


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Table 13 Appendix I - Frequency of weight change (larger than 0.0 kg) from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	From diagnosis to:			
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**
Number of participants included	99	100	74	62
Number of participants who had no weight change (0.0 kg[a]), n (%)[b]	7 (7.1%)	2 (2.0%)	0	0
Number of participants who had weight change (more than 0.0 kg[a]), n (%)[b]:	92 (92.9%)	98 (98%)	74 (100%)	62 (100%)
Lost weight (more than 0.0 kg[a]), n (%)[b]	32 (32.3%)	37 (37.0%)	21 (28.4%)	27 (43.5%)
Mean weight lost (kg[a]) (95% CI[a])	-4.08 (-5.47 to -2.70)	-4.62 (-5.78 to 3.13)	-4.26 (-5.93 to -2.59)	-4.20 (-5.79 to -2.60)
Gained weight (more than 0.0 kg[a]), n(%)[b]	60 (60.6%)	61 (61.0%)	53 (71.6%)	35 (56.5%)
Mean weight gained (kg[a]) (95% CI[a])	4.33 (3.68 to 4.97)	4.10 (3.21 to 4.98)	3.91 (2.91 to 4.91)	3.98 (2.74 to 5.23)

[a] Kg stands for kilograms, CI stands for confidence interval.
[b] Percentage based on total number of participants included in each period.

Table 14 Appendix I - Frequency of weight change (larger than 2.0 kg) from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	From diagnosis to:			
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**
Number of participants included	99	100	74	62
Number of participants who had no weight change (within 2.0 kg[a]), n (%)[b]	34 (34.3%)	38 (38.0%)	25 (33.8%)	20 (32.3%)
Number of participants who had weight change (more than 2.0 kg[a]), n (%)	65 (65.7%)	62 (62%)	49 (66.2%)	42 (67.7%)
Lost weight (more than 2.0 kg[a]), n (%)[b]:	18 (18.2%)	22 (22.0%)	13 (17.6%)	18 (29.0%)
From 2.1 to 5.0 kg[a]	8(8.1%)	8 (8.0%)	7 (9.5%)	11 (17.7%)
From 5.1 to 10.0 kg[a]	9(9. 1%)	10 (10.0%)	5 (6.8%)	5 (8.1%)
10.1 kg[a] and more	1(1.0%)	4 (4.0%)	1 (1.4%)	2 (3.2%)
Gained weight (more than 2.0 kg[a]), n(%)[b]:	47 (47.5%)	40 (40.0%)	36 (48.6%)	24 (38.7%)
From 2.1 to 5.0 kg[a]	26 (26.3%)	23 (23.0%)	26 (35.1%)	14 (22.6%)
From 5.1 to 10.0 kg[a]	20 (20.2%)	13 (13.0%)	7 (9.5%5%)	9 (14.5%5%)
10.1 kg[a] and more	1 (1.0%)	4 (4.0%)	3 (4.1%)	1 (1.6%)

[a] Kg stands for kilograms.
[b] Percentage based on total number of participants included in each period.

Table 15 Appendix I - Frequency of weight change (larger to 0.0% relative to weight at diagnosis), from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	From diagnosis to:			
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**
Number of participants included	99	100	74	62
Number of participants who had no weight change (0.0%), n (%)[a]	7 (7.1%)	2 (2.0%)	0	0
Number of participants who had weight change (more than 0.0%), n (%):	92 (92.9%)	98 (98%)	74 (100%)	62 (100%)
Lost weight (more than 0.0%), n (%)[a]:	32 (32.3%)	37 (37.0%)	21 (21.6%)	27 (43.5%)
From 0.1 to 5.0%	17 (17.2%)	21 (21.0%)	11 (14.9%)	16 (25.8%)
From 5.1 to 10.0%	11 (11.1%)	10 (10.0%)	6 (8.1%)	8 (12.9%)
From 10.1 to15.0%	3 (3.0%)	5 (5.0%)	4 (5.4%)	1 (1.6%)
From 15.1to 20.0%	1 (1.0%)	1 (1.0%)	0	2 (3.2%)
20.1 % and more	0	0	0	0
Gained weight (more than 0.0%), n (%)[a]:	60 (60.6%)	61 (61.0%)	53(59.5%)	35 (56.5%)
From 0.1 to 5.0%	25 (25.3%)	32 (32.0%)	33 (44.6%)	20 (32.3%)
From 5.1 to 10.0%	27 (27.3%)	21 (21.0%)	12 (16.2%)	10 (16.1%)
From 10.1to 15.0%	6 (6.1%)	5 (5.0%)	5 (6.8%)	2 (3.2%)
From 15.1 to 20.0%	2 (2.0%)	3 (3.0%)	0	2 (3.2%)
20.1 % and more	0	0	3 (4.0%)	1 (1.6%)

[a] Percentage based on total number of participants included in each period.

Table 16 Appendix I - Parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and breast cancer treatment

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Mean Weight change (kg[b]) (SD[b])**	**Mean difference, (95% CI[b]), p-value**	**n**	**Mean Weight change (kg[b]) (SD[b])**	**Mean difference, (95% CI[b]), p-value**	**n**	**Mean Weight change (kg[b]) (SD[b])**	**Mean difference, (95% CI[b]), p-value**	**n**	**Mean Weight change (kg[b]) (SD)**	**Mean difference, (95% CI[b]), p-value**
Chemotherapy[a]:												
Yes	84	1.34 (5.03)	0.25 (-2.46 to 2.96) p=0.86[c]	75	0.65 (5.14)	-0.79 (-3.31 to 1.73) p=0.53[c]	53	1.07 (4.68)	-1.84 (-4.48 to 0.81) p=0.17[c]	36	0.61 (6.22)	0.44 (-2.45 to 3.32) p=0.76
No	15	1.09 (3.82)		25	1.44 (6.45)		21	2.90 (6.19)		26	0.17 (4.61)	
Hormone therapy[a]:												
Yes	86	1.24 (4.93)	-0.49 (-3.37to 0.39) p=0.73[c]	90	0.98 (5.39)	1.23 (-2.60 to 5.06) p=0.52[c]	66	1.79 (5.35)	1.85 (-2.28 to 5.98) p=0.38[c]	55	0.28 (5.24)	-1.88 (-6.71 to 2.95) p=0.44[c]
No	13	1.73 (4.5)		9	-0.26 (6.81)		7	-0.06 (3.42)		6	2.17 (8.72)	

Tamoxifen[a]:												
Yes	41	2.19 (4.22)	1.51 (-0.44 to3.47) p=0.13[c]	45	2.87 (4.65)	3.68 (1.62 to 5.75) p<0.01[c]	42	2.62 (4.79)	2.38 (0.01 to 4.75) p=0.05[c]	35	1.52 (3.59)	2.53 (-0.70 to 5.33) p=0.08[d]
No	58	0.67 (5.21)		55	-0.81 (5.58)		32	0.23 (5.42)		27	-1.01 (7.22)	
Aromatase inh.[a]:												
Yes	47	0.82 (5.33)	-0.90 (-2.84 to 1.03) p=0.39[c]	59	-0.02 (5.36)	-2.45 (-4.64 to-0.26) p=0.03[c]	45	1.86 (6.06)	0.50 (- 2.05 to 3.05) p=0.70[c]	48	-0.01 (5.41)	-2.23 (- 5.86 to1.40) p=0.22[c]
No	52	1.73 (4.38)		39	2.43 (5.34)		27	1.36 (3.49)		12	2.24 (6.45)	
Anastrozole[a]:												
Yes	40	0.88 (5.42)	-0.71 (-2.69 to 1.27) p=0.47[c]	45	-0.27 (5.74)	-2.09 (-4.27 to 0.09) p=0.06[c]	33	1.38 (6.76)	-0.42 (-2.88 to 2.04) p=0.75[c]	33	-0.97 (5.97)	-3.13 (-5.92 to-0.34) p=0.03[c]
No	59	1.59 (4.45)		54	1.81 (5.15)		40	1.80 (3.53)		28	2.16 (4.69)	
Letrozole[a]:												
Yes	7	0.54 (5.19)	-0.09 (-4.61 to 2.97) p=0.67[c]	9	-0.06 (4.12)	-0.10 (-4.81 to 2.81) p=0.60[c]	3	2.48 (1.44)	0.93 (-5.19 to 7.05) p=0.76[c]	2	4.20 (1.69)	3.90 (-4.11to11.91) p=0.33[c]
No	92	1.36 (4.85)		91	0.94 (5.60)		71	1.55 (5.28)		60	0.29 (5.61)	
Exemestane[a]:												
Yes	4	1.12 (2.59)	-0.19 (-5.13 to 4.75) p=0.94[c]	10	0.73 (5.65)	-0.23 (-3.85 to 3.38) p=0.90[c]	15	3.45 (3.15)	2.26 (-0.70 to 5.24) p=0.13[c]	15	0.53 (5.11)	0.16 (-3.21 to 3.53) p=0.92[c]
No	95	1.31 (4.93)		89	0.96 (5.44)		58	1.18 (5.53)		46	0.37 (5.82)	

Gonadorelin analogues[a]:												
Yes	13	2.48 (4.06)	1.35 (-1.51 to 4.22) p=0.35[c]	15	2.74 (5.30)	2.23 (-0.80 to 5.25) p=0.15[c]	11	3.04 (4.82)	1.71 (-1.66 to 5.07) p=0.32[c]	9	2.88 (4.88)	2.88 (-1.09 to 6.86) p=0.16[c]
No	86	1.12 (4.96)		85	0.51 (5.47)		63	1.33 (5.23)		53	0.00 (5.61)	

[a] All participants had surgery and they might have also received other treatments (I.e. radiotherapy, biological therapy).
[b] Kg stands for kilograms, SD stands for standard deviation, CI stands for confidence interval.
[c] Independent sample t-test. Equal variances assumed.
[d] Independent sample t-test. Equal variances not assumed.

Table 17 Appendix I - Parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and 1) menopausal status at diagnosis and 2) change in menopausal status from diagnosis to 12, 24, 36 and 48 months post-diagnosis

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Mean Weight change (kg[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
At diagnosis:												
Pre &peri menopausal	36	1.90 (5.08)	0.93 (-1.08 to 2.94) p=0.36	31	1.92 (5.50)	1.55 (-0.79 to 3.89) p=0.19	20	2.58 (5.52)	1.35 (-1.34 to 4.05) p=0.32	15	3.90 (6.34)	4.59 (1.47 to 7.70) p<0.01
Postmenopausal	63	0.96 (4.73)		69	0.37 (5.44)		54	1.23 (5.04)		47	- 0.69 (4.86)	
Change in menopause status:												
Yes	29	2.92 (4.33)	2.29 (0.20 to 4.37) p=0.03	23	2.01 (4.95)	1.65 (-0.99 to 4.30) p=0.22	17	2.36 (5.81)	1.03 (-1.91 to 3.97) p=0.49	13	3.91 (6.81)	4.27 (1.08 to 7.45) p=0.01
No	70	0.63 (4.93)		65	0.35 (5.67)		49	1.33 (5.03)		41	-0.35 (4.30)	

[a] Kg stands for kilograms, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 18 Appendix I - Number of participants whose menopausal status changed from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis

	Menopausal status change from diagnosis to[a]:			
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**
Yes, n (%)	29 (29.3%)	23 (26.1%)	17 (25.7%)	13 (24.1%)
No, n (%)	70 (70.7%)	65 (73.9%)	49 (74.3%)	41 (75.9%)

[a] participants included in each period explored were those that are included in the analysis of weight change at 12, 24, 36 and 48 months post-diagnosis, and whose menopausal status was available at diagnosis and at each of the evaluation points explored (n=99, n=88, n=66 and n=54, respectively).

Table 19 Appendix I - Parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and genetic profile

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Mean Weight change (kg[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
FTO:												
AA + AT	45	0.97 (3.91)	-0.19 (-2.65 to 2.27) p=0.88	45	1.27 (5.05)	0.95 (-1.88 to 3.79) p=0.50	27	1.30 (3.26)	-2.00 (-4.10 to 1.11) p=0.06	26	1.00 (5.86)	0.24 (-4.59 to 5.07) p=0.92
TT	24	1.16 (6.32)		21	0.32 (6.02)		13	3.30 (2.65)		7	0.76 (4.14)	
Mc4R:												
CC +CT	27	0.51 (5.10)	-0.86 (-3.24 to 1.53) p=0.48	25	-0.16 (6.11)	-1.81 (-4.50 to 0.88) p=0.18	16	0.99 (2.80)	-1.61 (-3.65 to 0.43) p=0.12	11	-1.18 (5.65)	-3.19 (-7.22 to 0.84) p=0.12
TT	42	1.37 (4.68)		41	1.66 (5.78)		24	2.60 (3.32)		22	2.01 (5.20)	

[a] Kg stands for kilograms, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 20 Appendix I - Parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and biological, behavioural and tumour-related variables

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value**
Smoking status at diagnosis:												
Smoker	9	4.48 (5.90)	3.49 (0.18 to 6.80) p=0.04[b]	9	0.66 (6.73)	-0.21 (-4.03 to 3.61) p=0.91[b]	5	4.55 (11.05)	3.17 (-1.58 to 7.93) p=0.58[c]	9	3.84 (7.48)	4.00 (0.08 to 7.91) p=0.04[b]
Non smoker	90	0.99 (4.65)		91	0.86 (5.39)		69	1.38 (4.57)		53	-0.16 (5.03)	
	n	**Correlation coefficient[d] p-value**		**n**	**Correlation coefficient[d] p-value**		**n**	**Correlation coefficient[d] p-value**		**n**	**Correlation coefficient[d] p-value**	
Weight at diagnosis	99	r=-0.27 p<0.01		100	r=-0.19 p=0.05		74	r=-0.32 p<0.01		62	r=-0.18 p=0.17	
Age at diagnosis (years)	93	r=-0.13 p=0.21		95	r=-0.16 p=0.13		72	r=-0.06 p=0.63		57	r=-0.29 p=0.03	

Tumour Size (cm[a])	94	r=-0.05 p=0.61		96	r=0.10 p=0.32		72	r=-0.14 p=0.24		60	r=-0.01 p=0.97	
	n	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA[a]**
Stage of disease:												
Stage 1	30	1.15 (4.87)	F (2, 92)	36	0.31 (5.95)	F (2, 95)	24	2.99 (5.50)	F (2, 68)	26	-0.10 (4.45)	F (2, 56)
Stage 2	39	2.04 (5.03)	F=0.93	37	1.53 (5.55)	F=0.50	31	1.45 (5.04)	F=1.90	23	1.10(6.90)	F=0.36
Stage 3	26	0.34 (4.89)	p=0.40	25	0.51(4.88)	p=0.61	16	-0.20(5.59)	p=0.16	10	-0.31(4.93)	p=0.70

[a] Kg stands for kilograms, SD stands for standard deviation, CI stands for confidence interval, cm stands for centimetres, ANOVA stands for analysis of the variance.

[b] Independent sample t-test. Equal variances assumed.

[c] Independent sample t-test. Equal variances not assumed.

[d] Pearson's product moment correlation.

Table 21 Appendix I - Multiple linear regression analysis on factors associated with participants' weight change from breast cancer diagnosis to 12 months post-diagnosis

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	99	19.55 (10.46 to 28.63)	p<0.01	R=0.34 F (2, 63) F=8.88 p<0.01
Weight at diagnosis (kg[a])		-0.12 (-0.20 to -0.03)	p<0.01	
Smoking status at diagnosis[b]		-5.44 (-9.45 to -1.43)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) smokers, 1) non-smokers.

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Table 22 Appendix I - Multiple linear regression analysis on factors associated with participants' weight change from breast cancer diagnosis to 24 months post-diagnosis

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	95	12.27 (6.60 to 17.54)	p <0.01	R=0.45 F (2, 92) F=11.90 p<0.01
Weight at diagnosis (kg[a])		-0.08 (-0.14 to -0.01)	p=0.03	
Tamoxifen[b]		-4.05 (-5.99 to -2.11)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) smokers, 1) non-smokers.

Table 23 Appendix I - Multiple linear regression analysis on factors associated with participants' weight change from breast cancer diagnosis to 36 months post-diagnosis

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	74	13.52 (6.60 to 20.44)	p<0.012	R=0.38 F (2, 71)
Weight at diagnosis (kg[a])		-0.12 (-0.21 to -0.04)	p<0.01	F=6.2 p<0.01
Tamoxifen[b]		-2.23 (-4.50 to 0.04)	p=0.05	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) smokers, 1) non-smokers.

Table 24 Appendix I - Multilevel models for linear weight change after breast cancer diagnosis, using breast cancer treatments as time-invariant predictors

		Parameter	Model A[a]	Model B[a]	Model C[a]	Model D[a]	Model E[a]	Model F[a]
Number of participants			238	238	238	238	238	238
Fixed effects								
Initial status π_{0i}	Intercept (initial status)	Υ_{00}	72.8* (SE: 0.86)	72.2* (SE: 0.858)	79.4* (SE: 10.44)	69.3* (SE: 7.97)	66.2* (SE: 4.16)	72.9* (SE: 2.64)
	Chemotherapy	Υ_{01}	n/a	n/a	0.91 (SE: 1.79)	n/a	n/a	n/a
	Tamoxifen	Υ_{02}	n/a	n/a	-2.52 (SE: 1.92)	-2.22 (SE: 1.83)	-1.51 (SE: 1.78)	-0.46 (SE: 1.72)
	Anastrozole	Υ_{03}	n/a	n/a	-3.29 (SE: 1.77)	-2.81 (SE: 1.76)	n/a	n/a
	Letrozole	Υ_{04}	n/a	n/a	-5.59 (SE: 3.25)	n/a	n/a	n/a
	Exemestane	Υ_{05}	n/a	n/a	4.52* (SE: 2.19)	4.36* (SE: 2.20)	4.55* (SE: 2.21)	n/a
	Gonadorelin analogues	Υ_{06}	n/a	n/a	1.53 (SE: 3.09)	1.40 (SE: 3.02)	n/a	n/a
Rate of change π_{1i}	Months	Υ_{10}	n/a	0.030* (SE:0.007)	0.05 (SE: 0.09)	0.14* (SE: 0.07)	0.10* (SE: 0.04)	0.10* (SE: 0.02)
	Chemotherapy by months	Υ_{11}	n/a	n/a	0.01 (SE: 0.02)	n/a	n/a	n/a
	Tamoxifen by months	Υ_{12}	n/a	n/a	-0.03* (SE: 0.02)	-0.04* (SE: 0.02)	-0.04* (SE:0.02)	-0.05* (SE:0.01)
	Anastrozole by months	Υ_{13}	n/a	n/a	0.03* (SE: 0.01)	0.03 (SE: 0.01)	n/a	n/a
	Letrozole by months	Υ_{14}	n/a	n/a	0.05 (SE: 0.03)	n/a	n/a	n/a

	Exemestane by months	Υ_{15}	n/a	n/a	0.004 (SE: 0.02)	0.005 (SE: 0.02)	0.001 (SE: 0.02)	n/a
	Gonadorelin analogues by months	Υ_{16}	n/a	n/a	-0.06* (SE: 0.03)	-0.06* (SE: 0.03)	n/a	n/a
Estimates of Variance components								
Level-1	Within-person	σ_ε^2	8.9504* (SE: 0.2879)	5.7915* (SE: 0.1994)	5.7887* (SE:0.1989)	5.7919* (SE: 0.1992)	5.7900* (SE: 0.1991)	5.7900* (SE: 0.1991)
Level-2	In intercept	σ_0^2	173.4851* (SE:16.0975)	171.6079* (SE: 16.0528)	164.7607* (SE: 15.4235)	166.90 38* (SE: 15.6222)	168.66 51* (SE: 15.7866)	171.7578* (SE: 16.0693)
	In rate of change	σ_1^2	n/a	0.01017* (SE: 0.0014)	0.0089* (SE: 0.0012)	0.0090* (SE: 0.0013)	0.0097* (SE: 0.0014)	0.0097* (SE: 0.0014)
	Covariance	σ_{01}	n/a	-0.1297 (SE: 0.1067)	-0.0953 (SE: 0.1003)	-0.1109 (SE: 0.1013)	-0.1456 (SE: 0.1041)	-0.1447 (SE: 0.1051)
Goodness of fit of the total model								
	Deviance		12,067.30	11,554.19	11,525.04	11,530.20	11,540.72	11,545.08
	AIC (Akaike's Information Criterion)		12,073.30	11,566.19	11,561.04	11,558.20	11,560.72	11,561.08
	BIC (Schwarz's Bayesian Criterion)		12,090.35	11,600.29	11,663.33	11,637.763	11,617.55	11,606.54

[a] These linear models predict weight in the months following BC diagnosis as a function of the BC treatments analysed as time-invariant predictors. Model A is the unconditional means model. Model B is the unconditional growth model. Model C adds the main effects and the interaction between BC treatments and time passed since diagnosis (months) of all BC treatments explored in this study. Model D excluded the main effects and the interaction effects of those BC treatments that were not statistically significant in the preceding model. Model E includes the main effects and the interaction effects of the only two predictors that remained statistically significant: tamoxifen and exemestane. Model F excluded exemestane as its interaction effects were not significant in model E. The main effects of the BC treatments in all these models are treated as fixed effects. The values for the BC treatments are: 1) users; and 2) non-users.

* p-value <0.05.

Table 25 Appendix I - Final multilevel models for linear weight change after breast cancer diagnosis among participants, using breast cancer treatments as time-invariant predictors

		Parameter	Model E[a]	Model F[a]
Number of participants			238	238
Fixed effects				
Initial status π_{0i}	Intercept (initial status)	Υ_{00}	66.2 (SE[b]: 4.16) (95% CI[b]: 58.05 to 74.42), p<0.01	72.9 (SE[b]: 2.64) (95% CI[b]: 67.67 to 78.10), p<0.01
	Tamoxifen	Υ_{01}	-1.51 (SE[b]: 1.78) (95% CI[b]: -5.03 to 2.00), p=0.40	-0.46 (SE[b]: 1.72) (95% CI[b]: -3.86 to 2.94), p=0.79
	Exemestane	Υ_{02}	4.55 (SE[b]: 2.21) (95% CI[b]: 0.19 to 8.91), p=0.04	n/a
Rate of change π_{1i}	Months	Υ_{10}	0.10 (SE[b]: 0.04) (95% CI[b]: 0.02 to 0.17), p<0.01	0.10 (SE[b]: 0.02) (95% CI[b]: 0.05 to 0.14), p<0.01
	Tamoxifen by months	Υ_{11}	-0.04 (SE[b]: 0.02) (95% CI[b]: -0.08 to -0.01), p<0.01	-0.05 (SE[b]: 0.01) (95% CI[b]: -0.08 to -0.06), p<0.01
	Exemestane by months	Υ_{12}	0.001 (SE[b]: 0.02) (95% CI[b]: -0.04 to 0.04), p=0.09	n/a
Estimates of Variance components				
Level-1	Within-person	σ_ε^2	5.7900 (SE[b]: 0.1991) (95% CI[b]: 5.41 to 6.19), p<0.01	5.7900 (SE[a]: 0.1991) (95% CI[a]: 67.67 to 78.11), p<0.01

Level-2	In intercept	σ_0^2	168.66 51 (SE[b]: 15.7866) (95% CI[b]: 140.39 to 202.62), p<0.01	171.7578 (SE[b]: 16.0693) (95% CI[b]: 0.05 to 0.14), p<0.01
	In rate of change	σ_1^2	0.0097 (SE[b]: 0.0014) (95% CI[b]: 0.007 to 0.012), p<0.01	0.0097 (SE[b]: 0.0014) (95% CI[b]: -0.08 to -0.02), p<0.01
	Covariance	σ_{01}	-0.1456 (SE[b]: 0.1041) (95% CI[b]: -0.34 to 0.58), p=0.16	-0.1447 (SE[b]: 0.1051) (95% CI[b]: -3.86 to 2.94), p=0.79
Goodness of fit of the total model				
	Deviance		11,540.72	11,545.08
	AIC (Akaike's Information Criterion)		11,560.72	11,561.08
	BIC (Schwarz's Bayesian Criterion)		11,617.55	11,606.54

[a] These two linear models predict weight in the months following BC diagnosis as a function of the BC treatments analysed as time-invariant predictors. They are the same as in the previous table but additional information (95% CI and p-values have been added). Model E includes the main effects and the interaction effects of tamoxifen and exemestane. Model F included the main effects and the interaction effects of tamoxifen only. The main effects of the treatments in all these models are treated as fixed effects. The values for the BC treatments are: 1) users; and 2) non-users.

[b] SE stands for standard error, CI stands for confidence interval.

Table 26 Appendix I - Multilevel models for linear weight change after breast cancer diagnosis among participants, using breast cancer treatments as time-varying predictors

		Parameter	Model B[a]	Model 1[a]	Model 2[a]	Model 3[a]	Model 4[a]
Number of participants			**238**	**238**	**238**	**238**	**238**
Fixed effects							
Initial status π_{0i}	Intercept (initial status)	Υ_{00}	72.2* (SE[b]: 0.86)	73.1* (SE[b]: 2.49)	80.0* (SE[b]: 2.37)	70.3* (SE[b]: 2.02)	70.7* (SE[b]: 1.59)
	Chemotherapy	Υ_{10}	n/a	-0.01 (SE[b]: 0.02)	n/a	n/a	n/a
	Tamoxifen	Υ_{20}	n/a	-1.42* (SE[b]: 0.34)	-1.44* (SE[b]: 0.33)	-1.18* (SE[b]: 0.32)	-1.09* (SE[b]: 0.27)
	Anastrozole	Υ_{30}	n/a	-0.73* (SE[b]: 0.32)	0.74* (SE[b]: 0.32)	-0.83* (SE[b]: 0.32)	-0.85* (SE[b]: 0.32)
	Letrozole	Υ_{40}	n/a	3.01* (SE[b]: 0.85)	2.97* (SE[b]: 0.84)	2.85* (SE[b]: 0.85)	2.60* (SE[b]: 0.54)
	Exemestane	Υ_{50}	n/a	-1.30 (SE[b]: 0.75)	-1.28 (SE[b]: 0.74)	n/a	n/a
	Gonadorelin analogues	Υ_{60}	n/a	-0.21 (SE[b]: 0.57)	n/a	n/a	n/a
Rate of change π_{1i}	Months	Υ_{70}	0.030* (SE: 0.01)	-0.01 (SE[b]: 0.08)	-0.06 (SE[b]: 0.07)	-0.05 (SE[b]: 0.06)	-0.06* (SE[b]: 0.02)
	Chemotherapy by months	Υ_{80}	n/a	0.00 (SE[b]: 0.02)	n/a	n/a	n/a
	Tamoxifen by months	Υ_{90}	n/a	-0.02 (SE[b]: 0.02)	0.02* (SE[b]: 0.02)	0.01 (SE[b]: 0.02)	n/a

	Anastrozole by months	Υ_{100}	n/a	0.05* (SE[b]: 0.01)	0.05* (SE[b]: 0.01)	0.05* (SE[b]: 0.01)	0.05* (SE[b]: 0.01)
	Letrozole by months	Υ_{110}	n/a	-0.01 (SE[b]: 0.03)	-0.01 (SE[b]: 0.03)	-0.01 (SE[b]: 0.03)	n/a
	Exemestane by months	Υ_{120}	n/a	0.000 (SE[b]:0.02)	-0.00 (SE[b]: 0.02)	n/a	n/a
	Gonadorelin analogues by months	Υ_{130}	n/a	-0.03 (SE[b]:0.03)	n/a	n/a	n/a
Estimates of Variance components							
Level-1	Within-person	σ_ε^2	5.7915* (SE[b]: 0.1994)	5.5011* (SE[b]: 0.1905)	5.4957* (SE[b]: 0.1904)	5.63989* (SE[b]: 0.1947)	5.6427* (SE[b]: 0.1945)
Level-2	In intercept	σ_0^2	171.6079* (SE[b]:16.0528)	170.2522* (SE[b]: 16.0738)	169.7898* (SE[b]: 16.0076)	170.5178* (SE[b]: 15.9594)	170.56 28* (SE[b]: 15.9537)
	In rate of change	σ_1^2	0.01017* (SE[b]: 0.0014)	0.0095* (SE[b]: 0.0014)	0.0099* (SE[b]: 0.0014)	0.009429* (SE[b]: 0.0013)	0.0094* (SE[b]: 00013)
	Covariance	σ_{01}	-0.129799 (SE[b]: 0.1067)	-0.0339 (SE[b]: 0.1061)	-0.0340 (SE[b]: 0.1063)	-0.0715 (SE[b]: 0.1036)	-0.072954 (SE[b]: 0.1034)
Goodness of fit of the total model							
	Deviance		11,554.19	11,349.40	11,532.18	11,435.04	11,435.35
	AIC (Akaike's Information Criterion)		11,566.19	11,385.40	11,380.18	11,459.04	11,455.35
	BIC (Schwarz's Bayesian Criterion)		11,600.29	11,487.52	11,459.60	11,527.17	11,512.12

[a] These linear models predict weight post-diagnosis as a function of the BC treatments analysed as time-varying predictors. Model 1: the unconditional growth model. Model 2 adds the main effects and the interaction between BC treatments and time since diagnosis of BC all treatments in this study. Model 3 and 4 excluded the main effects and the interaction effects of those treatments that were not statistically significant in the preceding model. Model 5 excluded the interaction effects of tamoxifen and letrozole, as these were not significant in model 4. The main effects of the BC treatments in models 2, 3, 4 and 5 are treated as fixed effects. The values for the BC treatments are: 1) using/had used; and 2) non-users.

[b] SE stands for standard error.

* p-value <0.05.

Table 27 Appendix I - Final multilevel models for linear weight change after breast cancer diagnosis among participants, using breast cancer treatments as time-varying predictors

		Parameter	Model 4[a]	Model 5[a]
Number of participants			238	238
Fixed effects				
Initial status π_{0i}	Intercept (initial status)	Υ_{00}	70.7 (SE[b]: 1.59) (95% CI[b]: 67.56 to 73.81), p<0.01	70.6 (SE[b]: 1.57) (95% CI[b]: 67.47 to 73.64), p<0.01
	Tamoxifen	Υ_{10}	-1.09 (SE[b]: 0.27) (95% CI[b]: -1.63 to -0.56), p<0.01	-1.02 (SE[b]: 0.27) (95% CI[b]: -1.54 to -0.49), p<0.01
	Anastrozole	Υ_{20}	-0.85 (SE[b]: 0.32) (95% CI[b]: -1.48 to -0.21), p<0.01	-1.01 (SE[b]: 033) (95% CI[b]: -1.65 to -0.35), p<0.01
	Letrozole	Υ_{30}	2.60 (SE[b]: 0.54) (95% CI[b]: 1.53 to 3.67), p<0.01	2.72 (SE[b]: 0.53) (95% CI[b]: 1.67 to 3.76), p<0.01
Rate of change π_{1i}	Months	Υ_{40}	-0.06 (SE[b]: 0.02) (95% CI[b]: -0.11 to -0.02), p<0.01	-0.07 (SE[b]: 0.03) (95% CI[b]: -0.12 to -0.02), p=0.01
	Anastrozole by months	Υ_{50}	0.05 (SE[b]: 0.01) (95% CI[b]: 0.2 to 0.08), p<0.01	0.06 (SE[b]: 0.02) (95% CI[b]: 0.03 to 0.09), p<0.01
Estimates of Variance components				
Level-1	Within-person	σ_ε^2	5.6427 (SE[b]: 0.1945) (95% CI[b]: 5.27 to 6.04), p<0.01	5.5829 (SE[b]: 0.1963) (95% CI[b]: 5.21 to 5.98), p<0.01

Level-2	In intercept	σ_0^2	170.56 28 (SE[b]: 15.9537) (95% CI[b]: 141.99 to 204.88), p<0.01	168.4360 (SE[b]: 15.7521) (95% CI[b]: 140 to 202.32), p<0.01
	In rate of change	σ_1^2	0.0094 (SE[b]: 00013) (95% CI[b]: 0.007 to 0.012), p<0.01	0.03708 (SE[b]: 0.0109) (95% CI[b]: 0.02 to 0.06), p<0.01
	Covariance	σ_{01}	-0.072954 (SE[b]: 0.1034) (95% CI[b]: -0.28 to 0.13), p=0.48	-0.8030 (SE[b]: 0.3183) (95% CI[b]: -1.42 to -0.18), p=0.01
	In anastrozole	σ_2^2	n/a	0.5052 (SE[b]: 0.1897) (95% CI[b]: 0.13 to 0.88), p<0.01
	In anastrozole by months	σ_3^2	n/a	0.0122 (SE[b]: 0.0052) (95% CI[b]: 0.00 to 0.03), p=0.02
	Covariance	σ_{02}	n/a	-0.0196 (SE[b]: 0.0077) (95% CI[b]: -0.03 to-0.00), p=0.01
Goodness of fit of the total model				
	Deviance		11,435.35	11,414.88
	AIC (Akaike's Information Criterion)		11,455.35	11,440.88
	BIC (Schwarz's Bayesian Criterion)		11,512.12	11,514.69

[a] These linear models predict weight in the months following BC diagnosis as a function of the BC treatments analysed as time-varying predictors. Model 5 includes the main effects of tamoxifen, anastrozole and letrozole, and the interaction between anastrozole and time elapsed since diagnosis. The effects of the treatments are treated as fixed effects. Model 6 allows the interaction between anastrozole and time since diagnosis to have both fixed and random effects. The values of the BC treatments are: 1) using/had used; and 2) non-users.
[b] CI stands for confidence intervals, SE stands for standard error.

Table 28 Appendix I - Estimated body weight after breast cancer diagnosis of a woman diagnosed with breast cancer predicted by model 4

	Estimated weight (kg[a]) at:						
	12 months post-diagnosis	**24 months post-diagnosis**	**36 months post-diagnosis**	**48 months post-diagnosis**	**60 months post-diagnosis**	**72 months post-diagnosis**	**84 months post-diagnosis**
Tamoxifen							
Yes	71.4	72.1	72.9	73.6	74.3	75.0	75.7
No	70.3	71.0	71.8	72.5	73.2	73.9	74.6
Anastrozole							
Yes	71.4	72.1	72.9	73.6	74.3	75.0	75.7
No	71.1	72.4	73.8	75.1	76.4	77.7	79.0
Letrozole							
Yes	71.4	72.1	72.9	73.6	74.3	75.0	75.7
No	74.0	74.7	75.5	76.2	76.9	77.6	78.3

[a] Kg stands for kilograms. Estimated weight when the rest of the BC treatments are hold constant.

Table 29 Appendix I - Parametric univariable analysis on the association between participants' body adiposity parameters at the end of the follow up and breast cancer treatment

	Fat %			FM/FFM			Waist circumference		
	n	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (cm[a]) (SD[s])**	**Mean difference, (95% CI[a]), p-value[b]**
Chemotherapy:									
Yes	123	37.33 (6.50)	-0.98 (-2.64 to 0.67) p=0.24	118	0.62 (0.17)	-0.02 (-0.06 to 0.02) p=0.33	89	90.62 (11.40)	-3.30 (-6.75 to 0.16) p=0.06
No	99	38.31 (5.89)		97	0.64 (0.15)		91	93.92 (12.05)	
Hormone therapy:									
Yes	189	37.99 (6.27)	1.78 (-0.56 to 4.12) p=0.14	183	0.63 (0.16)	0.04 (-0.02 to 0.10) p=0.17	157	92.96 (11.84)	6.42 (1.22 to11.62) p=0.02
No	32	36.21(5.87)		31	0.59 (0.15)		22	86.54 (9.38)	
Tamoxifen:									
Yes	126	37.52 (6.66)	-0.50 (-2.17 to 1.17) p=0.56	122	0.62 (0.17)	-0.00 (-0.04 to 0.04) p=0.81	101	92.77 (12.52)	1.37 (-2.13 to4.86) p=0.44
No	95	38.02 (5.64)		92	0.63 (0.15)		78	91.40 (10.67)	
Aromatase inhibitors:									
Yes	138	38.46 (6.16)	1.92 (0.23 to 3.62) p=0.03	135	0.64 (0.17)	0.04 (-0.01 to 0.08) p=0.14	123	93.32 (11.46)	3.65 (-0.05 to 7.36) p=0.05
No	83	36.53 (6.22)		79	0.60 (0.15)		56	89.66 (12.04)	

Anastrozole:									
Yes	95	38.90 (5.99)	2.04 (0.39 to 3.69) p=0.02	92	0.65 (0.16)	0.05 (0.00 to 0.09) p=0.03	82	93.86 (10.82)	3.11 (-0.34 to 6.56) p=0.08
No	126	36.86 (6.30)		122	0.60 (0.16)		97	90.74 (12.33)	
Letrozole:									
Yes	17	39.38 (5.15)	1.78 (-1.32 to 4.88) p=0.26	17	0.66 (0.15)	0.04 (-0.04 to 0.12) p=0.33	14	95.11 (11.18)	3.19 (-3.25 to 9.64) p=0.33
No	204	37.60 (6.31)		197	0.62 (0.16)		165	91.92 (11.78)	
Exemestane:									
Yes	43	37.11 (6.21)	-0.77 (-2.86 to 1.32) p=0.47	42	0.59 (0.67)	-0.04 (-0.09 to 0.02) p=0.19	40	91.73 (11.58)	-0.57 (-4.73 to 3.60) p=0.79
No	178	37.88 (6.25)		172	0.63 (0.16)		139	92.30 (11.82)	
Gonadorelin analogues:									
Yes	19	38.58 (5.56)	0.93 (-2.03 to 3.88) p=0.93	19	0.64 (0.15)	0.018 (-0.06 to 0.09) p=0.65	12	91.58 (11.57)	-0.63 (-7.57 to 6.30) p=0.86
No	202	37.65 (6.30)		195	0.62 (0.16)		167	92.21 (11.78)	

[a] SD stands for standard deviation, CI stands for confidence interval, cm stands for centimetres.
[b] Independent sample t-test. Equal variances assumed.

Table 30 Appendix I - Parametric univariable analysis on the association between participants' body adiposity parameters at the end of the follow up and 1) menopausal status at diagnosis and 2) change in menopausal status after breast cancer diagnosis

	Fat %			FM/FFM			Waist circumference		
	n	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (cm[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Menopausal status at diagnosis:									
Pre + peri menopausal	60	36.00 (6.20)	-2.42 (-4.25 to -0.58) p=0.01	58	0.59 (0.14)	-0.05 (-0.10 to -0.00) p=0.03	42	88.88 (10.58)	-4.45 (-8.52 to -0.38) p=0.03
Postmenopausal	162	38.42 (6.14)		157	0.64 (0.16)		138	93.33 (12.01)	
Menopausal status change:									
Yes	55	35.95 (6.25)	-2.42 (-4.31 to -0.53) p=0.02	53	0.59 (0.14)	-0.05 (-0.10 to -0.00) p=0.04	39	89.44 (10.35)	-3.65 (-7.85 to -0.55) p=0.05
No	167	38.36 (6.14)		162	0.64 (0.16)		141	93.09 (12.11)	

[a] SD stands for standard deviation, CI stands for confidence interval, cm stands for centimetres.
[b] Independent sample t-test. Equal variances assumed.

Table 31 Appendix I - Parametric univariable analysis on the association between participants' body adiposity parameters at the end of the follow up and genetic profile

	Fat %			FM/FFM			Waist circumference		
	n	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[a]**	**n**	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[a]**	**n**	**Mean (cm[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value**
FTO:									
AA + AT	75	36.86 (6.31)	-0.29 (-3.01 to 2.42) p=0.83[b]	71	0.60 (0.16)	-0.02 (-0.89 to 0.05) p=0.60[b]	45	91.10 (11.01)	-3.71 (-9.93 to 2.49) p=0.24[b]
TT	31	37.15 (6.68)		30	0.62 (0.16)		21	94.82 (13.30)	
Mc4R:									
CC +CT	41	36.27 (6.32)	-1.10 (-3.63 to 1.43) p=0.39[b]	40	0.59 (0.15)	-0.02 (-0.09 to 0.04) p=0.44[b]	30	91.90 (13.69)	-0.70 (-6.58 to 5.12) p=0.82[c]
TT	65	37.37 (6.45)		61	0.62 (0.17)		36	92.60 (10.17)	

[a] SD stands for standard deviation, CI stands for confidence interval, cm stands for centimetres.
[b] Independent sample t-test. Equal variances assumed.
[c] Independent sample t-test. Equal variances not assumed

Table 32 Appendix I - Parametric univariable analysis on the association between participants' body adiposity parameters at the end of the follow up and biological, behavioural and tumour-related variables

	Fat %			FM/FFM			Waist circumference		
	n	**Mean (SD[a])**	**Mean difference, (95% CI), p-value[b]**	**n**	**Mean (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (cm[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Smoking status at diagnosis:									
Smoker	24	36.96 (7.62)	-0.87 (-3.53 to 1.79) p=0.52	22	0.64 (0.17)	0.02 (-0.06 to 0.09) p=0.69	16	93.96 (11.65)	1.87 (-4.27 to 8.00) p=0.55
Non-smoker	197	37.83 (6.07)		192	0.62 (1.16)		163	92.10 (11.88)	
	n	**Correlation coefficient[c] p-value**		**n**	**Correlation coefficient[c] p-value**		**n**	**Correlation coefficient[c] p-value**	
Time since diagnosis to the end of the follow up	222	r=0.09 p=0.20		215	r=0.06 p=0.42		180	r=-0.02 p=0.80	
Weight at diagnosis	221	r=0.70 p<0.01		215	r=0.72 p<0.01		180	r=0.75 p<0.01	
Weight at the end of the follow up)	222	r=0.75 p<0.01		215	r=0.77 p<0.01		180	r=0.81 p<0.01	

Weight change from diagnosis to the end of the follow up	221	r=0.16 p=0.02		215	r=0.17 p=0.02		180	r=0.21 p=0.01	
Age at diagnosis (years)	222	r=0.21 p<0.01		215	r=0.16 p=0.02		180	r=0.20 p=0.01	
Age at the end of the follow up (years)	222	r=0.21 p<0.01		215	r=0.15 p=0.03		180	r=0.17 p=0.02	
Tumour size (cm)	222	r=-0.04 p=0.57		215	r=-0.06 p=0.39		180	r=-0.06 p=0.44	
	n	**Mean (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean (cm[a])(SD[a])**	**One-way ANOVA[a]**
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 98 81 37	 37.91 (6.01) 37.94 (6.14) 37.03 (7.12)	 F (2, 206) F=0.68 p=0.94	 95 80 34	 0.62 (0.16) 0.63 (0.16) 0.61 (0.17)	 F (2, 206) F=0.68 p=0.94	 81 69 25	 93.67 (11.92) 91.12 (11.33) 90.80 (11.02)	 F (2, 172) F (1.13) p=0.32

[a] SD stands for standard deviation, CI stands for confidence interval, cm stands for centimetres, ANOVA stands for analysis of the variance.
[b] Independent sample t-test. Equal variances assumed.
[c] Pearson's product moment correlation test.
[d] Independent sample t-test. Equal variances not assumed.

Table 33 Appendix I - Multiple linear regression analysis on factors associated with participants' fat mass percentage at the end of the follow up

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	193	7.46 (2.76 to 12.67)	p<0.01	R=0.73 F (2, 190) F=108.57 p<0.01
Weight at diagnosis (kg[a])		0.34 (0.29 to 0.38)	p<0.01	
Age at diagnosis (years)		0.11 (0.05 to 0.16)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

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Table 34 Appendix I - Multiple linear regression analysis on factors associated with participants' fat mass/fat free mass ratio at the end of the follow up

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	186	-0.14 (-0.271 to -0.024)	p=0.02	R=0.74 F (2, 183) F=111.97 p<0.01
Weight at diagnosis (kg[a])		0.01 (0.008 to 0.010)	p<0.01	
Age at diagnosis (years)		0.002 (0.001 to 0.004)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

Table 35 Appendix I - Multiple linear regression analysis on factors associated with participants' waist circumference at the end of the follow up

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficient
Constant	158	29.17 (20.90 to 37.44)	p<0.01	R=0.83 F (3, 154) F=111.16 p<0.01
Weight at diagnosis (kg[a])		0.70 (0.56 to 0.96)	p<0.01	
Age at diagnosis (years)		0.19 (0.95 to 29.4)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

Table 36 Appendix I - Parametric univariable analysis on the association between participants' glucose and insulin levels at the end of the follow up and breast cancer treatment

	Glucose			Insulin		
	n	**Mean (mmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Chemotherapy:						
Yes	82	5.07 (0.91)	-0.14 (-0.37 to .10) p=0.25	71	33.92 (30.23)	-10.43 (-20.74 to -1.20) p=0.04
No	85	5.20 (0.60)		76	44.35 (32.83)	
Hormone therapy:						
Yes	144	5.17 (0.80)	0.23 (-0.11 to 0.58) p=0.18	130	39.35 (32.04)	2.28 (-14.49 to 19.06) p=0.79
No	22	4.94 (0.54)		16	37.07 (32.00)	
Tamoxifen:						
Yes	93	5.16 (0.68)	0.04 (-0.19 to 0.28) p=0.71	83	37.13 (30.54)	-4.56 (-15.11 to 5.99) p=0.39
No	73	5.11 (0.88)		63	41.69 (33.75)	
Aromatase inhibitors:						
Yes	112	5.20 (0.85)	0.19 (-0.06 to 0.44) p=0.15	103	39.41 (30.80)	1.06 (-10.43 to 12.56) p=0.85
No	54	5.01 (0.55)		43	38.35 (34.77)	

Anastrozole: **Yes** **No**	73 93	5.10 (0.60) 5.17 (0.09)	-0.07 (-0.30 to 0.17) p=0.57	66 80	40.97 (27.56) 37.56 (35.23)	3.40 (-7.11 to 13.92) p=0.53
Letrozole: **Yes** **No**	12 154	5.67 (1.68) 5.09 (0.65)	0.57 (-0.12 to 1.02) p=0.26[c]	11 135	61.31 (47.39) 37.29 (29.87)	24.03 (4.56 to 43.49) p=0.02
Exemestane: **Yes** **No**	38 128	5.28 (0.76) 5.10 (0.77)	0.18 (-0.10 to 0.46) p=0.12	37 109	29.42 (24.98) 42.39 (33.39)	-6.00 (-24.82 to -1.10) p=0.03
Gonadorelin analogues: **Yes** **No**	12 154	4.88 (0.37) 5.16 (0.79)	-0.28 (-0.73 to 0.18) p=0.23	12 134	36.17 (39.29) 39.36 (31.36)	-3.19 (-22.27 to 15.88) p=0.74

[a] Mmol/l stands for milimol per litre, pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 37 Appendix I - Parametric univariable analysis on the association between participants' glucose and insulin levels at the end of the follow up and 1) menopausal status at diagnosis and 2) change in menopausal status after breast cancer diagnosis

	Glucose			Insulin		
	n	**Mean (mmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Menopausal status at diagnosis:						
Pre + peri menopausal	41	4.92 (0.62)	-0.28 (-0.56 to -0.01) p=0.04	36	37.64 (32.33)	-4.72 (-16.83 to 7.41) p=0.44
Post-menopausal	126	5.21 (0.80)		111	40.47 (31.94)	
Menopausal status change:						
Yes	38	4.99 (0.55)	-0.19 (-0.41 to 0.09) p=0.18	35	36.57 (32.33)	-3.59 (-15.84 to 8.65) p=0.56
No	129	5.18 (0.82)		112	40.16 (31.94)	

[a] Mmol/l stands for milimol per litre, pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 38 Appendix I - Parametric univariable analysis on the association between participants' glucose and insulin levels at the end of the follow up and genetic profile

	Glucose			Insulin		
	n	**Mean (mmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
FTO:						
AA + AT	43	5.08 (0.40)	-0.11 (-0.58 to 0.36) p=0.65	41	43.01 (27.84)	9.94 (-4.12 to 24.00) p=0.17
TT	19	5.19 (.1.44)		19	33.07 (18.48)	
Mc4R:						
CC +CT	29	5.05 (0.61)	-0.12 (-0.56 to 0.31) p=0.57	27	42.80 (27.85)	5.33 (-7.97 to 18.63) p=0.43
TT	33	5.18 (1.12)		33	37.46 (23.61)	

[a] Mmol/l stands for milimol per litre, pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 39 Appendix I - Parametric univariable analysis on the association between participants' glucose and insulin levels at the end of the follow up and biological, behavioural and tumour-related variables

	Glucose			Insulin		
	n	**Mean (mmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a])(SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Smoking status at diagnosis:						
Smoker	16	5.13 (0.48)	-0.01 (-0.41 to 0.39) p=0.98	13	35.53 (32.79)	-3.95 (-22.36 to 14.46) p=0.67
Non-smoker	150	5.14 (0.98)		133	39.48 (31.48)	
	n	**Correlation coefficient[c] p-value**		**n**	**Correlation coefficient[c] p-value**	
Time since diagnosis to the end of the follow up	166	r=-0.01 p=0.22		146	r=-0.21 p=0.01	
Weight at diagnosis	153	r=0.34 p<0.01		133	r=0.34 p<0.01	
Weight at the end of the follow up	167	r=0.31 p<0.01		147	r=0.33 p<0.01	
Weight change from diagnosis to the end of the follow up	153	r=-0.09 p=0.25		133	r=-0.07 p=0.44	

Age at diagnosis (years)	160	r=0.13 p=0.09		141	r=0.10 p=0.25	
Age at the end of the follow up (years)	161	r=0.15 p=0.15		142	r=0.05 p=0.54	
Tumour size (cm)	163	r=-0.08 p=0.29		143	r=-0.04 p=0.67	
	n	**Mean (mmol/l[a]) (SD[a])**	**One Way ANOVA[a]**	**n**	**Mean (pmol/l[a])(SD[a])**	**One Way ANOVA[a]**
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 74 63 23	 5.07 (0.63) 5.17 (0.65) 5.32 (1.32)	 F(2, 158) F=0.58 p=0.39	 66 53 22	 42.59 (33.05) 36.35 (28.28) 39.72 (37.98)	 F (2, 138) F=0.55 p=0.57

[a] Mmol/l stands for milimol per litre, pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval, ANOVA stands for analysis of the variance.

[b] Independent sample t-tests. Equal variances assumed.

[c] Pearson's product moment correlation test.

Table 40 Appendix I - Multiple linear regression analysis on factors associated with participants' glucose levels at the end of the follow up

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	57	4.74 (4.058 to 5.430)	p<0.01	R=0.37 F (2, 54) F=4.24 p=0.02
Weight at diagnosis (kg[a])		0.01 (0.000 to 0.018)	p=0.05	
FTO[b]		-0.28 (-0.528 to -0.029)	p=0.03	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories FTO: 0) AA + AT and 1) TT.

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Table 41 Appendix I - Multiple linear regression analysis on factors associated with participants' insulin levels at the end of the follow up

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	56	-21.99 (-53.84 to 9.86)	p=0.17	R=0.63 F (2, 53) F=17.26 p<0.01
Weight at diagnosis (kg[s])		1.17 (0.75 to 1.59)	p<0.01	
FTO[a]		-15.20 (-27.12 to -3.28)	p=0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories FTO: 0) AA + AT and 1) TT.

APPENDIX II: EXPLORING MISSING DATA

Appendix II. Introduction

This appendix explores the number of weight records available for participants at different evaluation points. This information, as explained, was used to determine the appropriate window margins chosen for the investigated evaluation points post-diagnosis and had repercussions on the sample sizes for each evaluation point. The appendix then investigates the presence of missing data on the outcome of interest: body weight post-BC diagnosis.

Appendix II.A. Number of participants with available weight records

Table 42 (Appendix II, all the tables are found at the end of this appendix) reports on a relevant feature that should guide data analysis: the number of participants with available weight records at different evaluation points. Table 42 (Appendix II) shows the numbers of participants with weight measured at specific evaluation points: at BC diagnosis and at 12, 24, 36, 48, 60 and 72 months post-diagnosis. Initially, the window margin was arbitrarily chosen to be ± 0.5 months. However, only 59 participants (24.69%) had weight records within that window period at the time of diagnosis and less than 10% of the sample had weight records measured at any of the other exact evaluation points. In an attempt to explore whether participants had weights recorded near those points, the window margin was widened to ± 3 and ± 4 months, respectively. As expected, the number of participants with available data increased when the window margins were expanded.

The window margins were arbitrarily chosen as the literature in the area is not consistent. In some studies (i.e. Irwin et al. 2005; Kroenke et al. 2005) the window period for the review points was very large (i.e. weight taken from biannual surveys). Other researchers have reported using a smaller window-period for their follow up measurements, such as ± 1 month (Basaran et al. 2011) or ± 2 months (Makari-Judson et al. 2007; Reddy et al. 2013). Some studies provided vague information in

this regard, reporting that weight values were taken "approximately" at specific points (i.e. approximately at 18 months, 36 months after diagnosis) (Chen et al. 2011; Gu et al. 2011), yet other authors opted for reporting the average months/years at which the follow up point occurred in relation to a baseline measure (i.e. mean of 9.1 months from baseline), rather than providing the interval of time at which the weight measures were taken (Goodwin et al. 1999; Rock et al. 1999; Saquib et al. 2007; Tredan et al. 2010; Guinan et al. 2014). In the current study, selecting a ± 2 months period window would not raise the number of participants with available weight records to a much higher percentage than the original ± 0.5 month window (Table 42, Appendix II).

Appendix II.B. Number of weights across participants according to main predictors

The frequency of weight records available for each participant varied across BC treatment and menopause status (Figure 3, Appendix II). This variation could be attributed mainly to the use of chemotherapy. On average, during all follow up, participants who received chemotherapy were weighed more times than non-users (mean 13.57 times vs. 3.37 times, $p < 0.01$). Similarly, within each year, participants who received chemotherapy had more weights recorded than their counterparts (Table 43, Appendix II).

Participants who took AINs were also weighed more times than those that did not take AINs (10.14 vs. 7.33 times, $p < 0.01$). Those treated with tamoxifen were weighed an average of eight times after diagnosis to the point of study entry, whereas those who did not take tamoxifen were weighed a mean of 10.58 times ($p<0.01$). When looking at each year of follow up, the differences reached statistical significance in the first two years post-diagnosis for tamoxifen use, and during the fourth, fifth and sixth years when looking at AINs use (Table 43, Appendix II).

There were no statistically significant differences in the total number of weights available among the pre, peri and postmenopausal participant groups across the length of follow up. Nonetheless, during the first and fourth year post-diagnosis,

premenopausal women had a higher number of weight records compared with peri and postmenopausal women (Table 43, Appendix II).

Appendix II.C. Reasons for differences in number of weight records between chemotherapy users and non-users. Is this missing data?

The differences in number of weights recorded might lie in the clinical team looking after BC patients. All the patients were followed by the Surgery team, which, as part of routine care, does not assess patient's body weight. Conversely, the Oncology team only follows women who receive chemotherapy, and patients are weighed before each cycle of chemotherapy and yearly during their Oncology follow up. This suggests the presence of missing values among the chemotherapy group, but not among the non-treated group. Forty-five participants (34.6%) had the same number of weight records as cycles of chemotherapy, whereas 39 participants (30.1%) seemed to have missing weight records, as the number of cycles received was higher than the number of weights recorded in their medical reports during the length of the chemotherapy course. In addition, it was expected that chemotherapy-treated participants would have at least one weight record per year following BC diagnosis, however, at different evaluation points, between 25% to 35.1% of chemotherapy-treated participants had missing weight records beyond 12 months post-diagnosis.

On the other hand, because of the retrospective nature of the study and the unplanned measurement occasions from the time of BC diagnosis to the end of the follow up, it was difficult to ascertain when a weight had failed to be recorded in the group of women who did not receive chemotherapy. Therefore, it can be argued that the lower number of weights recorded in the chemotherapy non-treated group (Table 44, Appendix II) should not be considered missing data.

Appendix II.D. What are the reasons for differences in number of weight records between tamoxifen and/or aromatase inhibitors users and non-users? Are there missing data?

A plausible reason for the higher number of weight records among tamoxifen non-users compared with tamoxifen users and among AINs users vs. non-users could be related to chemotherapy use (Table 43, Appendix II). During the first and the second year post-diagnosis, the largest number of participants receiving chemotherapy (n=74) was found among participants who did not take tamoxifen. They hold the highest mean number of weights recorded (mean of 9.11 during first year and 1.82 during the second year) (Table 45, Appendix II). Similarly, the percentage of participants who received chemotherapy compared with those who did not is always higher in the subgroup of participants that received AINs (Table 46 – App II). No other plausible reason seems to explain the differences in number of weight records between the compared groups. However, it is possible that random effects could offer an alternative or complementary explanation for the effect of tamoxifen and AINs use on number of weights recorded following BC diagnosis.

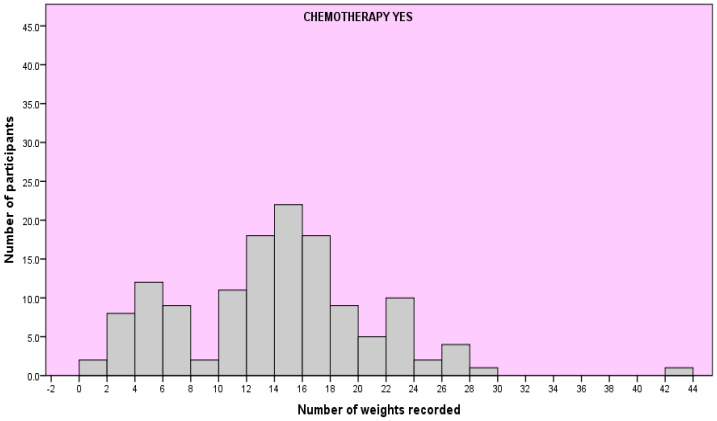
To summarise, results presented here suggest the presence of missing weight data, particularly among participants who received chemotherapy. The differences found seem to be linked to chemotherapy use rather than to other potential factors (i.e. tamoxifen or AINs use).

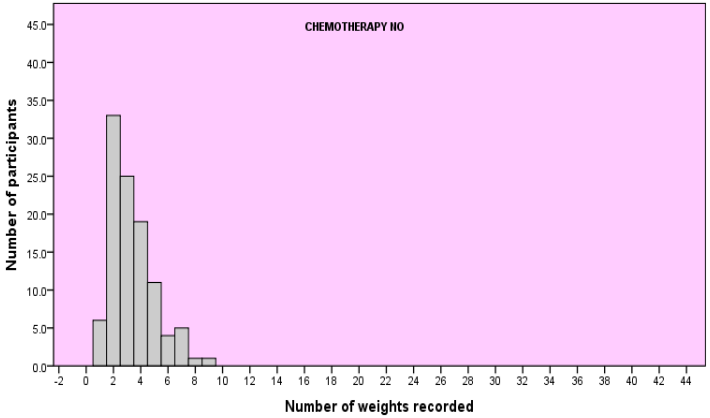
Table 42 Appendix II - Number of participants with weight records available at breast cancer diagnosis and at 12, 24, 36 and 48 months post-diagnosis, looking at different window periods (±0.5, ±3.0 and ±4.0 months)

Window (±0.5 months)	**At diagnosis (±0.5 months)**	**At 12 months post-diagnosis (±0.5 months)**	**At 24 months (±0.5 months)**	**At 36 months (±0.5 months)**	**At 48 months (±0.5 months)**	**At 60 months (±0.5 months)**	**At 72 months (±0.5 months)**	**At 84 months (±0.5 months)**
Total number of participants	239	237	215	172	121	78	41	16
Number of participants with weight records (%)	59 (24.69%)	20 (8.44%)	18 (8.37%)	11 (6.39%)	11 (9.1%)	7 (8.97%)	2 (4.88%)	4 (25%)
Number of participants without weight records (%)	180 (75.31%)	219 (91.56%)	197 (91.63%)	161 (93.61%)	110 (90.9%)	71 (91.03%)	39 (95.12%)	12 (75%)
Window (± 3.0 months)	**At diagnosis (From -2.0 to 3.0 months of diagnosis date)**	**At 12 months post-diagnosis (±3.0 months)**	**At 24 months (±3.0 months)**	**At 36 months (±3.0 months)**	**At 48 months (±3.0 months)**	**At 60 months (±3.0 months)**	**At 72 months (±3.0 months)**	**At 84 months (±3.0 months)**
Total number of participants	239	239	215	172	122	78	41	16

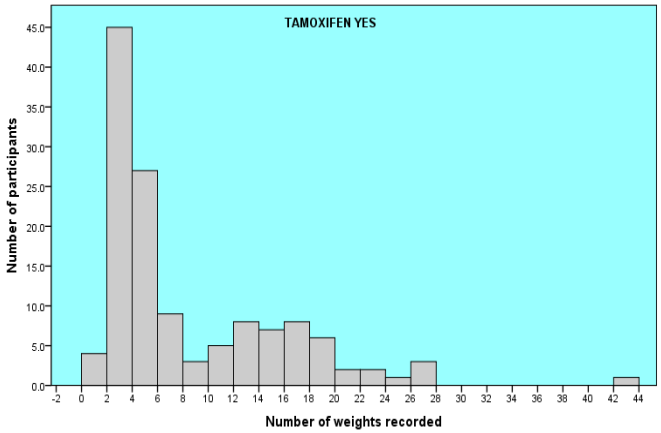
Number of participants with weight records (%)	217 (90.8%)	86 (36%)	88 (40.93%)	67 (38.95%)	53 (43.44%)	28 (35.9%)	20 (48.78%)	13 (81.25%)
Number of participants without weight records (%)	22 (9.2%)	153 (64%)	127 (59.07%)	105 (61.05%)	69 (56.56%)	50 (64.1%)	21 (51.22%)	3 (18.75%)
Window (± 4.0 months)	**At diagnosis (From -2.0 to 4.0 months of diagnosis date)**	**At 12 months post-diagnosis (±4.0 months)**	**At 24 months (±4.0 months)**	**At 36 months (±4.0 months)**	**At 48 months (±4.0 months)**	**At 60 months (±4.0 months)**	**At 72 months (±4.0 months)**	**At 84 months (±4.0 months)**
Total number of participants	239	239	224	178	128	82	44	16
Number of participants with weight records (%)	221 (92.5%)	101 (42.3%)	104 (46.4%)	78 (32.6%)	68 (53.1%)	39 (47.6%)	27 (61.4%)	16 (84.2%)
Number of participants without weight records (%)	18 (7.5%)	138 (57.7%)	120 (53.6%)	100 (56.2%)	60 (46.9%)	43 (52.4%)	17 (38.6%)	3 (15.8%)

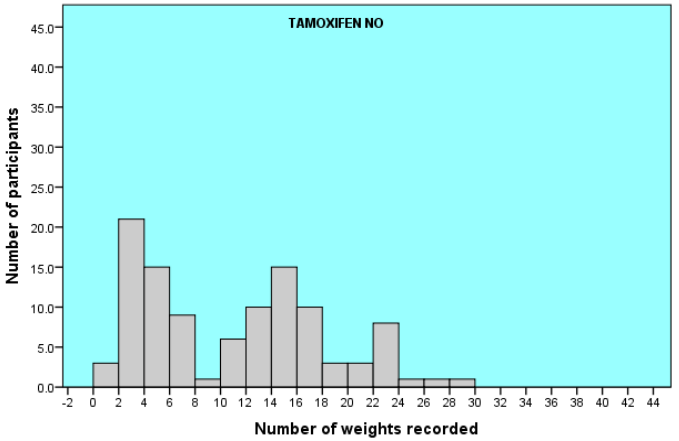
Figure 3 Appendix II - Histogram representing the total number of weight records hold by participants from breast cancer diagnosis to the end of the follow up, according to breast cancer treatments and menopausal status

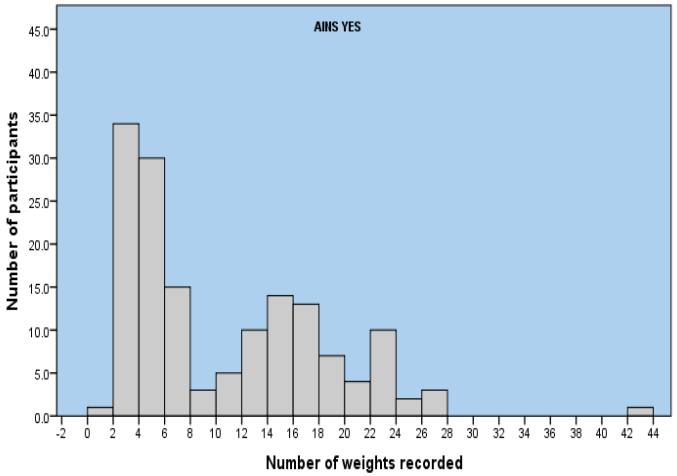


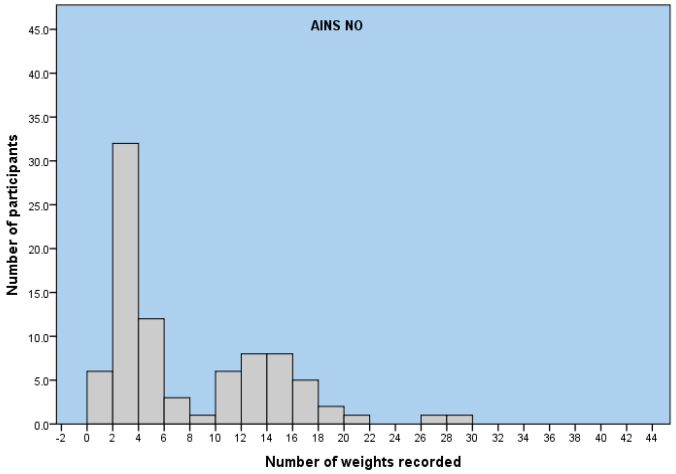


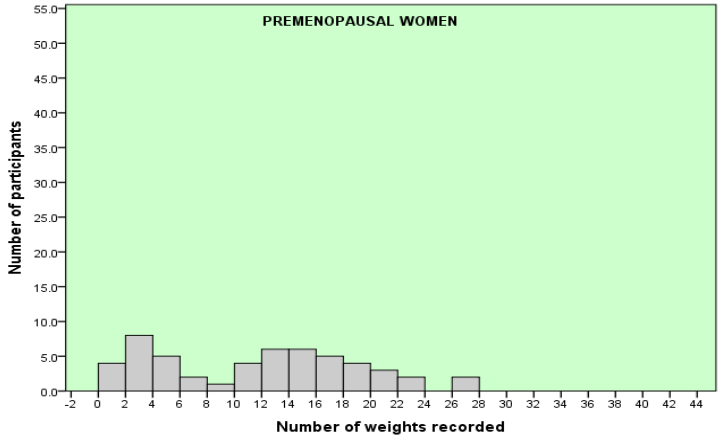
267

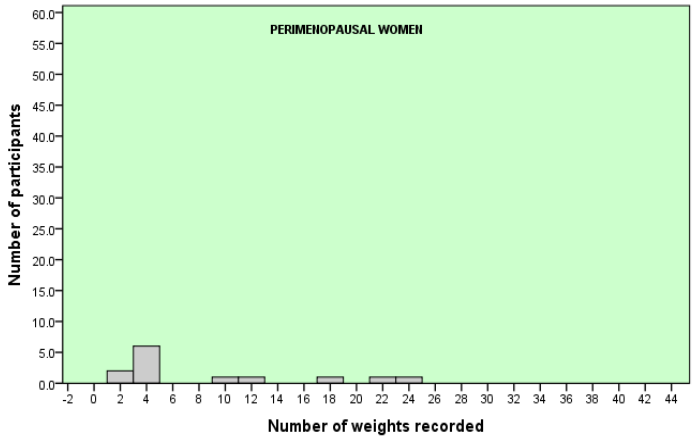

TAMOXIFEN YES
Number of participants
Number of weights recorded

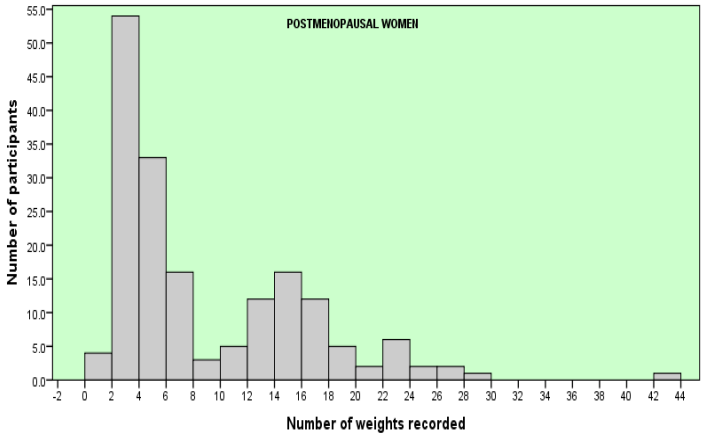

TAMOXIFEN NO
Number of participants
Number of weights recorded


AINS YES
Number of participants
Number of weights recorded


AINS NO
Number of participants
Number of weights recorded







Note: AINs stands for aromatase inhibitors

Table 43 Appendix II - Number of weight records hold by participants from breast cancer diagnosis to the end of the follow up, according to breast cancer treatment and menopausal status at diagnosis

	From diagnosis to the end of the follow up	During the 1st year[a]	During the 2nd year[a]	During the 3rd year[a]	During the 4th year[a]	During the 5th year[a]	During the 6th year[a]	During the 7th year[a]
Number of participants followed	239	239	236	202	161	109	64	37
Number of weight records	**n Mean (SD[b]) p-value of the difference**	**n Mean (SD[b]) p-value of the difference**						
Chemotherapy:								
Yes	134 1 3.57 (6.95)	134 8.43 (4.6)	132 1.67 (1.72)	114 1.60 (1.46)	90 1.38 1.58)	64 1.33 (1.62)	37 1.14 (1.03)	23 1.0 (1.00)
No	105 3.37 (1.65)	105 1.52 (0.94)	104 0.43 (0.73)	88 0.42 (0.71)	71 0.69 (0.77)	45 0.58 (0.58)	27 0.74 (0.71)	14 1.07 (0.92)
	p<0.01[c]	p<0.01[c]	p<0.01[c]	p<0.01[c]	p<0.01[c]	p<0.01[c]	p=0.07[c]	p=0.82[d]
Tamoxifen:								
Yes	131 7.93 (7.35)	131 4.31 (4.49)	128 0.85 (1.16)	114 1.01 (1.32)	92 1.15 (1.57)	64 1.13 (1.64)	39 1.00 (0.92)	23 0.96 (1.06)
No	107 10.58 (7.1)	107 6.78 (5.06)	107 1.47 (1.78)	87 1.20 (1.33)	68 0.97 (0.89)	45 0.87 (0.76)	25 0.92 (0.95)	14 1.14 (0.77)
	p=0.01[d]	p<0.001[c]	p<0.01[c]	p=0.32[d]	p=0.39[d]	p=0.33[d]	p=0.74[d]	p=0.57[d]

Aromatase inhibitors:								
Yes	152 10.14 (7.65)	152 5.78 (4.94)	152 1.25 (1.33)	134 1.15 (1.30)	111 1.23 (1.48)	79 1.24 (1.48)	41 1.15(0.99)	25 1.26 ((1.07)
No	86 7.33 (6.4)	86 4.79 (4.78)	83 0.92 (1.78)	67 0.97 (1.37)	49 0.71 (0.79)	30 0.43 (0.62)	23 0.65(0.71)	12 0.75 (0.62)
	p<0.01[c]	p=0.13[d]	p=0.11[d]	p=0.37[d]	p=0.05[d]	p<0.01[d]	p=0.02[d]	p=0.15[d]
Menopausal status at diagnosis:								
Premenopausal	52 11.25 (7.23)	52 7.23 (5.21)	50 1.24 (1.20)	42 1.52 (1.61)	32 1.19 (1.28)	20 1.2 (1.61)	12 1.0 (0.85)	7 0.57 (0.53)
Perimenopausal	13 8.38 (7.63)	13 5.77 (6.22)	13 0.85 (1.14)	11 0.64 (0.50)	7 1.43 (1.13)	4 0.5 (0.58)	3 0.33(0.58)	2 1.5 (0.71)
Postmenopausal	174 8.49 (7.26)	174 4.82 (4.58)	173 1.12 (1.61)	149 0.99 (1.25)	122 1.02 (1.35)	85 1.0 (1.31)	49 1.0 (0.96)	28 1.11 (1.03)
	p=0.05[e]	p<0.01[e]	p=0.69[e]	p=0.04[e]	p=063[e]	p=0.61[e]	p=0.48[e]	p=0.33[e]

[a] 1st year (from diagnosis to 12.50 months post-diagnosis); 2nd year (from 12.51 to 24.50 months post-diagnosis); 3rd year (24.51 to 36.50 months post-diagnosis); 4th year (36.51 to 48.50 months post-diagnosis); 5th year (48.51 to 60.50 months post-diagnosis); 6th year (60.51 to 72.50 months post-diagnosis); and 7th year (72.51 to 84.50 months post-diagnosis).

[b] SD stands for standard deviation.

[c] Independent sample t-test. Levene's Test for Equality of Variances significant.

[d] Independent sample t-test. Levene's Test for Equality of Variances no significant.

[e] One-Way analysis of the variance (ANOVA). Levene's Test for Equality of Variances not significant.

Table 44 Appendix II - Number of participants with available weight records from breast cancer diagnosis to 1st, 2nd, 3rd, 4th, 5th, 6thand 7th year post diagnosis and to the end of the follow up, stratified by chemotherapy use

	All follow up [a] (from diagnosis to the end of the follow up)		**During the 1st year (from diagnosis to 12.50 months post-diagnosis)**		**During the 2nd year (from 12.51 to 24.50 months)**		**During the 3rd year (from 24.51 to 36.50 months)**		**During the 4th year (from 36.51 to 48.50 months)**		**During the 5th year (from 48.51 to 60.50 months)**		**During the 6th year (from 60.51 to 72.50 months)**		**During the 7th year (from 72.51 to 84.50 months)**	
Chemotherapy																
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Number of participants	134	105	134	105	132	104	114	88	90	71	64	45	37	27	23	14
Total number of weight records	1,818	354	1,130	160	221	45	168	37	124	49	85	26	42	20	23	15
Mean number of weight records (SD[b])	13.7 (6.5)	3.37 (1.65)	8.43 (4.6)	1.52 (0.94)	1.67 (1.72)	0.43 (0.73)	1.59 (1.47)	0.42 (0.71)	1.38 (1.58)	0.69 (0.77)	1.33 (1.62)	0.58 (0.59)	1.14 (1.03)	0.74 (0.71)	1.0 (1.0)	1.07 (0.92)

Number of Participants (%)																
Number of weight records	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
0	0	0	4 (3)	10 (9.5)	34 (25.8)	71 (68.3)	29 (25.4)	60 (68.2)	23 (25.6)	33 (46.5)	16 (25)	21 (46.7)	13 (35.1)	10 (3.07)	7 (30.4)	2 (14.3)
1-5	22 (16.4)	94 (89.45)	32 (23.9)	95 (90.5)	97 (73.5)	33 (31.7)	83 (72.8)	28 (31.8)	65 (72.2)	48 (53.5)	46 (71.8)	24 (53.3)	24 (64.8)	17 (63.0)	17 (69.6)	12 (85.7)
6-10	18 (13.4)	11 (10.6)	46 (34.33)	0	0	0	2 (1.75)	0	1 (1.1)	0	2 (3.2)	0	0	0	0	0
11-15	44 (32.8)	0	45 (36.58)	0	1 (0.8)	0	0	0	1 (1.1)	0	0	0	0	0	0	0
16-20	29 (21.6)	0	7 (5.22)	0	0	0	0	0	0	0	0	0	0	0	0	0
21-25	15 (11.2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26-30	5 (3.7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	1 (0.7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Including eleven women with each one having 1 weight recorded more than 84.51 months post-diagnosis (nine received chemotherapy and two did not).
[b] SD stands for standard deviation.

Table 45 Appendix II - Number of weight records hold by participants during the 1st and 2nd year post breast cancer diagnosis stratified by tamoxifen and chemotherapy use

			During 1st year		During 2nd Year	
			Chemotherapy		Chemotherapy	
			Yes	No	Yes	No
Tamoxifen	Yes	Number of participants	59	72	57	71
		Mean number of weight records (SD[a])	7.73 (4.74)	1.51 (0.99)	1.51 (1.36)	0.32 (0.58)
	No	Number of participants	74	33	74	33
		Mean number of weight records (SD[a])	9.11 (4.35)	1.55 (0.83)	1.82 (1.95)	0.67 (0.96)

[a] SD stands for standard deviation.

Table 46 Appendix II - Number of weight records hold by participants during the 4th, 5th and 6th year post breast cancer diagnosis, stratified by aromatase inhibitors (AINs) and chemotherapy use

			During 4th year		During 5th year		During 6th year	
			Chemotherapy		Chemotherapy		Chemotherapy	
			Yes	No	Yes	No	Yes	No
AINs	Yes	Number of participants	61	50	47	32	47	32
		Mean number of weight records (SD[a])	1.59 (1.78)	0.8 (0.83)	1.57 (1.73)	0.72 (0.58)	1.57 (1.73)	0.72 (0.58)
	No	Number of participants	28	21	17	13	17	13
		Mean number of weight records (SD[a])	0.93 (0.9)	0.43 (0.51)	0.59 (0.71)	0.23 (0.44)	0.59 (0.71)	0.23 (0.44)

[a] SD stands for standard deviation.

APPENDIX III: SENSITIVITY ANALYSIS OF WEIGHT CHANGE AFTER BREAST CANCER DIAGNOSIS

Appendix III. Introduction

This appendix was designed as a complement to Chapter 4, with the aim of providing a rationale for the analytical approach used within the chapter. It evaluates the main results on weight change at 12, 24, 36 and 48 months post BC diagnosis. The appendix reviews the weight values of participants included in the main analysis presented in Chapter 4 and those participants who were excluded due to missing values. The appendix then provides details on the distribution of the main outcomes. The next two sections show the results of the analysis of weight change post-diagnosis and factors associated with it using a non-parametric approach. The following three sections deal with the presence of outliers and their impact on the findings regarding weight change post BC diagnosis. The appendix finishes by showing the repeated multiple regression analysis of weight change.

Appendix III.A. Similarities between participants included in, and participants excluded from the analysis of weight change after breast cancer diagnosis: Comparison of their weights at breast cancer diagnosis

Results from both parametric and non-parametric tests confirmed that there were no differences in weight at the time of BC diagnosis between those participants included in the analysis of weight change detailed in Chapter 4 and those excluded from the analysis due to missing weight records (Table 47, Appendix III). This provided a rationale for the complete-case data analysis approach used in the main analysis of Chapter 4.

Appendix III.B. Non-parametric approach for the analysis of weight change at 12, 24, 36 and 48 months post breast cancer diagnosis

Participants' weight values were not normally distributed. At 12, 24, 36 and 48 months post-diagnosis Shapiro-Wilk normality test's p-values were 0.025, 0.004, 0.131 and 0.018 respectively.

Similarly, weight change at 12, 24, 36 and 48 months post-diagnosis was also not normally distributed (Shapiro-Wilk normality test's p-values: 0.003, 0.018, 0.002 and 0.012 respectively). Although the departures from normality seemed small (Figure 4, Appendix III), the weight change analysis was conducted using a non-parametric approach (Wilcoxon signed-rank tests) (Table 47, Appendix III). Results were similar to those obtained from the parametric paired t-tests in Chapter 4 except at month 24 when, unlike the paired t-test's results, the Wilcoxon signed-rank test p-value reached significance. Nonetheless, the magnitude of the difference in p-values was very small (0.008). Hence, after considering the comparable results using parametric and non-parametric tests, the small differences in p-values between the two approaches and the sample size (n=100), the use of the parametric paired t-tests for the analysis of weight change at 12, 24, 36 and 48 months post BC diagnosis as presented in Chapter 4 was considered valid (Lund and Lund 2013a).

Appendix III.C. Factors associated with weight change at 12, 24, 36 and 48 months after breast cancer diagnosis analysed using a non-parametric approach

Results from the non-parametric tests conducted to analyse factors associated with weight change are presented in tables 48 to 51(Appendix III). Only the major differences between the results of the parametric and non-parametric tests will be detailed. Parametric and non-parametric analytical approaches showed that weight change post-diagnosis was not statistically associated with chemotherapy, the use of anastrozole, letrozole and exemestane, or with the use of gonadorelin analogues. Small differences were found between results of parametric and non-parametric tests in relation to tamoxifen use. The non-parametric test (table 48, Appendix III) showed

that tamoxifen use was significantly associated with weight change 24 months post-diagnosis ($p<0.01$), whereas the parametric tests presented in Chapter 4 did not indicate a statistically significant association ($p=0.08$). However, although the significance level changed when the non-parametric tests were used, the p-values were of nearly similar magnitude. Therefore, the results of both parametric and non-parametric test could be considered similar. Furthermore, the results of the parametric and non-parametric tests for the association of tamoxifen with weight change at other periods were similar. Hence the parametric results presented within the main results of Chapter 4 regarding weight change and its association with BC treatments were deemed valid.

Parametric and non-parametric tests revealed comparable findings when evaluating the association between weight change and menopausal status, change in menopausal status post-diagnosis, FTO and Mc4R genetic profile and weight change, as well as other factors (i.e. tumour size, age at BC diagnosis) (tables 49 to 51, Appendix III). Conversely, the statistically significant associations between weight at BC diagnosis and weight change at 12 and 36 months post-diagnosis in Chapter 4 were not supported under a non-parametric approach (Spearman's correlation p-values: 0.10 and 0.19 respectively). Arguably, the p-values were not very different and in both tests the correlation coefficients were of small magnitude (Pearson's product moment correlation r values: -0.27 and -0.32, vs. Spearman's correlation rho values: -0.16 and -0.15 respectively).

Smoking status was no longer statistically significantly associated with weight change at 48 months post-diagnosis when data were analysed non-parametrically (Table 51, Appendix III), although the p-value was small (Mann-Whitney U test $p=0.15$), a result similar to that found when using the parametric approach (independent sample t-test p-value: 0.04).

Overall, the results of the analysis of factors associated with weight change post-diagnosis using a parametric approach were not very different to the results of the non-parametric approach.

Appendix III.D. Outlier values in the analysis of weight change at 12, 24, 36 and 48 months post breast cancer diagnosis

A sensitivity analysis was conducted to explore the presence of outliers, the nature of these outliers and its impact of their presence in the findings. There were 12 participants whose weight change was more than 1.5 box-lengths from the edge of the box in a box-and-whisker plot, and one participant with an extreme weight gain at 36 months post-diagnosis (more than 3 box-lengths from the edge of the box). Seven of these participants had a large weight loss. Six participants had a large weight gain compared to other participants' weight change.

There were 30 outliers noted (ranging from 7 to 16) when exploring the association between weight change and different variables (i.e. treatments, menopausal status, etc). Data inspection suggested that the weights of these outliers were genuinely unusual values and not the consequence of data entry or measurement error. Therefore, they were not treated as invalid. Nonetheless, in order to see the impact of these outliers on the main results, the analysis was repeated excluding them from the analysis.

The magnitude of weight change was slightly greater at 12, 24, 36 and 48 months after removing the outliers from the analysis of weight change (Table 52, Appendix III). Statistically, the only difference was found at 24 months, when weight change became significant after removing outliers ($p=0.04$ vs. $p=0.12$). Nonetheless, the magnitude of the p-values was very close, and the magnitude of the difference in weight change in the two comparison groups was small before and after removing the outliers (0.85 kg vs. 0.90 kg). Moreover, it might not be clinically significant. Therefore, the main analysis on weight change post-diagnosis presented in Chapter 4 included all participants.

Similarly, the presence of outliers did not modify most of the results reported in Chapter 4 of factors associated with weight change (data not shown). The outliers did not create or remove any significant association of weight change with most variables (chemotherapy use, hormone therapy, letrozole, exemestane, FTO, tumour size, stage of disease, or weight and age at BC diagnosis). However, after removing the outliers, the statistically significant association between weight change and

tamoxifen, AINs, anastrozole, change in menopausal status and smoking status at diagnosis disappeared. Conversely, weight change became statistically significantly associated with use of gonadorelin analogues, menopause status at diagnosis and with Mc4R status.

Nonetheless, despite these changes, the participants with outlying weight change values were kept in the main analysis for several reasons. Firstly, the large number of outliers found could indicate that these values were informative. Secondly, the number of the outlying participants with large weight gain values (n=14) was similar to the number of outliers with large weight loss values (n=16). Thirdly, although the presence of the outliers modified the statistical impact of some factors of weight change post-diagnosis, the direction of the findings and the magnitude of the weight change was not altered greatly. Fourthly, a repeat analysis excluding the outliers produced few changes in the significance level of the associations found between different factors and weight change post BC diagnosis. Finally, these changes only affected one or two out of the four evaluation points explored for each variable.

Appendix III.E. Multivariable analysis of factors associated with weight change at 12, 24, 36 and 48 months post breast cancer diagnosis. Repeated analysis

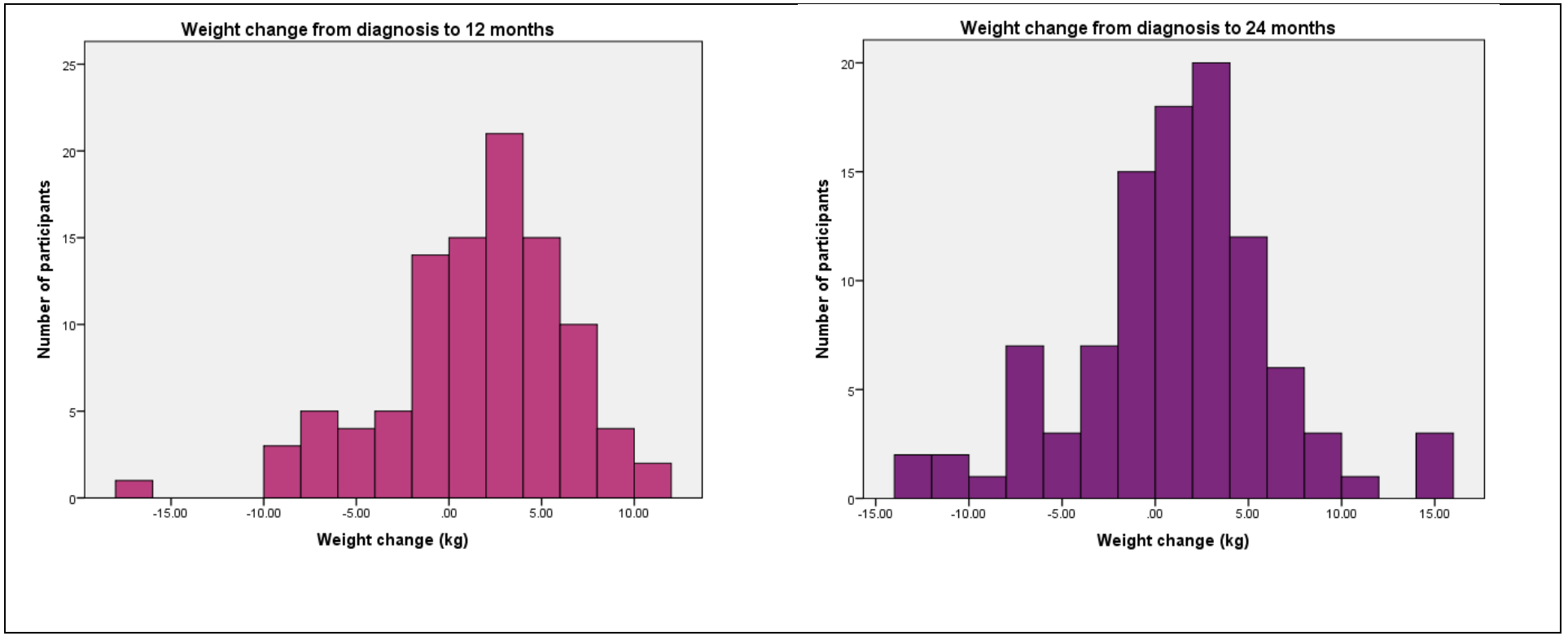
The multiple regression analysis presented in Chapter 4 met all the assumptions for the test, except for the presence of participants with unusually large studentized deleted residual values. Therefore, a repeat analysis of weight change was conducted after removing those participants (Tables 53 to 56, Appendix III). Most of the results showed coefficients of nearly similar magnitudes and comparable p-values to the ones in Chapter 4. This suggested that the use of multiple regression tests for the analysis presented in Chapter 4 was methodologically sound. The only exception was found in the analysis of weight change 48 months post-diagnosis (Tables 55 and 56, Appendix III) and it is detailed in Chapter 4.

Table 47 Appendix III - Differences in weight at diagnosis between participants included in, and those excluded from the analysis of weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis, using parametric and non-parametric tests

		12 months post-diagnosis		24 months post-diagnosis		36 months post-diagnosis		48 months post-diagnosis	
		Included	**Excluded**	**Included**	**Excluded**	**Included**	**Excluded**	**Included**	**Excluded**
Number of participants	**With follow up at:**	99	140	100	124	74	104	62	62
	With diagnosis weight at:	99	122[a]	100	106[b]	74	89[c]	66	54[d]
Mean weight at diagnosis (kg[e]) (SD)		70.8 (12.9)	73.3 (14.0)	72.6 (14.2)	71.3 (12.5)	70.3 (12.9)	72.1 (13.6)	73.4 (15.7)	69.5 (14.1)
Median weight at diagnosis (kg[e])		70.0	70.6	69.5	70.0	70.3	69.0	70.9	69.5
Mean difference, (95% CI[e]), p-values		-2.51 (-6.12 to 1.10) p=0.17[f]		1.33 (-2.36 to 5.01) p=0.48[g]		-1.74 (-5.87 to 2.39) p=0.41[e]		2.52 (-2.68 to 7.73) p=0.33[g]	
Mann-Whitney U test U, Z and p-values		U=5,540.50 Z=-1.05 p=0.29[h]		U=5,144.50 Z=-0.364 p=0.72[h]		U=3,126.50 Z=-0.555 p=0.58[h]		U=1,569.00 Z=-0.581 p=0.56[h]	

[a] Of those 140 participants excluded, weight at diagnosis was calculated with data from 122 of them, as weight records at the time of diagnosis were missing from 18 participants.
[b] From those 124 participants excluded from the analysis, weight records at the time of diagnosis were missing from 18 participants.
[c] From those 104 participants excluded from the analysis, weight records at the time of diagnosis were missing from 15 participants.
[d] From those 66 participants excluded from the analysis, weight records at the time of diagnosis were missing from 12 participants.
[e] Kg stands for kilograms, CI stands for confidence interval.
[f] Independent samples t-test, equal variances assumed.
[g] Independent samples t-test, equal variances not assumed.
[h] Asymptotic statistical significance level (2-tailed).

Figure 4 Appendix III - Distribution of weight change from diagnosis to 12, 24, 36 and 48 months post-diagnosis



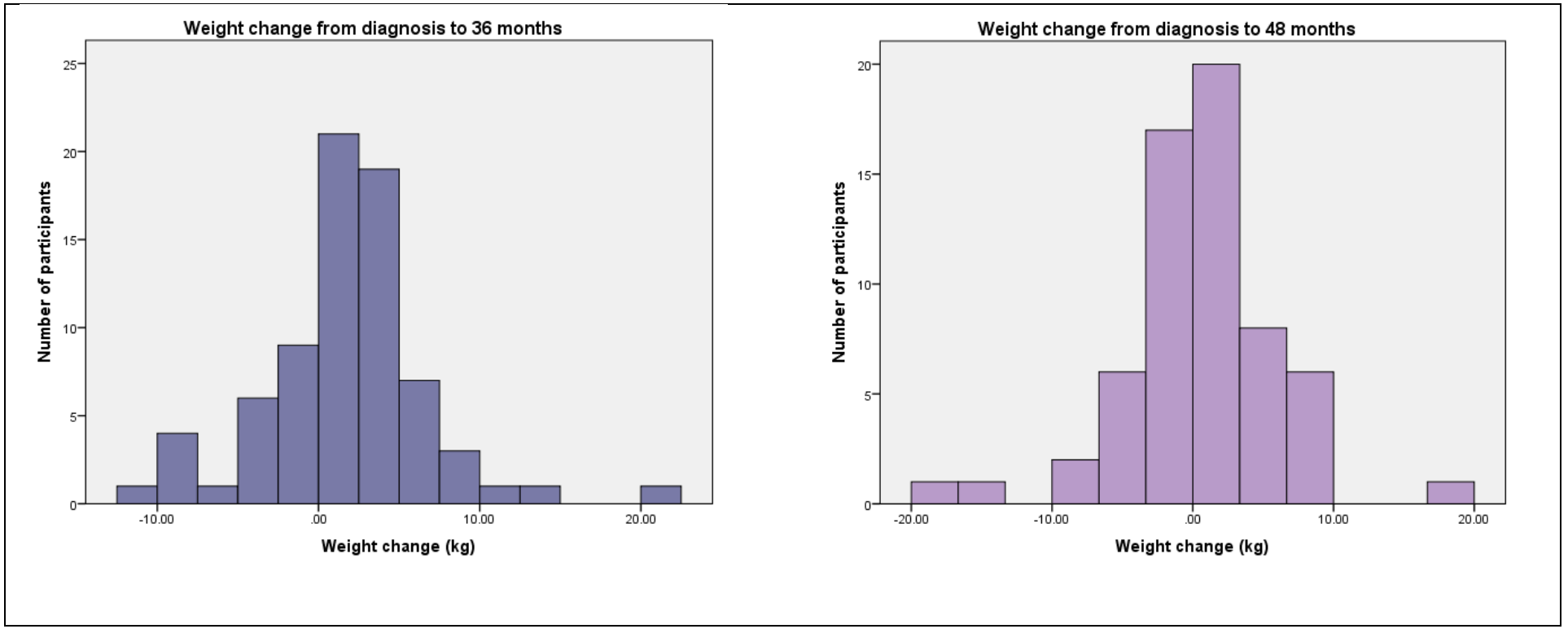

Weight change from diagnosis to 36 months
Number of participants
25
20
15
10
5
0
-10.00
.00
10.00
20.00
Weight change (kg)
Weight change from diagnosis to 48 months
Number of participants
20
15
10
5
0
-20.00
-10.00
.00
10.00
20.00
Weight change (kg)

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Table 48 Appendix III - Non-parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and breast cancer treatment

	Weight Change from diagnosis to:											
	12 months post-diagnosis			24 months post-diagnosis			36 months post-diagnosis			48 months post-diagnosis		
	N	Median Weight change (kg[b])	Mann-Whitney U test	n	Median Weight change (kg[b])	Mann-Whitney U test	n	Median Weight change (kg[b])	Mann-Whitney U test	n	Median Weight change (kg[b])	Mann-Whitney U test
Chemotherapy[a]:												
Yes	84	2.00	U=580.50 Z=-0.48	75	1.40	U=898.00 Z=-0.31	53	2.00	U=487.00 Z=-0.83	36	0.95	U=458.00 Z=-0.143
No	15	0.60	p=0.63	25	0.70	p=0.75	21	2.40	p=0.40	26	0.85	p=0.89
Hormone therapy[a]:												
Yes	86	2.00	U=535.00 Z=-0.25	90	1.67	U=299.00 Z=-1.29	66	2.35	U=158.50 Z=-1.36	55	1.0	U=157.50 Z=-0.18
No	13	2.00	p=0.80	9	-0.40	p=0.20	7	1.30	p=0.17	6	-1.60	p=0.86
Tamoxifen[a]:												
Yes	41	2.50	U=1,018.00 Z=-1.21	45	3.00	U=749.00 Z=-3.38	42	2.05	U=573.50 Z=-1.07	35	2.00	U=329.50 Z=-2.03
No	58	1.00	p=0.22	55	0.00	p <0.01	32	1.65	p=0.28	27	-1.70	p=0.04
Aromatase inhibitors[a]:												
Yes	47	1.30	U=1,139.00 Z=-0.58	59	1.0	U=917.00 Z=-1.69	45	2.40	U=540.00 Z=-0.78	48	0.85	U=253.50 Z=-0.64
No	52	2.20	p=0.56	39	2.00	p=0.09	27	1.50	p=0.43	12	1.25	p=0.52

Anastrozole[a]:												
Yes	40	1.75	U=1,126.50 Z=-0.38	45	1.00	U=1,019.50 Z=-1.37	33	2.40	U=640.50 Z=-0.22	33	-1.50	U=326.50 Z=-1.96
No	59	2.00	p=0.70	54	2.00	p=0.17	40	1.95	p=0.83	28	2.00	p=0.05
Letrozole[a]:												
Yes	7	0.00	U=292.50 Z=-0.40	9	-0.50	U=347.00 Z=-0.75	3	2.40	U=90.00 Z=-0.45	2	4.20	U=23.00 Z=-1.47
No	92	2.00	p=0.69	91	1.45	p=0.45	71	2.00	p=0.68	60	0.65	p=0.16
Exemestane[a]:												
Yes	4	1.50	U=171.00 Z=-0.34	10	3.20	U=402.00 Z=-0.50	15	2.40	U=317.00 Z=-1.61	15	2.00	U=318.00 Z=-0.45
No	95	2.00	p=0.74	89	1.00	p=0.62	58	1.65	p=0.11	46	0.30	p=0.65
Gonadorelin analogues[a]:												
Yes	13	4.40	U=460.00 Z=-1.03	15	3.00	U=480.00 Z=-1.52	11	4.00	U=233.50 Z=-1.71	9	2.80	U=166.50 Z=-1.44
No	86	1.75	p=0.30	85	1.00	p=0.13	63	1.90	p=0.09	53	0.50	p=0.15

[a] All participants had surgery and they might have also received other treatments (i.e. radiotherapy, biological therapy).
[b] Kg stands for kilograms.

Table 49 Appendix III - Non-parametric univariable analysis on the association between weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and 1) menopausal status at diagnosis and 2) change in menopausal status from diagnosis to 12, 24, 36 and 48 months post-diagnosis

	Weight Change from Diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**
Menopausal status at diagnosis:												
Pre &peri menopausal	36	3.25	U=958.50 Z=-1.27 p=0.20	31	2.00	U=904.50 Z=-1.23 p=0.22	20	2.80	U=421.00 Z=-1.45 p=0.15	15	3.00	U=195.50 Z=-2.58 p=0.01
Postmenopausal	63	0.70		69	0.70		54	2.95		47	0.10	
Change in menopause status:												
Yes	29	3.25	U=958.50 Z=-1.27 p=0.20	23	2.00	U=904.50 Z=-1.23 p=0.22	17	2.80	U=421.00 Z=-1.45 p=0.15	13	3.00	U=195.50 Z=-2.58 p=0.01
No	70	0.70		65	0.70		49	2.95		41	0.10	

[a] Kg stands for kilograms.

Table 50 Appendix III - Non-parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis and genetic profile

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median Weight change (kg[a])**	**Mann-Whitney U test**
FTO:												
AA + AT	45	0.20	U=456.50 Z=-1.05	45	1.65	U=438.00 Z=-0.47	27	1.80	U=114.00 Z=-1.78	26	0.70	U=85.50 Z=-2.42
TT	24	2.50	p=0.29	21	0.70	p=0.63	13	2.90	p=0.07	7	-1.50	p=0.81
Mc4R:												
CC +CT	27	0.00	U=521.50 Z=-0.56	25	1.20	U=428.50 Z=-1.11	16	1.40	U=119.50 Z=-2.03	11	-1.30	U=92.00 Z=-1.11
TT	42	2.00	p=0.58	41	1.40	p=0.28	24	2.85	p=0.04	22	0.70	p=0.28

[a] Kg stands for kilograms.

Table 51 Appendix III - Non-parametric univariable analysis on the association between participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis with biological, behavioural and tumour-related variables

	Weight Change from diagnosis to:											
	12 months post-diagnosis			**24 months post-diagnosis**			**36 months post-diagnosis**			**48 months post-diagnosis**		
	n	**Median weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median weight change (kg[a])**	**Mann-Whitney U test**	**n**	**Median weight change (kg[a])**	**Mann-Whitney U test**
Smoking status at diagnosis: Smoker	9	6.00	U=185.50 Z=-2.67 p<0.01	9	0.00	U=353.00 Z=-0.68 p=0.50	5	6.45	U=121.50 Z=-0.88 p=0.38	9	2.10	U=166.00 Z=-1.44 p=0.15
Non smoker	90	1.75		91	1.65		69	2.00		53	0.50	
	n	**Correlation coefficient[b] p-value**		**n**	**Correlation coefficient[b] p-value**		**n**	**Correlation coefficient[b] p-value**		**n**	**Correlation coefficient[b] p-value**	
Weight at diagnosis (kg[a])	99	r=-0.16 p=0.10		100	r=-0.14 p=0.18		74	r=-0.15 p=0.19		62	r=-0.16 p=0.22	
Age at diagnosis (years)	93	r=-0.12 p=0.25		95	r=-0.12 p=0.26		72	r=-0.05 p=0.65		57	r=-0.24 p=0.07	

Tumour Size (cm[a])	94	r=-0.14 p=0.17	96	r=0.10 p=0.31	72	r=-0.05 p=0.68	60	r=-0.05 p=0.71
	n	**Kruskal Wallis Test**	**n**	**Kruskal Wallis Test**	**n**	**Kruskal Wallis Test**	**n**	**Kruskal Wallis Test**
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 30 39 26	χ^2=2.69 d.f.[a]=2 p=0.26	 36 37 25	χ^2=2.90 d.f.[a]=2 p=0.23	 24 31 16	χ^2=4.08 d.f.[a]=2 p=0.13	 26 23 10	χ^2=1.05 d.f.[a]=2 p=0.59

[a] Kg stands for kilograms, cm stands for centimetres and d.f. stands for degrees of freedom.
[b] Spearman's correlation rho.

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Table 52 Appendix III - Repeated analysis of participants' weight change from breast cancer diagnosis to 12, 24, 36 and 48 months post-diagnosis, after excluding outliers

	12 months post-diagnosis		24 months post-diagnosis		36 months post-diagnosis		48 months post-diagnosis	
	Diagnosis[c]	**12 months[d]**	**Diagnosis[c]**	**24 monthsd**	**Diagnosis[c]**	**36 months[d]**	**Diagnosis[c]**	**48 months[d]**
Number of participants[a] (%)[b]	92 (40.7%)		93 (44.1%)		67 (39.9%)		58 (48%)	
Mean weight (SD) (kg[e])	69.7 (12.4)	71.2 (12.3)	71.5 (13.8)	72.4 (13.7)	69.2 (12.2)	70.9 (12.1)	72.3 (15.4)	73.0 (15.4)
Mean weight change (kg[e]), (95% CI[e]) and p-value	1.50 (0.62 to 2.37) p<0.01[f]		0.90 (0.05 to1.82) p=0.04[f]		1.70 (0.83 to 2.52) p<0.01[f]		0.70 (-0.36 to 1.81) p=0.19[f]	

[a] Number of participants with available weight records for both diagnosis and the explored evaluation point.
[b] Percentage based on the total number of participants available within each follow up period (at 12 months: 226, at 24 months: 211, at 36 months: 168 and at 48 months:121).
[c] Diagnosis date -2 months prior diagnosis to 4 months post diagnosis.
[d] Period window ±4 months.
[e] Kg stands for kilograms, CI stands for confidence interval.
[f] Paired t-test p-value.

Table 53 Appendix III - Repeated multiple linear regression analysis of participants' weight change from breast cancer diagnosis to 24 months post-diagnosis, after excluding outliers

Variables included	n	Unstandardised coefficients (95% CI[a]), p-value	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	94	12.81 (7.42 to 18.20)	p<0.01	R=0.47 F (2, 91) F=12.96 p<0.01
Tamoxifen[b]		-3.67 (-5.52 to -1.81)	p<0.01	
Weight at diagnosis (kg[a])		-0.09 (-0.16 to -0.03)	p<0.01	

[a] CI stands for confidence interval, kg stands for kilograms, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non – users.

Table 54 Appendix III - Repeated multiple linear regression analysis of participants' weight change from breast cancer diagnosis to 36 months post-diagnosis, after excluding outliers

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	73	13.49 (7.31 to 19.68)	p<0.01	R=0.43 F (2, 70) F=7.89 p<0.01
Tamoxifen[b]		-1.76 (-3.80 to 0.28)	p=0.09	
Weight at diagnosis (kg[a])		-0.14 (-0.21 to -0.06)	p<0.01	

[a] CI stands for confidence interval, kg stands for kilograms, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non – users.

Table 55 Appendix III - Multiple linear regression analysis of participants' weight change from breast cancer diagnosis to 48 months post- diagnosis, including outliers

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	57	15.97 (6.96 to 24.98)	p<0.01	R=0.64 F (3, 53) F=6.52 p<0.01
Weight at diagnosis (kg[a])		-0.09 (-0.18 to -0.002)	p=0.04	
Age at diagnosis (years)		-0.09 (-0.21 to 0.03)	p=0.14	
Tamoxifen[b]		2.78 (-5.40 to -0.17)	p=0.04	

[a] CI stands for confidence interval, kg stands for kilograms, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non – users.

Table 56 Appendix III - Repeated multiple linear regression analysis of participants' weight change from breast cancer diagnosis to 48 months post-diagnosis, after excluding outliers

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	56	12.18 (6.96 to 24.98)	p<0.01	R=0.38 F (3, 52) F=2.92 p=0.04
Weight at diagnosis (kg[a])		-0.04 (-0.18 to -0.002)	p=0.03	
Age at diagnosis (years)		-0.10 (-0.21 to 0.03)	p=0.08	
Tamoxifen[b]		-1.98 (-5.40 to 0.17)	p=0.11	

[a] CI stands for confidence interval, kg stands for kilograms, ANOVA stands for analysis of the variance.
[b] Categories: 0) users and 1) non-users.

APPENDIX IV: SAMPLE RESIDUALS IN THE MULTILEVEL MODELS

A visual inspection of the normal quantile-quantile plot (Q-Q Plot) and scatter/dot plot for the residuals of the time-invariant model F suggests that they were normally distributed and homoscedastic (Figure 5, Appendix IV).

On the other hand, the normal Q-Q Plot of the residuals in time-varying model 4 suggested that the residuals were normally distributed. The scatter/dot plot indicates that the residual variability was approximately equal at each tamoxifen and anastrozole value (Figure 6, Appendix IV). The variability found in letrozole could be due the small number of participants that received that treatment (n=18).

Figure 5 Appendix IV - Normal Q-Q plot and scatter/dot plot of the residuals in model F

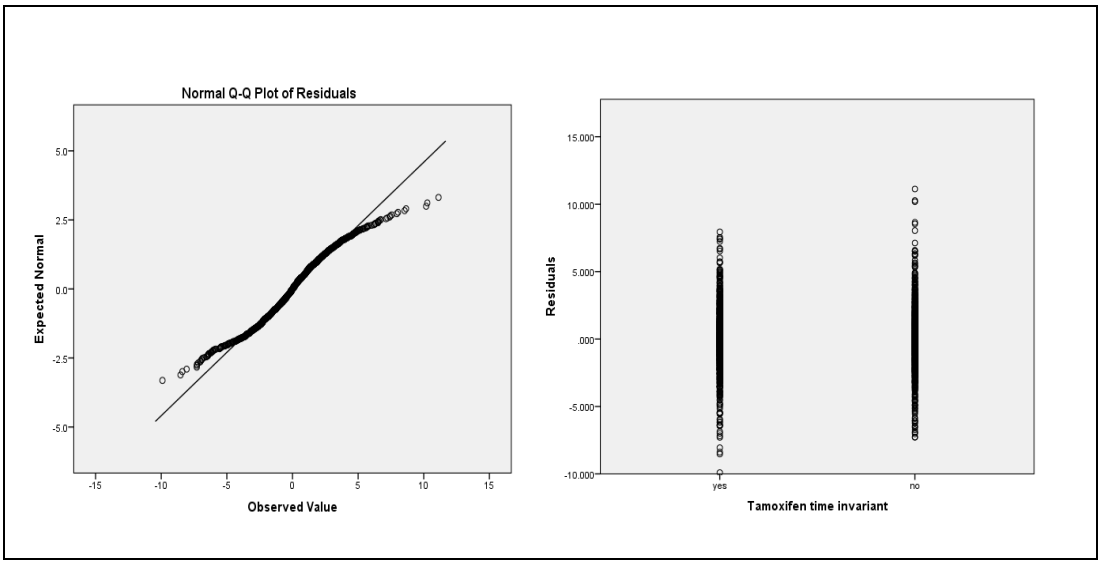
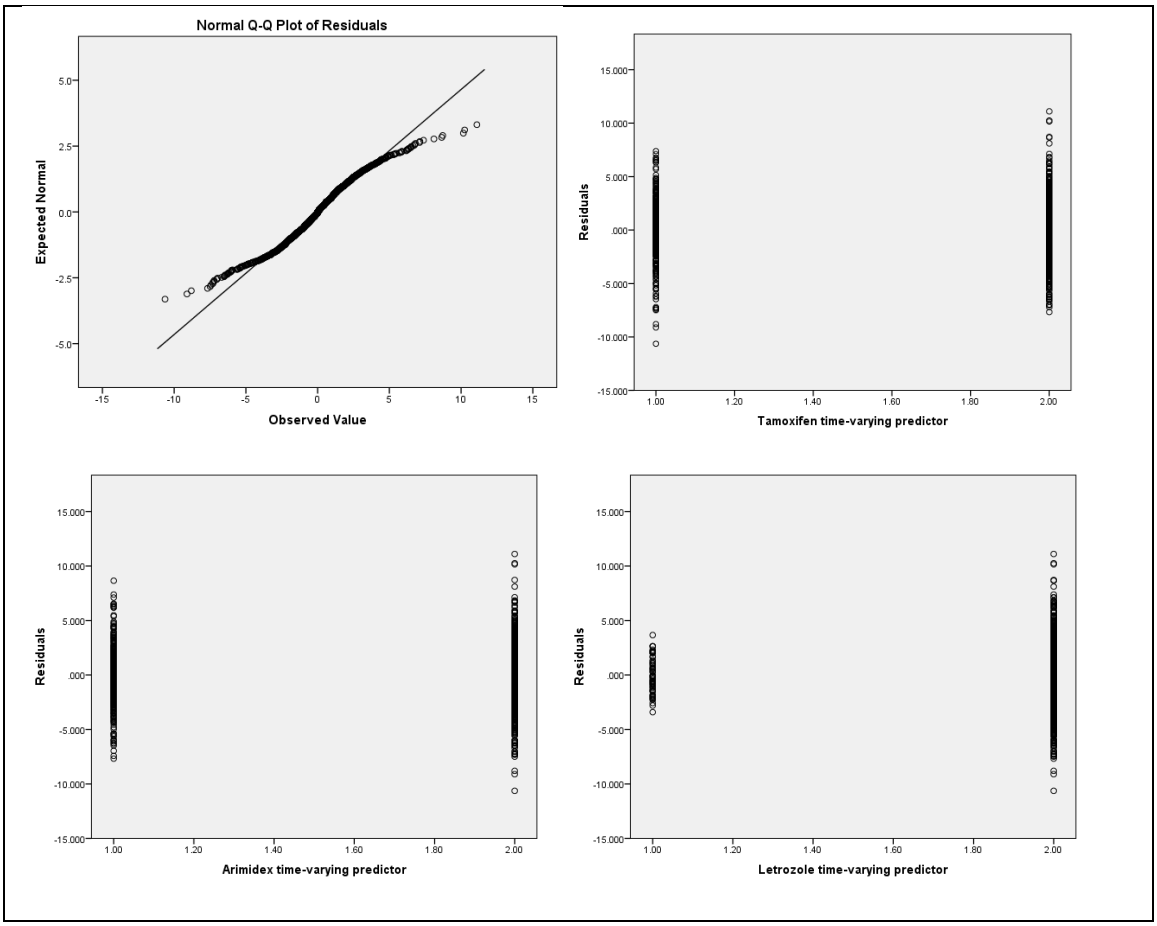


Figure 6 Appendix IV - Normal Q-Q plot and scatter/dot plots of the residuals in model 4



APPENDIX V: SENSITIVITY ANALYSIS OF THE RESULTS OF BODY ADIPOSITY AND METABOLIC PARAMETERS

Appendix V. Introduction

This appendix provides a rationale for the analytical approach used in Chapter 4 in relation to metabolic and adiposity results. It begins by providing a rationale for the values chosen to represent insulin levels in participants with initial values of less than 13.89 pmol/l. It then presents the distribution of the metabolic and body adiposity parameters and continues with an analysis of factors associated with those metabolic and body adiposity outcomes using a non-parametric approach. It follows by exploring the impact of outliers on the results. The final section is a repeat of the multiple regression analyses presented in Chapter 4.

Appendix V.A. Exploring insulin level as a numerical variable

Thirty-two participants had insulin levels below the limit the assay could detect (13.89 pmol/l). Therefore, three parallel analyses were conducted using three different presumed values for the insulin levels of these 32 participants. A value of 13.89 pmol/l was used for the first analysis, 7 pmol/l for the second analysis and a value of 1 pmol/l for the third analysis. The results of these three analyses were very similar (Tables 57 to 60, Appendix V) (all tables are presented at the end of this appendix). Therefore these participants were presumed to have an insulin level of 7 pmol/l for the main analyses presented in Chapter 4.

Appendix V.B. Factors associated with body adiposity and metabolic parameters at the end of the follow up, using a non-parametric approach

Body fat percentage and FM/FFM ratio were the only two normally distributed variables (Shapiro-Wilk normality test p-values: 0.25 and 0.22 respectively). The distribution of the rest of the outcomes (waist circumference and fasting glucose and insulin) is presented in Figure 7 (Appendix V). Nonetheless, in spite of these departure from normality, the value of the mean and the median were comparable (waist circumference: mean: 92.3cm and median: 91.7 cm, glucose: mean 5.14 mmol/l and median: 5.2 mmol/l and insulin: mean: 39.31 pmol/l and median: 31.5 pmol/l). Consequently, the value of these and all metabolic and adiposity parameters were summarised in Chapter 4 with the mean and SD.

A sensitivity analysis was conducted to evaluate the appropriateness of using a parametric approach to analyse those non-normally distributed outcomes (waist circumference, glucose and insulin levels). The body adiposity and metabolic outcome values and p-values resulting from the non-parametric tests were of similar magnitude to those from the parametric tests presented in Chapter 4 (tables 61 to 64, Appendix V, for waist circumference results and tables 65 to 69, Appendix V for metabolic results). Hence, parametric tests were chosen to explore body adiposity and metabolic parameters in the sample.

Appendix V.C. Outlier values in body adiposity and metabolic parameters at the end of the follow up. Repeated analysis

Six participants had higher glucose levels compared to the average in the sample and two others had lower glucose levels. In addition, eight participants had higher insulin levels than the mean. On the other hand, there was only one participant whose FM/FFM ratio and waist circumference values were larger than the mean. All of them were considered outliers.

An inspection of the body adiposity and metabolic outcome values of these participants suggested that these were natural values. However, a repeated analysis

was conducted excluding them. The results (data not shown) suggested that excluding the outliers did not produce major changes in the results presented in Chapter 4, therefore, they were included in the main analysis.

Appendix V.D. Multiple regression analysis of body adiposity and metabolic parameters at the end of the follow up. Repeated analysis

A sensitivity analysis was conducted as the assumptions of the multiple linear regression analysis presented in Chapter 4 were not met due to the presence of participants with unusually large (>3) studentized deleted residual values. The repeated analysis excluding participants with unusual values showed similar results to those presented in Chapter 4, suggesting that the findings of the regression analysis were statistically valid (Tables 69 to 71 for body adiposity parameters and tables 72 to 74 for metabolic parameters, Appendix V). The only exception was when evaluating glucose levels (Tables 72 and 73, Appendix V), as was explained in Chapter 4.

Table 57 Appendix V - Sensitivity analysis of the association between participants' insulin levels at the end of the follow up and breast cancer treatment, using three hypothetical values for those insulin levels whose magnitude was below the limit the assay could detect

	Insulin (value: 13.88 pmol/l[a])			Insulin (value: 7 pmol/l[a])			Insulin pmol/l (value: 1 pmol/l[a])		
	n	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Chemotherapy:									
Yes	71	35.86 (28.60)	-9.49 (-19.36 to 0.39) p=0.06	71	33.92 (30.23)	-10.43 (-20.74 to -12) p=0.04	71	32.23 (31.83)	-11.25 (-21.99 to 0.51) p=0.04
No	76	45.35 (31.76)		76	44.35 (32.83)		76	43.48 (33.89)	
Hormone therapy:									
Yes	130	40.73 (30.72)	1.51 (-14.54 to 17.56) p=0.85	130	39.35 (32.04)	2.28 (-14.49 to 19.06) p=0.79	130	38.15 (33.33)	2.96 (-14.53 to 20.44) p=0.74
No	16	39.22 (29.95)		16	37.07 (32.00)		16	35.19 (33.95)	
Tamoxifen:									
Yes	83	38.71 (29.07)	5.11 (-14.40 to 5.80) p=0.40	83	37.13 (30.54)	-4.56 (-15.11 to 5.99) p=0.39	83	35.76 (31.98)	-4.79 (-15.80 to 6.21) p=0.39
No	63	43.01 (32.47)		63	41.69 (33.75)		63	40.55 (35.00)	

Aromatase inhibitors:									
Yes	103	40.75 (29.52)	0.63 (-10.36 to 11.64) p=0.91	103	39.41 (30.80)	1.06 (-10.43 to 12.56) p=0.85	103	38.25 (32.14)	1.43 (-10.55 to 13.41) p=0.81
No	43	40.11 (33.24)		43	38.35 (34.77)		43	36.82 (36.25)	
Anastrozole:									
Yes	66	42.01 (26.34)	2.64 (-7.42 to 12.71) p=0.60	66	40.97 (27.56)	3.40 (-7.11 to 13.92) p=0.53	66	40.06 (28.76)	4.07 (-6.88 to 15.03) p=0.46
No	80	39.37 (33.75)		80	37.56 (35.23)		80	35.99 (36.69)	
Letrozole:									
Yes	11	61.32 (47.39)	22.45 (3.81 to 41.08) p=0.02	11	61.31 (47.39)	24.03 (4.56 to 43.49) p=0.02	11	61.32 (47.39)	25.40 (-5.12 to 45.68) p=0.01
No	135	38.87 (28.36)		135	37.29 (29.87)		135	35.91 (31.35)	
Exemestane:									
Yes	37	31.65 (23.06)	-11.94 (-23.30 to -0.57) p=0.04	37	29.42 (24.98)	-12.97 (-24.82 to -1.10) p=0.03	37	27.48 (26.86)	-13.87 (-26 to -1.51) p=0.03
No	109	43.59 (32.24)		109	42.39 (33.39)		109	41.34 (34.61)	

Gonadorelin analogues:									
Yes	12	37.89 (38.00)	-2.91 (-21.16 to 15.34) p=0.75	12	36.17 (39.29)	-3.19 (-22.27 to 15.88) p=0.74	12	34.67 (40.58)	-3.44 (-23.32 to 16.45) p=0.73
No	134	40.80 (29.96)		134	39..36 (31.36)		134	33.11 (46.91)	

[a] Pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 58 Appendix V - Sensitivity analysis of the association between participants' insulin levels at the end of the follow up and 1) menopausal status at diagnosis and 2) change in menopausal status after breast cancer diagnosis, using three hypothetical values for insulin levels whose magnitude was below the limit the assay could detect

	Insulin (value: 13.88 pmol/l[a])			**Insulin (value: 7 pmol/l[a])**			**Insulin (value: 1 pmol/l[a])**		
	n	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Menopausal status at diagnosis:									
Pre + peri menopausal	36	37.48 (30.79)	-4.35 (-15.95 to 7.24) p=0.46	36	37.64 (32.33)	-4.72 (-16.83 to 7.41) p=0.44	36	34.26 (33.53)	-5.02 (-17.65 to 7.61) p=0.43
Postmenopausal	111	41.83 (30.59)		111	40.47 (31.94)		111	39.28 (33.26)	
Menopausal status change:									
Yes	35	38.15 (30.77)	-3.43 (-15.15 to 8.28) p=0.56	35	36.57 (32.33)	-3.59 (-15.84 to 8.65) p=0.56	35	35.01 (33.52)	-3.73 (-16.50 to 9.04) p=0.56
No	112	41.58 (30.56)		112	40.16 (31.94)		112	38.94 (33.31)	

[a] Pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 59 Appendix V - Sensitivity analysis of the association between participants' insulin levels at the end of the follow up and genetic profile, using three hypothetical values for insulin levels whose magnitude was below the limit the assay could detect

	Insulin (value: 13.88 pmol/l[a])			**Insulin (value: 7 pmol/l[a])**			**Insulin (value: 1 pmol/l[a])**		
	n	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
FTO:									
AA + AT	41	43.18 (27.64)	9.38 (-4.47 to 23.24) p=0.18	41	43.01 (27.84)	9.94 (-4.12 to 24.00) p=0.17	41	42.87 (28.05)	10.42 (-3.85 to 24.77) p=0.15
TT	19	33.80 (17.50)		19	33.07 (18.48)		19	32.32 (19.49)	
Mc4R:									
CC +CT	27	43.05 (27.54)	5.16 (-7.91 to 18.26) p=0.43	27	42.80 (27.85)	5.33 (-7.97 to 18.63) p=0.43	27	42.58 (28.17)	5.47 (-8.04 to 18.99) p=0.42
TT	33	37.88 (23.11)		33	37.46 (23.61)		33	37.10 (24.14)	

[a] Pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval.
[b] Independent sample t-test. Equal variances assumed.

Table 60 Appendix V - Sensitivity analysis of the association between participants' insulin levels at the end of the follow up and biological, behavioural and tumour-related variables, using three hypothetical values for insulin levels whose magnitude was below the limit the assay could detect

	Insulin (value: 13.88 pmol/l[a])			Insulin (value: 7 pmol/l[a])			Insulin (value: 1 pmol/l[a])		
	n	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**	**n**	**Mean (pmol/l[a]) (SD[a])**	**Mean difference, (95% CI[a]), p-value[b]**
Smoking status at diagnosis:									
Smoker	13	37.65 (30.91)	-3.23 (-20.84 to 14.38) p=0.72	13	35.53 (32.79)	-3.95 (-22.36 to 14.46) p=0.67	13	33.68 (34.61)	-4.58 (-23.77 to 14.61) p=0.64
Non-smoker	133	40.88 (30.64)		133	39.48 (31.48)		133	38.37 (33.29)	
	n	**Correlation coefficient[c]**	**p-value**	**n**	**Correlation coefficient[c]**	**p-value**	**n**	**Correlation coefficient[c]**	**p-value**
Time since diagnosis to the end of the follow up (months)	146	r=-0.20	p=0.01	146	r=-0.21	p=0.01	146	r=-0.21	p=0.01
Weight at diagnosis (kg[a])	133	r=0.34	p<0.01	133	r=0.34	p<0.01	133	r=0.34	p<0.01

Weight at the end of the follow up (kg[a])	147	r=0.33	p<0.01	147	r=0.33	p<0.01	147	r=0.33	p<0.01
Weight change from diagnosis to the end of the follow up (kg[a])	133	r=-0.06	p=0.46	133	r=-0.07	p=0.44	133	r=-0.07	p=0.43
Age at diagnosis (years)	141	r=0.09	p=0.27	141	r=0.10	p=0.25	141	r=0.10	p=0.25
Age at the end of the follow up (years)	142	r=0.05	p=0.55	142	r=0.05	p=0.54	142	r=0.05	p=0.54
Tumour size (cm[a])	143	r=-0.03	p=0.68	143	r=-0.04	p=0.67	143	r=-0.04	p=0.67
	n	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVA[a]**	**n**	**Mean Weight change (kg[a]) (SD[a])**	**One-way ANOVaA**
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 66 53 22	 43.73 (31.87) 37.90 (26.73) 41.28 (36.66)	 F(2, 138) F=0.52 p=0.59	 66 53 22	 42.59 (33.06) 36.35 (28.28) 39.71 (37.98)	 F (2, 138) F=0.55 p=0.56	 66 53 22	 41.59 (34.20) 34.99 (29.79) 38.35 (39.28)	 F(2, 138) F=0.57 p=0.56

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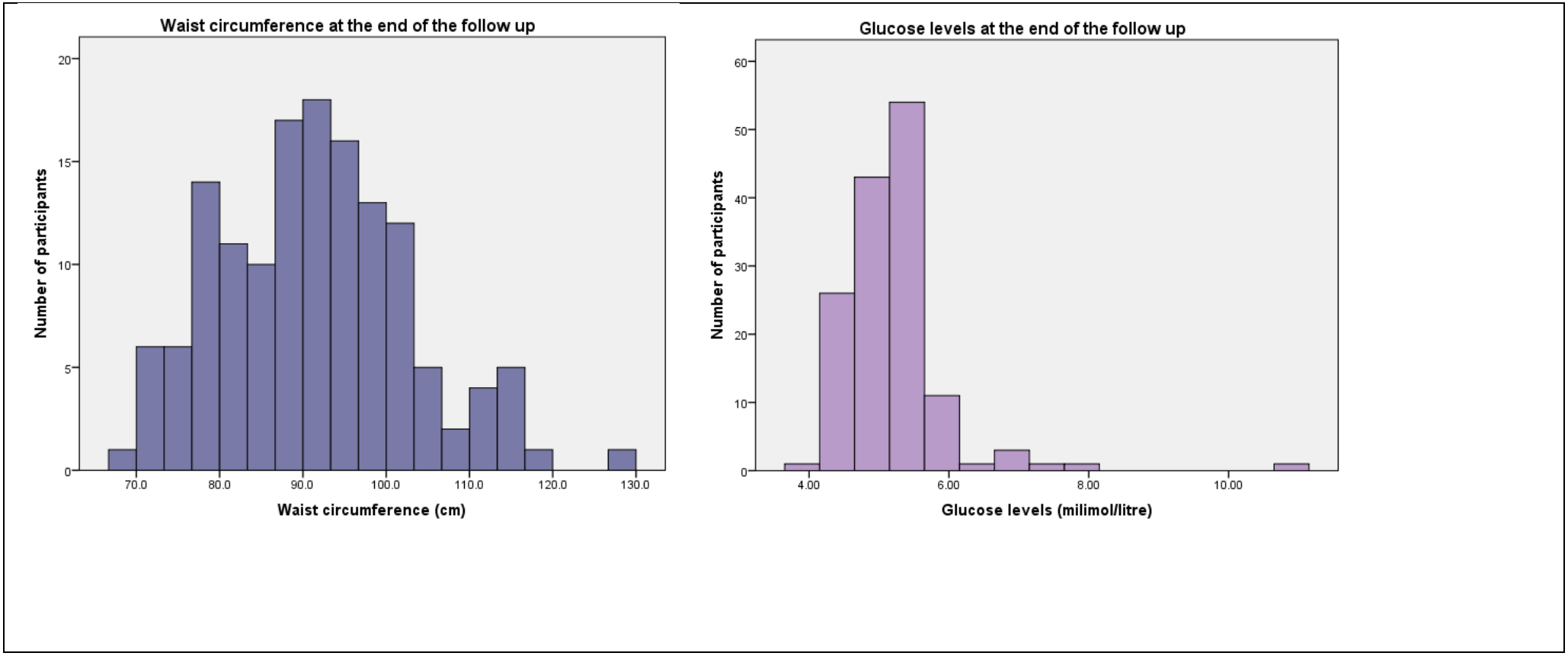
[a] Pmol/l stands for picomole per litre, SD stands for standard deviation, CI stands for confidence interval, kg stands for kilograms, cm stands for centimetres, ANOVA stands for analysis of the variance.

[b] Independent sample t-test. Equal variances assumed.

[c] Pearson's product moment correlation test.

Figure 7 Appendix V - Histograms representing the distribution of waist circumference, glucose and insulin levels at the end of the follow up



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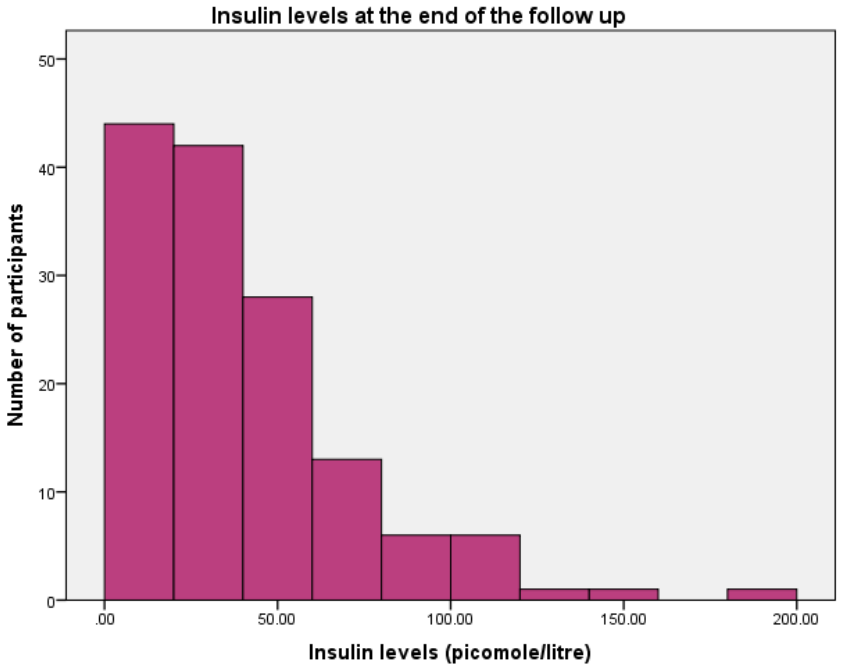
Insulin levels at the end of the follow up
Number of participants
50
40
30
20
10
0
.00
50.00
100.00
150.00
200.00
Insulin levels (picomole/litre)

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Table 61 Appendix V - Non-parametric univariable analysis on the association between participants' body waist circumference at the end of the follow up and breast cancer treatment

	Waist circumference		
	n	**Median (cm[b])**	**Mann-Whitney U test**
Chemotherapy[a]:			
Yes	89	91.00	U=3,406.50 Z=-1.84 p=0.07
No	91	95.00	
Hormone therapy[a]:			
Yes	157	92.50	U=1,193.00 Z=-2.35 p=0.02
No	22	84.50	
Tamoxifen[a]:			
Yes	101	92.00	U=3,749.50 Z=-0.55 p=0.58
No	78	92.00	
Aromatase inhibitors[a]:			
Yes	123	93.00	U=2,827.50 Z=-1.92 p=0.055
No	56	90.35	

Anastrozole[a]: **Yes** **No**	 82 97	 94.60 90.70	 U=3,283.50 Z=-2.01 p=0.04
Letrozole[a]: **Yes** **No**	 14 165	 95.00 92.00	 U=961.50 Z=-1.04 p=0.30
Exemestane[a]: **Yes** **No**	 40 139	 92.50 92.00	 U=2,737.00 Z=-0.15 p=0.88
Gonadorelin analogues[a]: **Yes** **No**	 12 167	 92.50 92.00	 U=960.50 Z=-0.24 p=0.81

[a] All participants had surgery and they might have also received other treatments (i.e. radiotherapy, biological therapy).
[b] Cm stands for centimetres.

Table 62 Appendix V - Non-parametric univariable analysis on the association between participants' waist circumference at the end of the follow up and 1) menopausal status at diagnosis and 2) change in menopausal status after breast cancer diagnosis

	Waist circumference		
	n	**Median (cm[a])**	**Mann-Whitney U test**
Menopausal status at diagnosis:			
Pre + perimenopausal	42	89.50	U=2,257.00 Z=-2.17
Postmenopausal	138	93.50	p=0.03
Menopausal status change:			
Yes	39	90.00	U=2,236.50 Z=-1.78
No	141	93.00	p=0.07

[a] Cm stands for centimetres.

Table 63 Appendix V - Non-parametric univariable analysis on the association between participants' waist circumference at the end of the follow up and genetic profile

	Waist circumference		
	n	**Median (cm[a])**	**Mann-Whitney U test**
FTO:			
AA + AT	45	92.25	U=396.50 Z=-1.05
TT	21	92.00	p=0.29
Mc4R:			
CC +CT	30	92.25	U=493.00 Z=-0.60
TT	36	88.25	p=0.54

[a] Cm stands for centimetres.

Table 64 Appendix V - Non-parametric univariable analysis on the association between participants' waist circumference at the end of the follow up and biological, behavioural and tumour-related variables

	Waist circumference		
	n	**Median (cm[a])**	**Mann-Whitney U test**
Smoking status at diagnosis: **Smoker** **Non-smoker**	 16 163	 95.00 92.00	 U=1,570.00 Z=-0.66 p=0.51
	n	**Correlation coefficient[b]**	**p-value**
Time since diagnosis to the end of the follow up	180	r=-0.03	p=0.70
Weight at diagnosis (kg[a])	180	r=0.74	p<0.01
Weight at the end of follow up (kg[a])	180	r=0.78	p<0.01
Weight change from diagnosis to the end of the follow up (kg[a])	180	r=0.17	p=0.03
Age at diagnosis (years)	180	r=0.19	p=0.01
Age at the end of the follow up (years)	180	r=0.17	p=0.02
Tumour size (cm[a])	180	r=-0.06	p=0.45

	n	Kruskal Wallis Test	p-value
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 81 69 25	χ^2=2.10 d.f.[b]=2	p=0.35

[a] Kg stands for kilograms, cm stands for centimetres and d.f. stands for degrees of freedom.
[b] Spearman's correlation rho.

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Table 65 Appendix V - Non-parametric univariable analysis on the association between participants' glucose and insulin levels at study entry, and breast cancer treatment

		Glucose			Insulin	
	n	**Median (mmol/l[b])**	**Mann-Whitney U test**	**n**	**Median (pmol/l[b])**	**Mann-Whitney U test**
Chemotherapy[a]:						
Yes	82	5.00	U=2,689.50 Z=-2.55	71	26.60	U=2,057.00 Z=-2.49
No	85	5.20	p=0.01	76	40.31	p=0.01
Hormone therapy[a]:						
Yes	144	5.10	U=1,260.00 Z=-1.55	130	31.46	U=976.50 Z=-0.40
No	22	4.90	p=0.12	16	27.95	p=0.69
Tamoxifen[a]:						
Yes	93	5.20	U=3,037.00 Z=-1.16	83	27.78	U=2,387.00 Z=-0.90
No	73	5.10	p=0.24	63	36.25	p=0.37
Aromatase inhibitors[a]:						
Yes	112	5.10	U=2,819.00 Z=-0.708	103	33.82	U=2,054.00 Z=-0.69
No	54	5.05	p=0.48	43	24.72	p=0.49

Anastrozole[a]:						
Yes	73	5.10	U=3,392.50 Z=-0.01	66	38.27	U=2,227.00 Z=-1.63
No	93	5.10	p=0.99	80	26.56	p=0.10
Letrozole[a]:						
Yes	12	5.20	U=682.50 Z=-1.51	11	47.85	U=469.50 Z=-2.03
No	154	5.10	p=0.13	135	29.52	p=0.04
Exemestane[a]:						
Yes	38	5.15	U=2,156.50 Z=-1.06	37	21.39	U=1,500.00 Z=-2.33
No	128	5.10	p=0.29	109	34.86	p=0.02
Gonadorelin analogues[a]:						
Yes	12	5.00	U=679.00 Z=-1.531	12	21.18	U=682.50 Z=-0.87
No	154	5.10	p=0.13	134	32.36	p=0.38

[a] All participants had surgery and they might have also received other treatments (i.e. radiotherapy, biological therapy).
[b] Mmol/l stands for milimol per litre and pmol/l stands for picomole per litre.

Table 66 Appendix V - Non-parametric univariable analysis on the association between participants' glucose and insulin levels at study entry and 1) menopausal status at diagnosis and 2) change in menopausal status after breast cancer diagnosis

	Glucose			Insulin		
	n	**Median (mmol/l[a])**	**Mann-Whitney U test**	**n**	**Median (pmol/l[a])**	**Mann-Whitney U test**
Menopausal status at diagnosis:						
Pre + perimenopausal	41	5.00	U=1,954.00 Z=-2.34	36	25.90	U=1,754.00 Z=-1.10
Postmenopausal	126	5.20	p=0.02	111	34.86	p=0.27
Menopausal status change:						
Yes	38	5.00	U=2,019.00 Z=-1.66	35	26.60	U=1,774.00 Z=-0.85
No	129	5.10	p=0.09	112	34.48	p=0.39

[a] Mmol/l stands for milimol per litre and pmol/l stands for picomole per litre.

Table 67 Appendix V - Non-parametric univariable analysis on the association between participants' glucose and insulin levels at study entry, and genetic profile

	Glucose			Insulin		
	n	**Median (mmol/l[a])**	**Mann-Whitney U test**	**n**	**Median (pmol/l[a])**	**Mann-Whitney U test**
FTO:						
AA + AT	43	5.10	U=364.00 Z=-0.68	41	35.00	U=328.00 Z=-0.98
TT	19	5.00	p=0.49	19	28.75	p=0.33
Mc4R:						
CC +CT	29	5.10	U=446.00 Z=-0.46	27	34.10	U=382.00 Z=-0.94
TT	33	5.00	p=0.65	33	35.00	p=0.34

[a] Mmol/l stands for milimol per litre and pmol/l stands for picomole per litre.

Table 68 Appendix V - Non-parametric univariable analysis on the association between participants' glucose and insulin levels at study entry, and biological, behavioural and tumour-related variables

	Glucose			Insulin		
	n	**Median (mmol/l[a])**	**Mann-Whitney U test**	**n**	**Median (pmol/l[a])**	**Mann-Whitney U test**
Smoking status at diagnosis:						
Smoker	16	5.20	U=1,084.00 Z=-0.64	13	33.68	U=777.00 Z=-0.60
Non-smoker	150	5.10	p=0.52	133	31.32	p=0.55
	n	**Correlation coefficient[a]**	**p-value**	**n**	**Correlation coefficient[b]**	**p-value**
Time since diagnosis to the end of the follow up (months)	166	r=-0.02	p=0.77	146	r=-0.19	p=0.02
Weight at diagnosis (kg[a])	153	r=0.29	p<0.01	133	r=0.37	p<0.01
Weight at the end of follow up (kg[a])	167	r=0.30	p<0.01	147	r=0.38	p<0.01
Weight change from diagnosis to the end of the follow up (kg[a])	153	r=0.05	p=0.55	133	r=-0.07	p=0.42
Age at diagnosis (years)	160	r=0.12	p=0.12	141	r=0.13	p=0.12
Age at the end of the follow up (years)	161	r=0.13	p=0.12	142	r=0.09	p=0.28

Tumour size (cm[a])	163	r=-0.06	p=0.41	143	r=-0.01	p=0.87
	n	**Kruskal Wallis Test**	**p-value**	**n**	**Kruskal Wallis Test**	**p-value**
Stage of disease: **Stage 1** **Stage 2** **Stage 3**	 74 63 24	 χ^2=0.34 d.f.[b]=2	 p=0.84	 66 53 22	 χ^2=1.66 d.f.=2	 p=0.44

[a] Mmol/l stands for milimol per litre, pmol/l stands for picomole per litre, kg stands for kilograms, cm stands for centimetres, d.f. stands for degrees of freedom.
[b] Spearman's correlation rho.

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Table 69 Appendix V - Multiple linear regression analysis of participants' body fat percentage at the end of the follow up, after excluding an outlier

Variables included	**n**	**Unstandardised coefficients (95% CI[a])**	**p-value**	**Correlation coefficient ANOVA[a] coefficients**
Constant	192	6.87 (2.30 to 11.43)	p<0.01	R=0.75 F (2, 189) F=119.79 p<0.01
Weight at diagnosis (kg[a])		0.34 (0.30 to 0.39)	p<0.01	
Age at diagnosis (years)		0.11 (0.05 to 0.17)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

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Table 70 Appendix V - Multiple linear regression analysis of participants' fat mass/fat free mass ratio, after excluding two outliers

Variables included	**n**	**Unstandardised coefficients (95% CI[a])**	**p-value**	**Correlation coefficient ANOVA[a] coefficients**
Constant	184	-0.15 (-0.273 to -0.042)	p<0.01	R=0.76 F (2, 181) F=127.92 p<0.01
Weight at diagnosis (kg[a])		0.01 (0.008 to 0.013)	p<0.01	
Age at diagnosis (years)		0.002 (0.001 to 0.004)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

Table 71 Appendix V - Multiple linear regression analysis of participants' waist circumference at the end of the follow up, after excluding one outlier

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	157	31.68 (22.39 to 40.97)	p<0.01	R=0.77 F (2, 154) F=115.66 p<0.01
Weight at diagnosis (kg[a])		0.70 (0.60 to 0.79)	p<0.01	
Age at diagnosis (years)		0.18 (0.07 to 0.30)	p<0.01	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.

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Table 72 Appendix V - Multiple linear regression analysis of participants' glucose level at the end of the follow up, including outliers

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	153	4.67 (3.54 to 5.87)	p<0.01	R=0.37 F (2, 150) F=12.38 p<0.01
Weight at diagnosis (kg[a])		0.02 (0.01 to 0.29)	p<0.01	
Letrozole[b]		-0.49 (-0.93 to -0.05)	p=0.03	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non-users.

Table 73 Appendix V - Multiple linear regression analysis of participants' glucose level at the end of the follow up, after excluding two outliers

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	151	4.22 (3.342 to 5.097)	p<0.01	R=0.32 F (2, 158) F=8.54 p<0.01
Weight at diagnosis (kg[a])		0.01 (0.008 to 0.022)	p<0.01	
Letrozole[b]		-0.10 (-0.456 to 0.249)	p=0.56	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non-users.

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Table 74 Appendix V - Multiple linear regression analysis of participants' insulin level at the end of the follow up, excluding one outlier

Variables included	n	Unstandardised coefficients (95% CI[a])	p-value	Correlation coefficient ANOVA[a] coefficients
Constant	55	-10.73 (-39.70 to 18.23)	p=0.46	R=0.57 F (2, 52) F=12.67 p<0.01
Weight at diagnosis (kg[a])		0.94 (0.54 to 1.33)	p<0.01	
FTO[b]		-12.47 (-23.18 to -1.76)	p=0.02	

[a] Kg stands for kilograms, CI stands for confidence interval, ANOVA stands for analysis of the variance.
[b] Categories: 0) users, 1) non-users.

APPENDIX VI: PARTICIPANTS INFORMATION SHEET, CONSENT FORM AND DECLINE SLIP

Appendix VI. Introduction

This appendix contains a copy the information letter received by participants, as well as a copy of the consent form, and the decline sip.

Appendix VI.A. Participants information sheet

PARTICIPANT INFORMATION SHEET

LREC Number: 08/H0201/35

Version 2

Date: 28th April 2008

Title of the research project: Excess weight gain among breast cancer patients.

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Take time to decide whether or not you wish to take part.

PART ONE

What is the purpose of the study?

Many women with breast cancer change their weight after the diagnosis. It has been suggested that this weight change depends on the type of the tumour, the treatment received, and whether or not the woman has gone through her menopause. Furthermore, weight change might also be related to the presence of some genes.

Our study aims to determine weight changes after breast cancer diagnosis. We will also investigate whether weight change is related to the tumour, the treatment, the menopausal status and some genes.

This study is being used in part towards a PhD qualification for one member of the team (Ana Maria Barberia).

Why have I been chosen?

We are interested in breast cancer patients who have been diagnosed more than a year ago.

Participation in the study is entirely voluntary. You are completely free to decide whether you want to participate. Your decision will not determine the quality of care that you are receiving.

What will happen to me if I take part?

Should you decide to take part, you will be asked to complete and send to us the consent form, detailing a contact telephone number. Once we receive it, a member of the research team (Ana Maria Barberia) will contact you, at the time and date you indicate us in the consent form, to confirm that you want to participate in the study, to answer possible questions that you might want to ask us in relation to the study, and to arrange the date and time for data collection.

Data for the study will be collected by phone, with a blood test and from your medical notes. Ana Maria Barberia will contact you by phone at an agreed time, and will ask you basic relevant questions about your education, ethnic origin, past medical history, medication, whether you smoke, and whether you have been through the menopause yet.

She will also invite you to book an appointment to come to the hospital to give us a blood sample, so we can assess the presence of some genes and whether these are related to weight change after breast cancer diagnosis.

Finally, we will need to obtain further data relevant to this study, such as diagnosis, treatment received or weight. We can collect this information directly from your hospital medical notes. However, it is possible that we might need to phone you again to confirm or complete some of this information.

Your normal treatment and/or medication will not be withheld during the study. We will refund your travelling expenses when you come to the hospital for this blood sample test.

If you want, you can contact us to get the results of data that we collect from you, and to get a summary of the research findings.

Are there any risks associated with taking part?

The only risk from taking part in this study is the risk associated with a normal blood test. You might develop an infection in the place where the blood sample is being collected that could lead to lymphoedema.

Lymphoedema is a swelling that occurs when the amount of fluid in an area (e.g. your arm) is greater than the capacity of the lymphatic system to transport it away. Lymphoedema can occur after surgery, when lymph nodes are removed after treatment for breast cancer, and after radiotherapy, as it might cause scar tissue that interrupts the normal activity of the lymphatic system. An infection also could damage the lymph vessels leading to lymphoedema.

A study has found that the risk of developing lymphoedema is 2.4 times higher in those who have venepuncture than in those who do not have it. This risk should be known by the phlebotomist (health professional specialised in taking blood samples), who will be requested to collect the blood sample from the arm opposite to the affected breast

What are the possible benefits of taking part?

We cannot promise the study will help you but you might find comfort by knowing that the information we get from this study will help improve the treatment and care of women with breast cancer in the future.

Will my taking part in the study be kept confidential?

Yes, all information which is collected about you during the course of this research will be kept strictly confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

What will happen if I do not want to carry on with the study?

You are free to withdraw at any time, without giving a reason. You will just need to let us know. This would not affect the standards of care you receive. If you withdraw from the study, we will not collect further data, but we may need to use data collected up to your withdrawal, with your permission.

In addition, we would like to let you know that with your permission, we intend to store your blood samples in order for us or other researchers to conduct further medical research to look at some genes and proteins that could be related to breast cancer. It is likely that results from these further studies will not have a predictive value or clinical implication for you, but they will contribute to our understanding of the biological causes of breast cancer. Ethical approval and your consent will be sought before conducting any further research using your donated blood sample. No test of known clinical value for diagnosis or predicting disease on a sample that can be linked to you will be done without your consent. It is intended that blood samples collected will be stored in the NHS trust and later transferred to a long term research tissue bank, where the samples will be kept anonymous but linked to the donor by assigning them a unique code. The samples will be stored for a minimum period of five years and while it has a potential use. Afterwards, they will be disposed according to existing policy.

Will my taking part in this study be kept confidential?

Yes, all information that is collected about you during the course of the research will be kept strictly confidential. This information will be safely stored at the hospital and only the research team will have access to it. A back up copy of your data will be stored anonymously in other computers used for data analysis.

Our procedures for handling, processing, storing and destroying data relating to your participation in the study are compliant with the Data Protection Act 1998. With your

consent we will inform your GP of your participation in our study. However, we will not share with them data about you that we obtain from your participation in the research.

In the event that you are unable to confirm that you still wish to participate in this study, we will not collect more data from you. Nonetheless, any identifiable data already collected will be used for the purpose of this study.

What will happen to the results of the research study?

Once the study is completed we intend to publish the results for other researchers to read. You will not be identified in any report / publication.

Who is organising and funding the research?

The researchers are a group of doctors and a research student from Royal Bournemouth Hospital, Poole Hospital and Bournemouth University who have an interest in the topic being explored.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people called Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favourable opinion by the Dorset Research Ethics Committee, and by the School of Health and Social Care Research Committee at Bournemouth University. A Patients' Partnership Panel has also provided positive feedback about this study.

Together with this letter, you will find a consent form, a decline form and a pre-paid envelope. If you decide to take part in the study, please tick the boxes in the informed consent, sign it and send it to us in the envelope provided. Once we receive it, we will contact you by phone at the time and date you indicate us, to agree a time and date for data collection.

If you do not want to participate, please complete the decline form and send it to us.

Ask us if there is anything that is not clear or if you would like more information and we will be happy to answer your questions. Take time to decide whether or not you wish to take part.

What if something goes wrong?

There is only a small risk associated to this study: having a blood test.

However, if any negligent harm occurs to you because of the management, the design, or the conduct of this study, you will be covered by the NHS liability insurance.

Dr Hickish

Tel. 01202 704789

Ana Maria Barberia

Tel. 075 074 02595

Thank you very much for reading this information and considering taking part in this study.

If you need independent information or advice about your rights as a research subject or about being involved in this particular research study you can ask other members of the Oncology or Surgery team, or you can contact the local NHS Patient Advisory Liaison Service (PALS) and the local NHS Research &Development office.

PALS:

Royal Bournemouth Hospital: tel. 01202 704886

Poole Hospital: tel. 01202 448499

R&D office:

Poole Hospital: tel. 01202 665511 (Ext 8489)

Appendix VI.B. Consent form

CONSENT FORM

LREC Number: 08/H0201/35

Version 2

Date: 28th April 2008

Title of the research project: Excess weight gain among breast cancer patients.

Research Team: Prof. Hickish, Prof. Kerr, Prof. Thomas, Dr. Begley, Mr. Skene, Mr. Perry, Mr. Pain, Miss Evans, Ana M. Barberia

This consent form has two parts. Part 1 refers to the study cited above, detailed in the Patient Information Sheet. Part 2 refers to additional research studies that might be conducted in the future. Please, read the following lines and initial the box when appropriate. If you have any questions, do not hesitate to contact us.

Part 1

1. I confirm that I have read and understand the information sheet dated 28th April 2008, version 2, for the above study which is part of an educational project. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that

I am free to withdraw at any time without giving any reason, without my medical or legal rights being affected. ☐

3. I understand and consent that relevant sections of my medical notes and data collected during the study will be stored and kept confidential for the purpose of this study. ☐

4. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the research team. I give my permission for these individuals to have access to my records. ☐

5. I understand and consent that any identifiable data/tissue collected during the study will be retained and used for the purpose of this study in the event of me loosing capacity to consent. ☐

6. I understand and consent that if I decide to withdraw from the study, data already collected up to my withdrawal might be used for the purpose of this study. ☐

7. I agree to my GP being informed of my participation in the study. ☐

8. I agree to take part in the above study. ☐

Part 2

9. I confirm that I consent that researchers store my blood sample to be used in further medical research projects which would have to be approved by a properly constituted research ethics committee, and that I will be asked for consent before my blood sample will be used. ☐

Please, complete the following information and sign the consent

Telephone number at which we can contact you:

Most convenient time and dates to contact you at this number

Days of the week:

Time:

Your Name: Date: Your Signature:

Please return these two pages to us in the envelope provided and we will contact you.

For us to complete:

Person taking consent : Date: Signature:

DECLINE SLIP

LREC Number: 08/H0201/35

Version 1

Date: 26th January 2008

Title of the research project: Excess weight gain among breast cancer patients

If you don't wish to participate in this research project, please complete and return this decline slip.

This is to confirm that I do not wish to participate in the study titled "Excess weight gain among breast cancer patients".

_____	_____	_____
Name	Date	Signature

To help us plan research projects in the future it would be helpful if you could tell us why you do not want to participate.

The reason is (optional):

GLOSSARY

Term	Definition
Abdominal area	The area of the body between the thorax and the pelvis with the exception of the back—called also belly.
Adiponectin	A substance secreted by the adipose tissue that increases insulin sensitivity, helping cells process sugar more effectively.
Adipose tissue	Tissue in which fat is stored.
Adjusted R^2	It indicates how well terms fit a curve or line, but adjusts for the number of terms in a model.
Aetiology	The cause or causes of a disease or abnormal condition.
Agonist	A chemical substance (as a drug) capable of combining with a receptor on a cell and initiating the same reaction or activity typically produced by the binding of an endogenous substance.
All-cause mortality	All of the deaths that occur in a population, regardless of the cause.
Allele	Any of the alternative forms of a gene that may occur at a given locus.
Alpha-melanocyte-stimulating hormone	A group of polypeptide hormones derived from prepro-opiomelanocorticotropin. It stimulates production and release of melanin by melanocytes in the skin and hair and in the brain; affects the appetite and sexual arousal.
Amenorrhea	Abnormal absence or suppression of menstruation.
Anaemia	A condition in which the blood is deficient in red blood cells, in haemoglobin, or in total volume.
Anaesthesia	The branch of medicine concerned with administration of anaesthetics (a drug or agent used to abolish the sensation of pain, to achieve adequate muscle relaxation during surgery, to calm fear and allay anxiety, and to produce amnesia for the event).

Analysis of the variance (ANOVA)	The separation of variance attributable to one variable from the variance attributable to others. By partitioning the total variance of a set of observations into parts due to particular factors, for example, sex, treatment group etc., and comparing variances (mean squares) by way of F-tests, differences between means can be assessed. The simplest analysis of this type involves a one-way design, in which n subjects are allocated, usually at random, to the k different levels of a single factor.
Angiogenesis	The formation and differentiation of blood vessels.
Anorexia nervosa	A serious eating disorder that is characterized especially by a pathological fear of weight gain leading to faulty eating patterns, malnutrition, and usually excessive weight loss.
Anthracycline	A class of antineoplastic drugs (as doxorubicin and epirubicin) used for inhibiting or preventing the growth and spread of neoplasms or malignant cells.
Apoptosis	The cessation of all physical and chemical processes that invariably occurs in all living organisms.
Appetite	The desire to eat.
Area under the receiver operating characteristic curve (AUC)	Often a useful way of summarizing the information from a series of measurements made on an individual over time. Often a predictor of biological effects such as toxicity or efficacy.
Aromatase	An enzyme or complex of enzymes that promotes the conversion of an androgen (as testosterone) into oestrogens (as oestradiol).
Assays	An analysis (as of a drug) to determine the presence, absence, or quantity of one or more components.
Autocrine	Of, relating to, promoted by, or being a substance secreted by a cell and acting on surface receptors of the same cell.
Automated chemiluminescence	Electronic process that produce luminescence (as bioluminescence) due to chemical reaction usually at low temperatures.

Axilla	The cavity beneath the junction of the arm or anterior appendage and shoulder or shoulder girdle.
Basal metabolic rate	An expression of the rate at which oxygen is utilized in a fasting subject at complete rest as a percentage of a value established as normal for such a subject.
Bias	Any influence or action at any stage of a study that systematically distorts the findings.
Bioactive substances	Substances that have an effect on a living organism.
Bioelectrical Impedance Analysis	A method that determines the opposition to the flow of an electric current through body tissues which can then be used to calculate an estimate of total body water (TBW). TBW can be used to estimate fat-free body mass and, by difference with body weight, body fat.
Biopsy	The removal and examination of tissue, cells, or fluids from the living body.
Bivariate	The use of two responses or outcomes per observation.
Body composition	The relative proportions of protein, fat, water, and mineral components in the body.
Body Mass Index (BMI)	A measure of body fat that is the ratio of the weight of the body in kilograms to the square of its height in meters.
Bone marrow	A soft highly vascular modified connective tissue that occupies the cavities and cancellous part of most bones.
Bonferroni correction	A procedure for guarding against an increase in the probability of a type-one error (see below) when performing multiple significance tests.
Breast cancer specific mortality	A term used for death rate, or the number of deaths in a certain group of people in a certain period of time, attributed to a condition.
Breast cancer progression	The course of a disease, such as cancer, as it becomes worse or spreads in the body.

Breast cancer recurrence	Reappearance of a breast tumour after previous removal.
Breast cancer survival rate	The portion of people with the same type and stage of cancer are still alive a certain amount of time (usually 5 years) after they were diagnosed.
Breast second primary cancer	A term used to describe a new primary cancer that occurs in a person who has had cancer in the past.
Bulimia nervosa	A serious eating disorder characterized by compulsive overeating usually followed by self-induced vomiting or laxative or diuretic abuse, and is often accompanied by guilt and depression.
Caloric intake	The number of calories received orally and/or parenterally into the body.
Cardiology	The study of the heart and its action and diseases.
Cardiovascular disease	Any abnormal condition characterized by dysfunction of the heart and blood vessels.
Carcinogenesis	The production of cancer.
Categorical variables	Qualitative variables, variables that cannot meaningfully be expressed in numbers. i.e. skin colour.
Centrifuges	A machine using centrifugal force for separating substances of different densities, for removing moisture, or for simulating gravitational effects.
Cervix	The narrow lower or outer end of the uterus.
Chemical ovarian ablation	Chemotherapy to block ovarian hormonal activity.
Cholecystokinin	A hormone secreted especially by the duodenal mucosa that regulates the emptying of the gallbladder and secretion of enzymes by the pancreas.
Clinical trial	Medical experiments designed to evaluate which (if any) of two or more treatments is the more effective.
Coactivators	A type of nuclear proteins that participate in the potentiation of transcription of genes that are responsive to ligand-dependent transcription factors.

Coefficient of determination (R^2)	The square of the correlation coefficient between two variables. Gives the proportion of the variation in one variable that is accounted for by the other.
Colorectal cancer	Cancer relating to or affecting the colon and the rectum.
Comorbid	Existing simultaneously with and usually independently of another medical condition.
Complete-case data analysis	An analysis that uses only individuals who have a complete set of measurements. An individual with one or more missing values is not included in the analysis.
Computed axial tomography (CT)	A method of producing a three-dimensional image of an internal body structure by computerized combination of two-dimensional cross-sectional X-ray images.
Confidence intervals (CI)	A range of values, calculated from the sample observations that is believed, with a particular probability, to contain the true parameter value. A 95% confidence interval, for example, implies that were the estimation process repeated again and again, then 95% of the calculated intervals would be expected to contain the true parameter value.
Confounding variable	An extra variable not accounted for. They can ruin an experiment and give useless results.
Continuous variables	A measurement not restricted to particular values except in so far as this is constrained by the accuracy of the measuring instrument. For such a variable equal sized differences on different parts of the scale are equivalent.
Convergence	The property or manner of approaching a limit, such as a point, line, function, or value.
Cook's distance	An influence statistic designed to measure the shift in the estimated parameter vector, ^β, from fitting a regression model when a particular observation is omitted. It is a combined measure of the impact of that observation on all regression coefficients.
Corepressors	A small molecule that activates a particular genetic repressor by combining with it.

Correlation coefficient (r)	An index that quantifies the linear relationship between a pair of variables.
Covariance matrix	A matrix whose element in the i, j position is the covariance between the i^{th} and j^{th} elements of a random vector.
Cross-sectional data	Data collected by observing many subjects at the same point of time, or without regard to differences in time.
Cytology	The microscopic examination of cells obtained from the body for diagnostic purposes.
Cytotoxic	Toxic to cells.
Dementia	A usually progressive condition (as Alzheimer's disease) marked by the development of multiple cognitive deficits (as memory impairment, aphasia, and inability to plan and initiate complex behaviour).
Deoxyribonucleic acid (DNA)	A nucleic acid of complex molecular structure occurring in cell nuclei as the basic structure of the genes.
Deviance	A measure of the extent to which a particular model differs from the saturated model for a data.
Dexamethasone	A synthetic glucocorticoid also used in the form of its acetate or sodium phosphate especially as an anti-inflammatory and antiallergic agent.
Diabetes	Type-one diabetes is a diabetes of a form that is characterized by a severe deficiency of insulin secretion resulting from atrophy of the islets of Langerhans and causing hyperglycemia and a marked tendency toward ketoacidosis. Type-two diabetes is a diabetes of a common form that develops especially in adults and most often in obese individuals and that is characterized by hyperglycemia resulting from impaired insulin utilization coupled with the body's inability to compensate with increased insulin production.
Dichotonomous variable	Observations which occur in one of two possible states, these often being labelled 0 and 1.
Dispersion	The amount by which a set of observations deviate from their mean.

Ductal carcinoma	A type of tumour that primarily presents in the ducts of a gland (i.e. mammary gland).
Dummy variable	The variables resulting from recoding categorical variables with more than two categories into a series of dichotonomous variables.
Durbin-Watson test	A test that the residuals from a linear regression or multiple regression are independent.
Effect size	A measure of the strength of a phenomenon.
Electrode	Is an electrical conductor used to make contact with a non-metallic part of a circuit.
Endocrine	Producing secretions that are distributed in the body by way of the bloodstream.
Endogenous	Relating to or produced by metabolic synthesis in the body.
Fat mass	That portion of the human body that is composed strictly of fat (as opposed to fat-free mass).
Fat free mass	The lean body mass plus the skeletal mass.
Fatty acid	Any of the saturated or unsaturated organic acids that have a single carboxyl group and usually an even number of carbon atoms and that occur naturally in the form of glycerides in fats and fatty oils.
Fenretinide	A synthetic vitamin A derivative that exhibits apoptotic and anti-invasive properties.
Fixed effects	The effects attributable to a finite set of levels of a factor that are of specific interest.
F-test	Test for the equality of the variances of two populations having normal distributions, based on the ratio of the variances of a sample of observations taken from each. Most often encountered in the analysis of variance (ANOVA).
Gastro-intestinal system	The system that makes food absorbable into the body.
Genes	A specific sequence of nucleotides in DNA or RNA that is located usually on a chromosome and that is the functional unit of inheritance

	controlling the transmission and expression of one or more traits by specifying the structure of a particular polypeptide and especially a protein or controlling the function of other genetic material.
Genotype	All or part of the genetic constitution of an individual or group.
Ghrelin	A 28-amino-acid peptide hormone that is secreted primarily by stomach cells with lesser amounts secreted by other cells (as of the pancreas) and acts to stimulate appetite and the secretion of growth hormone.
Glycosylation	The reaction in which a carbohydrate is attached to a hydroxyl or other functional group of another molecule
Glucose	An optically active sugar $C_6H_{12}O_6$ that has an aldehydic carbonyl group.
Glucose intolerance	Inability to properly metabolize glucose.
Glucose metabolism	The process by which simple sugars found in many foods are processed and used to produce energy.
Growth factor	A substance that promotes growth and especially cellular growth.
Haemolysed sample (Haemolysis)	Alteration, dissolution, or destruction of red blood cells in such a manner that haemoglobin is liberated into the medium in which the cells are suspended.
Hazard ratio (HR)	The ratio of the hazard rates corresponding to the conditions described by two levels of an explanatory variable.
Histopathology	A branch of pathology concerned with the tissue changes characteristic of disease.
Holm–Bonferroni method	It is a way to deal with family wise error rates (FWER) for multiple hypothesis tests. It is a modification of the Bonferroni correction. The Holm-Bonferroni method is fairly simple to calculate, but it is more powerful than the single-step Bonferroni.
Homeostasis	The tendency of biological systems to maintain relatively constant conditions in the internal environment while continuously interacting with

	and adjusting to changes originating within or outside the system.
Homeostasis Model Assessment (HOMA)	It estimates steady state beta cell function (%B), and insulin sensitivity (%S), as percentages of a normal reference population.
Homoscedasticity	A condition when all random variables in the sequence or vector have the same finite variance.
Homozygous	Having the two genes at corresponding loci on homologous chromosomes identical for one or more loci.
Hormone replacement therapy	The administration of oestrogen often along with a synthetic progestin especially to ameliorate the symptoms of menopause and reduce the risk of postmenopausal osteoporosis.
Hot-flushes	Waves of sudden widespread relaxation of the muscle walls of skin blood vessels so that they dilate and cause the skin to become warm.
Hypertriglyceridemia	The presence of an excess of triglycerides in the blood.
Hypothalamus	A basal part of the diencephalon that lies beneath the thalamus on each side, forms the floor of the third ventricle, and includes vital autonomic regulatory centres (as for the control of food intake).
Hypothesis testing	A general term for the procedure of assessing whether sample data is consistent or otherwise with statements made about the population.
Hysterectomy	Surgical removal of the uterus.
Iliac crest	The thick curved upper border of the ilium.
Impedance	The apparent opposition in an electrical circuit to the flow of an alternating current that is analogous to the actual electrical resistance to a direct current and that is the ratio of effective electromotive force to the effective current.
Immunoassay	A technique or test (as the enzyme-linked immunosorbent assay) used to detect the presence or quantity of a substance (as a protein) based on its capacity to act as an antigen or antibody.

Incidence	The rate of occurrence of new cases of a particular disease/outcome in a population being studied.
Independent sample t-test	A student ‘s t tests used for assessing hypotheses about population means when independent samples are available from each population.
Inference	The process of drawing conclusions about a population on the basis of measurements or observations made on a sample of units from the population.
Inflammatory bowel disease	Either of two inflammatory diseases of the bowel: a Crohn’s disease or Ulcerative colitis.
Insulin	A protein hormone that is synthesized in the pancreas from proinsulin and secreted by the beta cells of the islets of Langerhans, that is essential for the metabolism of carbohydrates, lipids, and proteins, that regulates blood sugar levels by facilitating the uptake of glucose into tissues, by promoting its conversion into glycogen, fatty acids, and triglycerides, and by reducing the release of glucose from the liver, and that when produced in insufficient quantities results in diabetes mellitus.
Insulin resistance	Reduced sensitivity to insulin by the body's insulin-dependent processes (as glucose uptake, lipolysis, and inhibition of glucose production by the liver) that results in decreased activity of these processes or an increase in insulin production or both and that is typical of type-two diabetes but often occurs in the absence of diabetes.
Insulin sensitivity	The systemic responsiveness to glucose.
Intercept	The parameter in an equation derived from a regression analysis corresponding to the expected value of the response variable when all the explanatory variables are zero.
Internal validity	The extent to which the effects detected in a study are truly caused by the treatment or exposure in the study sample, rather than being due to other biasing effects of extraneous variables.
Intra-class correlation	A descriptive statistic that describes how strongly units in the same group resemble each other.
Invasive carcinoma	A neoplasm in which collections of epithelial cells infiltrate or destroy the surrounding tissue.

Kruskal Wallis Test	A distribution free method that is the analogue of the analysis of variance of a one-way design. It tests whether the groups to be compared have the same population median.
Leptin	A peptide hormone that is produced by fat cells and plays a role in body weight regulation by acting on the hypothalamus to suppress appetite and burn fat stored in adipose tissue.
Leverage	A term used in regression analysis for those observations that have an extreme value on one or more explanatory variables. The effect of such points is to force the fitted model close to the observed value of the response leading to a small residual.
Ligand	A group, ion, or molecule coordinated to a central atom or molecule in a complex.
Linear function	A polynomial function of degree zero or one.
Linear regression	The statistical procedure in which a straight line is established through a data set that best represents a relationship between two subsets or two methods.
Linearity	Describing, described by, or related to a straight line.
Lipid catabolism	The breakdown processes that generate energy and primary metabolites from fatty acids.
Liver	A large very vascular glandular organ of vertebrates that secretes bile and causes important changes in many of the substances contained in the blood which passes through it (as by converting sugars into glycogen which it stores up until required and by forming urea).
Lymph nodes	Small oval or bean-shaped bodies situated in groups along the course of the lymph drainage vessels. They offer defence against the spread of infection by producing antibodies, and become involved in the spread of cancer.
Lymphoedema	An abnormal excess accumulation of serous fluid in connective tissue or in a serous cavity due to faulty lymphatic drainage.

Malabsorption syndrome	A syndrome resulting from a faulty absorption of nutrient materials from the digestive tract that is typically characterized by weakness, diarrhoea, muscle cramps, oedema, and loss of weight.
Mammary gland	An organ in female mammals that produces milk to feed young offspring. They are situated in the breasts.
Mann-Whitney U test	A distribution free test used as an alternative to the Student's t-test for assessing whether two populations have the same median. The test statistic U is calculated from comparing each pair of values, one from each group, scoring these pairs 1 or 0 depending on whether the first group observation is higher or lower than that from the second group and summing the resulting scores over all pairs.
Mastectomy	Surgical removal of all or part of the breast and sometimes associated lymph nodes and muscles.
Mean	An average; a number that in some sense represents the central value of a set of numbers.
Measurement error	The difference between the true value of something being measured and the value obtained by measurement. Measurement error can be the result of one or more of several different factors, including random error, and systematic error.
Melanin-concentrating hormone	A cyclic 19-residue orexigenic hypothalamic neuropeptide, which plays a key role in regulating feeding behaviour, mood, the sleep-wake cycle and energy balance. It also has an autocrine role in regulating beta-cell mass dynamics and pancreatic islet cell secretion. Overexpression of MCH is associated with obesity.
Menopause	The cessation of menstruation.
Menstrual period	A single cyclic occurrence of menstruation (a discharging of blood, secretions, and tissue debris from the uterus that recurs in non-pregnant human and other primate females of breeding age at approximately monthly intervals and that is considered to represent a readjustment of the uterus to the non-pregnant state).
Median	The value in a set of ranked observations that divides data into two parts of equal size.

Meta-analysis	Any systematic method that uses statistical analysis to integrate data from a number of independent studies.
Metabolic syndrome	A syndrome marked by the presence of usually three or more of a group of factors (as high blood pressure, abdominal obesity, high triglyceride levels, low HDL levels, and high fasting levels of blood sugar) that are linked to an increased risk of cardiovascular disease and type-two diabetes.
Metabolism	The sum of the processes by which a particular substance is handled in the living body.
Metastasis	The spread of a disease-producing agent (as cancer cells or bacteria) or disease from the initial or primary site of disease to another part of the body.
Mitogen-activated protein kinase (MAPK)	A highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival.
Missing data	Observations missing from a set of data for some reason.
Multicollinearity	The condition occurring when two or more of the independent variables in a regression equation are correlated.
Multilevel model	Regression models for multilevel or clustered data where units i are nested in clusters j, for instance a cross-sectional study where students are nested in schools or longitudinal studies where measurement occasions are nested in subjects.
Multiparity	The status of a mother of more than one child.
Multiple linear regressions	A term usually applied to models in which a continuous response variable, y, is regressed on a number of explanatory variables.
Multivariable analysis	Refers to statistical models in which there are multiple independent or explanatory variables.
Muscle	A body tissue consisting of long cells that contract when stimulated and produce motion.
Neutropenia	Leukopenia in which the decrease in white blood cells is chiefly in neutrophils.

Nicotine	The chief active principle of tobacco.
Non-melanoma skin cancer	A malignant growth of the external surface or epithelial layer of the skin.
Non-parametric tests	A test that does not assume anything about the underlying distribution.
Normal distribution	A symmetrical distribution of scores with the majority concentrated around the mean.
Normality	A term used to indicate that some variable of interest has a normal distribution.
Nuclear receptors	Any of a "superfamily" of soluble (non-membrane-bound) receptors for a constellation of physiologically active compounds (ligands). When nuclear receptors are activated by their cognate ligand, they form dimers, bind DNA and activate transcription of relevant primary target genes.
Null hypothesis	It is the commonly accepted fact; it is the opposite of the alternate hypothesis.
Odds ratio (OR)	A measure of association in a case-control study which quantifies the relationship between an exposure and health outcome from a comparative study.
Oesophagus	A muscular tube connecting the throat with the stomach.
Oestrogens	Any of various natural steroids (as oestradiol) that are formed from androgen precursors, that are secreted chiefly by the ovaries, placenta, adipose tissue, and testes, and that stimulate the development of female secondary sex characteristics and promote the growth and maintenance of the female reproductive system.
Oophorectomy	The surgical removal of one or both ovaries (bilateral).
Oral contraceptives	Medicines taken by mouth to help prevent pregnancy.
Ordinal variable	A variable that allows for rank order by which data can be sorted, but still does not allow for relative degree of difference between them.

Ordinary least squares regression	A method used for estimating parameters by minimizing the difference between the observed response and the value predicted by the model.
Outlier	An observation so distant from the central mass of data that it noticeably influences results.
Ovarian ablation	Ovarian suppression to block ovarian hormonal activity.
Overexpression	Excessive expression of a gene (as that caused by increasing the frequency of transcription.
Paired t-tests	A Student's t-test for the equality of the means of two populations, when the observations arise as paired samples.
Pancreas	A lobulated gland that in humans lies in front of the upper lumbar vertebrae and behind the stomach. It functions in the breakdown of proteins, fats, and carbohydrates, and secretes the hormones insulin and glucagon.
Paracrine	Of, relating to, promoted by, or being a substance secreted by a cell and acting on adjacent cells.
Parameter	A numerical characteristic of a population or a model.
Parametric tests	Procedures for testing hypotheses about parameters in a population described by a specified distributional form, often, a normal distribution.
Pearson's product moment correlation	Measures of the strength of a linear association between two variables.
Peptides	Any of various amides that are derived from two or more amino acids by combination of the amino group of one acid with the carboxyl group of another and are usually obtained by partial hydrolysis of proteins.
Pharmacokinetics	The characteristic interactions of a drug and the body in terms of its absorption, distribution, metabolism, and excretion.
Phenotype	The observable properties of an organism that are produced by the interaction of the genotype and the environment.

Physiology	The organic processes and phenomena of an organism or any of its parts or of a particular bodily process.
Phosphatidylinosito-3 kinase (PI3K/atk)	A signalling pathway that plays a critical role in regulating diverse cellular functions including metabolism, growth, proliferation, survival, transcription and protein synthesis. Dysregulation of the PI3K/Akt pathway is implicated in a number of human diseases including cancer, diabetes, cardiovascular disease and neurological diseases.
Phosphorylation	To cause (an organic compound) to take up or combine with phosphoric acid or a phosphorus-containing group.
Pituitary gland	An endocrine gland located at the base of the brain. It secretes several hormones which regulate the growth, development, and proper functioning of other endocrine glands and are of vital importance to the growth, maturation, and reproduction of the individual.
Polymorphism	A variation in a specific sequence of DNA.
Polynomial	Is an expression constructed from variables and constants, using only the operations of addition, subtraction, multiplication and non-negative integer exponents.
Power	The probability of rejecting the null hypothesis when it is false. Power gives a method of discriminating between competing tests of the same hypothesis, the test with the higher power being preferred. It is also the basis of procedures for estimating the sample size needed to detect an effect of a particular magnitude.
Precision	The extent to which a measurement procedure gives the same results each time it is repeated under identical conditions.
Prevalence	The percentage of a population that is affected with a particular disease/factor at a given time.
Prognosis	The prospect of survival and recovery from a disease as anticipated from the usual course of that disease or indicated by special features of the case.
Proliferation	Rapid and repeated production of new parts or of

	offspring (as in a mass of cells by a rapid succession of cell divisions).
Pseudo-R^2 statistics	An index sometimes used in assessing the fit of specific types of models particularly logistic regression and those used for modelling survival times.
P-value	The probability of the observed data (or data showing a more extreme departure from the null hypothesis) when the null hypothesis is true.
Pyromark	A specially formulated coating for metal surfaces intended for high heat exposure.
Pyrosequencing	A real-time DNA sequencing technique.
Quadratic function	A polynomial function, whose graph is a parabola whose axis of symmetry is parallel to the y-axis.
Quantile-quantile plot (Q-Q Plot)	A plot of the points whose coordinates are the quantiles for different values of p.
Random effects	The effects attributable to a (usually) infinite set of levels of a factor, of which only a random sample occur in the data.
Random error	Error which occurs due to chance.
Randomised selection of the sample (Random sample)	A sample of n individuals selected from a population in such a way that each sample of the same size is equally likely.
Reagent	A substance used (as in detecting or measuring a component, in preparing a product, or in developing photographs) because of its chemical or biological activity.
Receptors	A chemical group or molecule (as a protein) on the cell surface or in the cell interior that has an affinity for a specific chemical group, molecule, or virus.
Reliability	The extent to which repeated measurements on units (for instance people) yield similar results.
Renal cancer	Cancer relating to, involving, affecting, or located in the region of the kidneys.
Residual	The difference between the observed value of a response variable and the value predicted by some model of interest.

Resting energy expenditure (REE)	Energy expenditure measured under resting, although not necessarily basal, conditions.
Resting metabolic rate	A synonymous of resting energy expenditure.
Retrospective study	A general term for studies in which all the events of interest occur prior to the onset of the study and findings are based on looking backward in time. Commonly encountered is the retrospective cohort study, in which a past cohort of individuals are identified from previous information and their subsequent mortality or morbidity determined and compared with the corresponding experience of some suitable control group.
Rib	Any of the paired curved bony or partly cartilaginous rods that stiffen the lateral walls of the body of most vertebrates and protect the viscera, that occur in mammals exclusively or almost exclusively in the thoracic region.
Risk ratio (RR)	For a disease, death, or other outcome, the ratio of the incidence rate among individuals with a given risk factor to the incidence rate among those without it.
Sarcopenic obesity	An increase in body fat mass accompanied by decreased or no changes in fat free mass.
Scatter/dot plot	Is a type of mathematical diagram using Cartesian coordinates to display values for two variables for a set of data. Data is displayed as a collection of points, each having the value of one variable determining the position on the horizontal axis and the value of the other variable determining the position on the vertical axis.
Screening programme	The systematic offering of a screening test—i.e. , faecal occult blood test, cervical cytology, breast examination—to a population or a specified segment of a population, with the aim of identifying a disease at an early and more treatable stage.
Shapiro-Wilk normality test	Tests that a set of random variables arise from a specified probability distribution. Most commonly used to test for departures from the normal distribution and the exponential distribution.
Serum	The clear yellowish fluid that remains from blood

	plasma after fibrinogen, prothrombin, and other clotting factors have been removed.
Šidák correction	A variant of Bonferroni which uses a Taylor expansion.
Significance level	The level of probability at which it is agreed that the null hypothesis will be rejected. Conventionally set at 0.05.
Single nucleotide polymorphism (SNP)	Although all humans share 99.9 percent of their genetic material, single-letter differences in our DNA sequences—known as single nucleotide polymorphisms—ensure that each individual is unique.
Skinfold thickness	A measure of the amount of subcutaneous fat, obtained by inserting a fold of skin into the jaws of a caliper. The skinfolds are usually measured on the upper arm, thigh, or upper abdomen.
Slope	The gradient of the line or the regression coefficient of the relationship. A positive slope implies that increasing one variable will increase the other.
Social desirability bias	The tendency of respondents to answer questions in a manner that will be viewed favourably by others. It can take the form of over-reporting "good behaviour" or under-reporting "bad," or undesirable behaviour.
Spearman’s correlation rho	A rank correlation coefficient (a correlation coefficients that depend only on the ranks of the variables not on their observed values).
Standard deviation (SD)	The difference between a sample value and the mean.
Standard error (SE)	An estimate of the standard deviation of the means of many samples, calculated as the standard deviation (*s*) divided by the square root of the number of individuals in a sample.
Steatohepatitis	Liver disease characterized by fatty change of hepatocytes, accompanied by intralobular inflammation and fibrosis. Most often caused by alcohol abuse, diabetes, or obesity but may be linked to adverse drug reactions, gastrointestinal and pancreatic disorders or total parenteral nutrition.

Steroids	Substances that are naturally produced in the body. They help reduce inflammation and control different functions in our bodies such as the immune system or the way the body uses food. Steroids can also be man-made and used as part of cancer treatment.
Studentized deleted residual	An alternative criterion for identifying outliers. The basic idea is to delete the observations one at a time, each time refitting the regression model on the remaining n–1 observations. Then, we compare the observed response values to their fitted values based on the models with the ith observation deleted. This produces (unstandardised) deleted residuals. Standardizing the deleted residuals produces studentized deleted residuals.
Subcutaneous adipose Tissue	Fat deposits beneath the skin.
Survey	A study that collects planned information from a sample of individuals about their history, habits, knowledge, attitudes or behaviour in order to estimate particular population characteristics.
Synthesise	To produce by synthesis (the formation of a chemical compound by the union of its elements or from other suitable components.
Systematic error	A non-random statistical error that affects the mean of a population of data and defines the bias between the means of two populations.
Systematic review	A review of a clearly formulated question which uses systematic and explicit methods to identify, select and critically appraise relevant research, and collect and analyse data from the studies that are included in the review. Statistical methods (e.g., meta-analysis), may or may not be used to analyse and summarise the results of the included studies.
Systemic	Pertaining to or affecting the body as a whole.
Taxane	Any of various tricyclic compounds (as docetaxel and paclitaxel) with anticancer activity that are obtained from yew trees or are made synthetically.
Thermogenesis	The production of heat especially in the body (as by oxidation).

Thyroid gland	The largest of the endocrine glands. It is located in the front and sides of the neck just below the thyroid cartilage and produces hormones that are vital in maintaining normal growth and metabolism.
Transcriptional activity	The synthesis of RNA using a DNA template catalyzed by an RNA polymerase.
Type-one error	The error that results when the null hypothesis is falsely rejected.
Type-two error	The error that results when the null hypothesis is falsely accepted.
Tyrosine kinase receptors	Single-pass, transmembrane proteins that bind extracellular polypeptide ligands and cytoplasmic effector and adaptor proteins to regulate biological processes.
Umbilical level	Of or relating to the central abdominal region that is situated between the right and left lumbar regions and between the epigastric region above and the hypogastric region below.
Unbalance design	A design which has an unequal number of observations.
Univariable analysis	The statistical procedures that involve the use of one explanatory variable.
Univariate	The use of one response variable or outcome per observation.
Unstandardised coefficient	It represents the amount by which dependent variable changes if we change independent variable by one unit keeping other independent variables constant.
Urinary bladder	A distensible membranous sac that serves for the temporary retention of the urine.
Validity	It means that a test or instrument is accurately measuring what it’s supposed to.
Variance components	Variances of random effects terms in multilevel models.
Venepuncture	Entry into a vein, usually with a hollow needle so as to gain access to the bloodstream for the purpose of obtaining a sample of blood.

Waist	The part of the body between the thorax and hips.
Wilcoxon signed-rank test	A distribution free method for testing the difference between two populations using matched samples. The test is based on the absolute differences of the pairs of observations in the two samples, ranked according to size, with each rank being given the sign of the original difference. The test statistic is the sum of the positive ranks.

Word count: 79,074.