B-AHEAD 2

Protocol	4th August 2017	Version 9.0

Breast - Activity and Healthy Eating After Diagnosis 2 during chemotherapy

Contents

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This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) guidelines and regulatory requirements.

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Dr Michelle Harvie - 13th February 2013

1. Purpose of investigation

Excess weight at the time of breast cancer diagnosis and weight gain during adjuvant chemotherapy increases the risk of breast cancer recurrence and death. We and others have demonstrated that continuous energy restriction (CER) and exercise is only partially effective at limiting the 2.5-3kg weight gain which occurs during chemotherapy, and for promoting weight loss for overweight women. Our other studies in non cancer patients have shown intermittent energy restriction (IER) is equivalent or superior to continuous restriction (2, 3) for weight control, and our pilot studies indicate that IER could be useful amongst chemotherapy patients because the days after chemotherapy administration can be avoided.

The purpose of this study is to formally assess the feasibility and effectiveness of IER to prevent chemotherapy induced weight gain and promote weight loss for overweight women compared with continuous energy restriction in a randomised trial (n=170). This study will re test whether continuous energy restriction can be effective, and whether the novel IER is better. The trial aims to identify a much needed regimen for weight control and toxicity reduction for breast cancer patients receiving chemotherapy. If either of our test regimes are succesful, this would then be tested in a larger phase III trial to evaluate its effects on relapse free and overall survival.

1.1 Background

In the UK over 16,000 women receive adjuvant chemotherapy for breast cancer annually. Significant numbers (55-65%) of these women are overweight or obese at the time of diagnosis. About 55% of overweight women, and 70% of women of normal weight at diagnosis, gain weight during the period of adjuvant chemotherapy (1;1;2). Excess weight at diagnosis and weight gain afterwards are linked to risk of recurrence and death in women of any age receiving a range of adjuvant treatments in most (3;4) but not all studies (5). The only randomised trial in this area (The Women's Intervention Nutrition Study [WINS] adjuvant dietary trial) demonstrated a 24% reduction in recurrence with weight loss of 2.1kg compared with gains of 0.6 kg in the control group in women receiving a range of adjuvant treatments(6).

During the first year after breast cancer diagnosis adjuvant chemotherapy leads to greater increases in weight compared with endocrine therapy (3-7 kg vs. 1-4 kg respectively) (1;7). We and others have demonstrated weight gain is associated with gains in body fat and reductions in lean body mass, known as sarcopenic obesity (7;8). Gains in adiposity occur with increased energy intakes and decreased energy expenditure during chemotherapy, due to psychosocial and anxiety related factors (9), chemotherapy-induced menopause (10), and the use of steroids as anti-emetics. Chemotherapy patients need to reduce calorie intake to compensate for reductions in resting energy expenditure and exercise levels (8). During chemotherapy women are less able to adhere to a daily calorie controlled regimen partly because of increased hunger and carbohydrate cravings and nausea induced by treatment that can frequently be relieved by eating. We demonstrated recently that increased carbohydrate intake by 50g from 200g/day to 250g/day accounts for most body fat gain. Weight gain is a particular problem amongst normal weight patients who usually control weight by restricting intake and being physically active but develop disinhibited eating patterns and lethargy as a result of chemotherapy (11).

The reason for initiating the new study outlined below is that we have found that CER was only partially effective for controlling weight in chemotherapy-treated patients in our

ongoing randomised trial of CER vs. standard care (B-AHEAD -1). Over the first six month period the intervention was very effective in non-chemotherapy patients, where weight gain was seen in only 20% receiving the intervention compared to 55% of the control group (P < 0.01) (n = 260). In contrast there was a minimal reduction of weight gain with CER in women on chemotherapy: 45% of overweight and 60% of normal weight women in both our intervention and control groups treated with adjuvant chemotherapy gained weight (n = 130), compared to the usual 70% of women that we see gaining weight in the clinic. Non-chemotherapy patients lost 1.8 kg (+/-2.5kg SD) of body fat as a result of six months CER whereas chemotherapy-treated patients gained on average 0.74 kg (+/-3.0kg SD). Average gains in body fat amongst weight gaining patients were 2.8 kg (+/-1.9 kg) SD. Failure to control weight persisted in the 6 month period post chemotherapy, so that at one year post diagnosis chemotherapy patients gained 0.6 kg (+/-3.0kg SD) body fat whereas non-chemotherapy patients lost 2.1kg on CER (=/-3.1kg SD). Limited efficacy of CER and exercise to control weight in patients treated with adjuvant chemotherapy has also been reported by others (12). In order to be effective it is important to institute weight control before or at the time of initiation of chemotherapy. In our current study, 48% of women approached who were scheduled to have adjuvant chemotherapy agreed to be randomised in the period between surgery and the start of chemotherapy. Soon after primary diagnosis is a 'teachable moment' and an important opportunity to promote behaviour change (13). Controlling appetite early on during chemotherapy seems particularly important, as it otherwise appears difficult to re-establish normal eating patterns post-chemotherapy (14).

In the new study outlined here we wish to assess whether IER will be more effective than CER in preventing weight gain during chemotherapy, and promoting weight loss for overweight women. We recently demonstrated in a randomised trial in 115 overweight women at increased risk of breast cancer that this novel IER regimen (2 days per week of a low carbohydrate diet [<50 g carbohydrate and approximately 800-1000 kcal/day]) is superior to CER for induction of weight loss. Over a 3 month period women on the two day IER diet lost 4.3 (+/-4.6 SD) kg body fat compared with 2.2 (+/-4.2) kg on a standard CER diet. IER leads to greater weight loss through increased satiety and reduced carbohydrate on both restricted days (15), as well as an important carry over satiety effect for the remaining days of the week. This satiety effect could be extremely beneficial for chemotherapy patients. The average energy deficit of 300kcal / day seen with IER would prevent chemotherapy associated weight gain and, in some women may promote weight loss.

1.2 Other potential benefits of IER for breast cancer patients

IER improves insulin sensitivity to a greater extent than CER

The adverse effects of excess weight and weight gain on prognosis in breast cancer patients are thought to be largely mediated by reduced glucose tolerance and hyperinsulinemia (16). Insulin sensitivity is compromised during chemotherapy because of weight gain, direct cellular effects of chemotherapy and the standard use of steroids as anti-emetics for at least two days with each cycle of therapy (48). Treatment with metformin to circumvent these effects to improve outcome has been suggested (16). Insulin sensitivity was improved to a much greater extent by IER compared with CER in our randomised trial (17). In healthy overweight women, insulin sensitivity (HOMA) increased by 50% on the 2 restricted days and by 25% on the 5 non restricted days compared with 18% on a standard CER regimen (P <0.05).

Potential to reduce chemotherapy toxicity

Chemotherapy toxicity is a major concern and a source of anxiety and depression in patients (18). Both animal studies and small case series suggest that IER during adjuvant chemotherapy may reduce chemotherapy toxicity (19;19-21). Animal studies suggest IER may reduce normal tissue damage by reducing oxidative stress, and by upregulation of stress response mechanisms (19). There is an increasing interest in whether inhibitors of glycolysis (which may be seen as equivalent to energy restriction) such as 2 deoxy-glucose and lonidamine can increase the efficacy and decrease the toxicity of chemotherapy treatments (24). Ten patients undergoing 48 hour spells of restriction, including four breast cancer patients receiving cyclophosphamide, carboplatin and docetaxel reported reductions in fatigue, nausea, sore mouth, and abdominal symptoms (21). These data are intriguing but limited as they are self reported and prone to bias. Our planned study is an opportunity to collect data on the effects of IER and CER on chemotherapy induced toxicity using subjective self report data, including assessment of any anti inflammatory effects of IER which could reduce the well documented arthralgia and myalgia seen with docetaxel (22). We also plan to assess two novel objective blood biomarkers which assess epithelial toxicity and myelosuppression in a sub set of patients in both the IER and CER groups as energy restriction has been linked to enhanced immune function (23) (3.3 Sub-study 1).

The potential of IER to either increase or decrease the effectiveness of chemotherapy.

Energy restriction has been shown to reduce both tumour and normal cell proliferation (25). This may be beneficial for reducing toxicity for normal tissues (see above), but reduced tumour cell proliferation raises a theoretical concern that it could limit the effectiveness of chemotherapy. A number of cell line and patient studies do not support these concerns and have shown enhanced chemotherapeutic response with either fasting (32), the energy restriction mimetic metformin (26), and the inhibitors of glycolysis 2 deoxy-glucose (27;28) and lonidamine (29).

The most studied energy restriction regimen involves 2 days of fasting immediately prior to chemotherapy. This regimen produces a synergistic response with cyclophosphamide in a murine cancer model, although no obvious effect in a human MDA-MB231 xenograft model. This regimen is currently being tested in 3 randomised clinical trials in the USA and the Netherlands (30).

Two other studies have shown rapid reductions in proliferation of tumour and normal cells during 48 hours of fasting, rapid induction of proliferation and synchronisation of cell cycling in tumours within 6 hours of refeeding, but delayed induction of proliferation in other healthy body tissues after refeeding (31;32).

Given these data, we will administer our 2 day restriction immediately prior to chemotherapy. We will deliberately avoid the immediate post chemotherapy period when women receive steroids and often have an increased appetite which will make compliance to energy restriction difficult.

1.3 Pilot data of IER in chemotherapy patients

In a small pilot study, 6 of our adjuvant chemotherapy breast cancer patients all managed to include the 2 day restriction for 2 days before 2 chemotherapy cycles. These limited data suggest the diet is achievable in our target population. The general acceptability of

the IER regimen will be rigorously tested in the proposed study.

1.4 Preventing weight gain and promoting weight loss

We anticipate a significant proportion of patients will be overweight or obese at the time of diagnosis (~60% of B-AHEAD-1 patients). As well as preventing weight gain our IER and CER regimens are also designed to promote gradual weight loss of 0.5 –1kg amongst these overweight or obese women. This weight loss will be achieved by asking women in both the IER and CER groups to adhere to an overall 25% energy restriction. This will be achieved in the CER group with a daily 25% energy restricted diet, and in the IER group with the 2 day restriction and by advising a maximum amount of healthy Mediterranean diet foods allowed on the 5 remaining days (see 2.6).

We will not be specifically asking healthy weight women to lose weight. Weight loss in normal weight women is a possibility with the IER and CER regimens, we do not see this as a problem, as weight loss within a normal range i.e. reducing from a BMI of 25 to 20 kg / m^2 could be beneficial. We will limit the possibility of inappropriate weight loss in the study by excluding women with a sub optimal BMI (<19 kg / m^2). Three weekly weight monitoring in the trial, at chemotherapy visits, will allow us to identify and manage any excessive or inappropriate weight loss in the two diet groups. IER women with excessive or inappropriate weight loss will be asked to increase intake on non restricted days or reduce to just 1 restricted day per week whilst CER women with excessive or inappropriate weight loss will be asked to increase their daily intake.

1.5 Exercise component of the weight control intervention

We will test IER and CER diets in combination with a home based exercise programme since numerous studies have shown both diet and exercise are required for successful weight management. Exercise during and after chemotherapy is linked to improved health and well-being (33), is important for maintaining lean body mass (8) and for reducing overall mortality in breast cancer patients (34). Furthermore we have recently demonstrated that a telephone supported home exercise programme is as effective as supervised exercise classes, and the former will thus be used in this study (35).

1.6 Future studies

If either the IER and exercise or CER and exercise interventions prove effective in terms of weight control, improving insulin sensitivity and reducing toxicity, this study could bring about a paradigm shift in cancer chemotherapy. The best intervention would need to be tested in a large scale follow up randomised study to assess its effects on breast cancer recurrence and death. Adherence to the regimen and effects on biomarkers of prognosis in the current study will inform the size and statistical power for a survival trial.

2. Plan of investigation

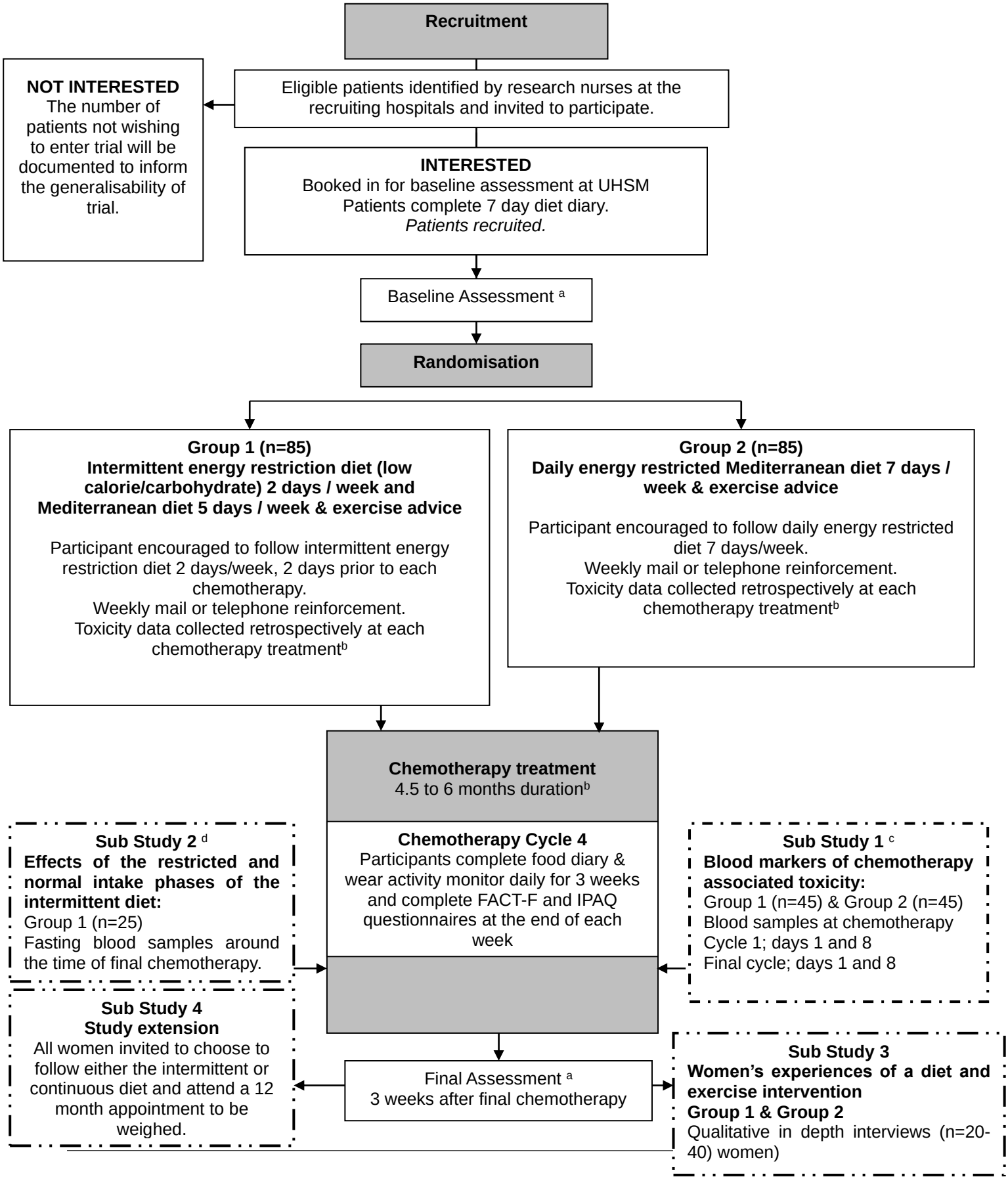
2.1 Trial Design

Randomisation will be 1:1 comparing:
1. An intermittent energy restriction (2 days/week) plus exercise weight control intervention.
2. A comparison group receiving a daily continuous energy restriction plus exercise weight control intervention.

Intervention group (n = 85)
2 consecutive days / week of energy restriction with an intermittent low energy diet (<50g carbohydrate / day and ad lib protein diet [self limits to approximately 800-1000 kcal/day]), and a Mediterranean diet for 5 days/week, plus an exercise intervention (2.5 hours/ moderate activity week) – detailed below.

Comparison group (n = 85)
Continuous energy restricted Mediterranean diet plus an exercise intervention (2.5 hours/ moderate activity week) – detailed below.

Figure 1 – B –AHEAD 2 -Trial Schema



[a] Baseline and final assessments:

1. Weight, body fat (DXA, impedence), waist and hips.
2. Quality of life and fatigue (functional assessment of cancer therapy; FACT-B, FACT-ES and FACT-F scales)
3. Serum markers of breast cancer risk prognosis (fasting serum insulin, glucose, insulin sensitivity [HOMA], adiponectin, leptin)
4. Serum markers of cardiovascular disease: fasting total, LDL and HDL cholesterol, triglycerides, systolic/diastolic blood pressure and overall prognosis (high reactive C-reactive protein)
5. Fitness: exercise test on treadmill
6. Blood markers of oxidative stress (fructosamine, advanced oxidation protein products (40), glutathione, thioredoxin, ORAC [total antioxidant capacity], uric acid, homocysteine, catalase, glutathione peroxidise, superoxide dismutase [SOD])
7. Motivational, stage of behaviour change, health beliefs, and self-efficacy scales
8. Dietary intake (7 day food diary) and International Physical Activity Questionnaire (IPAQ) long version
9. Study experiences (final appointment only)

[b] Completed routinely by chemotherapy nurses and collected at end by research nurses at site:

1. Differences in self reported chemotherapy toxicity (Common Terminology Criteria for Adverse Events)

[c] Optional Sub study 1. Blood markers of chemotherapy associated toxicity:

- Serum markers for Cytokeratin 18 (CK18) and FMS Like Tyrosine Kinase 3 ligand (FLT 3 ligand)

[d] Optional Sub-study 2. Examining the effects of the restricted and normal intake phases of the intermittent diet.

- Serum markers of breast cancer risk on the intermittent energy restricted diet days; insulin sensitivity, IGF-I and oxidative stress markers

Outcome measures
Relative changes in the following outcomes between the IER and comparison CER group over the period of chemotherapy treatment (4.5 – 6 months):

Primary
Weight, body fat and fat free mass (DXA)

Secondary

- Other measures of adiposity: waist and hip circumference, body fat and fat free mass assessed with bioelectrical impedence scales (Tanita 180)

- Serum markers of breast cancer risk and prognosis (36) (fasting serum insulin, glucose, insulin sensitivity [HOMA] (37), adiponectin, leptin) (38)

- Serum markers of cardiovascular disease (fasting total, LDL and HDL cholesterol, triglycerides, systolic/diastolic blood pressure) and overall prognosis (high sensitivity C–reactive protein) (49)

- Fitness; exercise test on treadmill (62)

- Quality of life & fatigue (FACT-B, FACT-ES, FACT-F scales)

- Blood markers of oxidative stress (fructosamine, advanced oxidation protein products (40), glutathione, thioredoxin, ORAC [total antioxidant capacity], uric acid, homocysteine, catalase, glutathione peroxidise, superoxide dismutase [SOD])

- Differences in self reported chemotherapy toxicity (Common Terminology Criteria for Adverse Events)

- Changes in blood markers of chemotherapy toxicity (CK18 and FLT3) between groups (sub-study 1)

- Adherence to the diet and exercise regimens (7-day diet diaries), accelerometer (Actigraph USA) and International Physical Activity Questionnaire (IPAQ) long version (41)

- Motivational, stage of behaviour change, health beliefs, and self-efficacy scales (42) to understand the process of behaviour change amongst partcipants.

- Acute changes in insulin sensitivity, IGF and oxidative stress markers on restricted days of the intermittent diet (sub-study 2)

2.2 Eligibility

Inclusion criteria

1. Scheduled to have adjuvant or neoadjuvant chemotherapy
2. Breast cancer stage I–III
3. Any age >18 years: weight affects prognosis amongst pre- and post menopausal women
4. BMI>19 Kg / m^2 (Using IER/ CER and exercise we aim to avoid weight gain in all patients; this may lead to some weight loss in normal weight individuals).
5. Ability to understand written instructions.
6. Resident within Greater Manchester or Cheshire area or expressed a willingness to travel if further afield in order to maximise uptake and retention to interventions and study.
7. Written informed consent.
8. Pre-operative or pre-chemotherapy haemoglobin level > 11 g/dl
9. Clear routine staging CT scan (relevant only to patients scheduled to receive neoadjuvant chemotherapy, patients with 4 or more axillary nodes involved, and patients scheduled to have an ANC after chemotherapy)

Exclusion criteria

1. Metastatic disease
2. Completed previous chemotherapy for breast or other cancers within the last 2 years
3. Physical or psychiatric conditions which may impair compliance to the diet or physical activity interventions assessed from medical history by recruitment nurse/clinician i.e.
   - Serious digestive and/or absorptive problems, including inflammatory bowel disease.
   - Cardiovascular or respiratory problems (determined from, for example recent pre-operative ECG, chest X–ray)
   - Musculoskeletal disease or joint problems.
   - Psychiatric disorders or conditions, e.g. untreated major depression, psychosis, substance abuse, severe personality disorder.
4. Medications affecting weight e.g. continuous daily steroids (2-3 days with chemotherapy allowed)
5. Diabetics on insulin or sulphonylureas (glibenclamide, gliclazide, glimepiride, glipizide, tolbutamide) as they could cause hypoglycaemia on restricted days of the intermittent diet (diabetics treated with diet alone or with any other medication are eligible).
6. Already commenced this course of chemotherapy.

2.3 Recruitment

170 early breast cancer patients scheduled to have standard adjuvant or neoadjuvant chemotherapy (see below) will be recruited via consultants and National Cancer Research Network nurses from 11 Manchester and Cheshire Cancer network breast units (University Hospital of South Manchester [UHSM], The Christie, Oldham, Stepping Hill, North Manchester, Tameside, Wigan, Salford, Macclesfield, Leighton and Bolton hospitals). Hospitals within other cancer networks can also recruit. Women who are scheduled to have chemotherapy will be identified by National Cancer Research Network nurses within their unit multidisciplinary team meeting. These women will be invited to enter the study at their first post-operative or chemotherapy assessment appointment by the appropriate doctor or specialist nurse. Eligible women will be given a detailed patient information sheet and given the opportunity to ask questions concerning the trial. If it not possible to see women in clinic, they will be telephoned by the research nurse or other member of the

research team. The purpose of this phone call will be to briefly introduce the study and get consent to post an information sheet if the participant is interested. We will seek permission for the research nurse to contact them 48 hours after receipt of the information sheet to determine their interest in entering the trial. Potential participants will be informed they can enter the trial immediately or at any time before they commence chemotherapy (usually 3 weeks later). Women will also be made aware of the trial at the time of surgery from literature available on the wards in these hospitals. Posters and leaflets to raise awareness of the study will also be displayed in patient areas. This information will outline the study and tells women they may be approached again at their post surgery appointment. There will also be a simple website to advertise the study from which the PIS can be downloaded.

All research nurses in participating centres will be fully informed about the study and recruitment procedure. Interested women will be asked by the research nurse whether they consent to their details being securely transferred on the trial proforma (by fax or nhs.net e-mail) to the B-AHEAD 2 research team at UHSM. This verbal consent will be documented. Once the proforma has been received, the B-AHEAD 2 research team at UHSM will then contact the patient to arrange a baseline appointment and consent will take place at that appointment.

Using this approach we successfully recruited 48% (163 of the 340 approached agreed to enter) of eligible chemotherapy patients from the Network into our previous randomised trial in the same hospitals outlined above (B-AHEAD 1 study). Approximately 550 early breast cancer patients in these hospitals commence chemotherapy each year of which, we anticipate 70% will be eligible. Assuming a conservative 35% uptake, recruitment should be completed within 16 months.

All participants will be categorised during their baseline and final appointments using the Adult Pre-exercise Screening System (APSS) Tool (Exercise and Sports Science Australia [ESSA], Fitness Australia and Sports Medicine Australia [SMA]) (74). A satisfactory pre-op or pre-chemotherapy ECG or a low/moderate risk outcome on the questionnaire is sufficient to prove that the participant is suitable for the 6 minute walk test (6MWT) and moderate exercise advice. If a participant has not had a satisfactory recent ECG and/or is deemed high risk on the questionnaire (it is predicted that this will be an unlikely occurrence), their GP's opinion will be sought before walk test and exercise advice are performed. The questionnaire will be repeated at the final appointment to ensure that it is still safe to perform the 6MWT and provide advice on moderate exercise.

2.4 Adjuvant and neoadjuvant chemotherapy

All women will be scheduled to have adjuvant or neoadjuvant chemotherapy. The most commonly used regimen is currently (FEC-Docetaxel) and includes fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2 (FEC) intravenously on day 1 every 21 days for 3-4 cycles followed by Docetaxel 100 mg/m^2 intravenously on day 1 every 21 days for a further 3-4 cycles. IER/ CER will extend until the end of chemotherapy to cover any chemotherapy associated treatment delays. The chemotherapy period of treatment is projected to be 4.5 – 6 months.

Chemotherapy toxicity will be managed according to standard protocols within the Christie NHS trust and the other hospitals. All patients will have access to the 24 hour Christie

chemotherapy hot line for advice as to the management of toxicity, as well as their breast care nurse and the research nurses.

2.5 Randomisation

Women will be randomised to each of the 2 study arms using computer minimisation programme, stratifying for three factors:

1. Whether women are scheduled to receive adjuvant or neoadjuvant chemotherapy.
2. Whether women are a healthy weight or overweight. (respectively 45% and 55% in our B-AHEAD-1 cohort) .
3. Whether women are pre- (and peri) or post menopausal to ensure groups are matched for chemotherapy induced menopause.

This minimisation programme will be created by the trial statistician (Julie Morris) at the University Hospital of South Manchester. The programme will be located on a department computer and used by the trial administrator to determine group allocation.
Laboratory staff undertaking serum assays and the researcher analysing the motivational and quality of life questionnaires will be blinded to the participant study arm. It is not possible to blind other staff.

2.6 Interventions

Both groups

Normal weight women aiming to maintain weight will be prescribed a diet which meets their estimated energy requirements, whilst overweight / obese women will be prescribed a 25% energy restriction to promote steady weight loss of 0.5 – 1kg / week.
Baseline energy requirements for both diet groups will be assessed from estimated basal metabolic rate [based on estimated requirements from the Henry equations] (44) multiplied by their reported activity levels [Metabolic equivalents].

Intervention group: Intermittent diet

Women will be asked to follow the intermittent diet for 2 days/week throughout the trial beginning immediately prior to the first chemotherapy cycle. The two restricted days must be consecutive and should be the 2 days immediately prior to chemotherapy on chemotherapy weeks. If this is not possible alternative days should be undertaken, as this is preferable to none or a reduced number of restricted days. The restricted days limit carbohydrate (< 50 g) but allow generous amounts of protein and have been found to be effective and successful in a non cancer population as the diet is palatable and satiating. The two restricted days include generous amounts of lean meat, fish, eggs, tofu, monounsaturated (MUFA) and polyunsaturated (PUFA) fat, limited Quorn and limited dairy foods, 5 portions of vegetables and 1 portion of fruit, at least 2 litres of low energy fluids and a multivitamin and mineral supplement. The diet typically self limits to 70–90 g protein and <60 g fat/day and is limited in saturated fat which has possible links with breast cancer (43). We will encourage a high fluid intake which is important in this population who may suffer chemotherapy-induced constipation.
Women will be asked to follow a Mediterranean diet for the remaining 5 days of the week. The amounts of this diet on these days will be tailored according to their estimated energy requirements, and whether they are aiming to maintain or lose weight. The Mediterranean diet provides 30% energy from fat (15% MUFA, 8% PUFA, 7% saturated), 25% energy from protein and 45% from low glycaemic load carbohydrate and includes 4 portions of vegetables and 2 portions of fruit per day, and is limited in alcohol (<10 units per week).

Comparison group: Daily continuous energy restricted diet.
This group will be asked to follow an energy restricted Mediterranean diet (see above). The quantities of food from the range of permitted food groups on these days will be tailored according to their estimated energy requirements, and whether they are aiming to maintain or lose weight.

Initial dietary and exercise advice for both groups
Both groups will receive a face to face dietary consultation with one of the study dietitians (40 minutes). Each patient will receive guidance on appropriate food choices, portion sizes, menus, recipes and appropriate behavioural techniques to enhance adherence. The diet will be self-selected by the patients and not provided by the study team. All women will receive advice for dealing with chemotherapy related side effects such as nausea, dry and sore mouth, constipation, or diarrhoea.

Both groups will also receive face to face exercise advice from the study exercise specialist (40 minutes). Women will be advised to gradually increase the frequency and intensity of exercise with the aim of undertaking 2.5 hours (5 x 30 minutes) of moderate activity/week (at 60–80% maximum heart rate) according to published guidelines for patients receiving adjuvant chemotherapy (45). This advice will be individualised and tailored according to current activity levels, participant preferences, abilities, co-morbidities and energy levels. Many breast cancer patients in our current studies are keen to undertake walking regimens. They will be provided with pedometers (Omron HJ 113) to promote adherence to the prescribed regimens.

Support and monitoring in both diet groups
Our previous B-AHEAD-1 study demonstrated that a phone and mail intervention is as effective with respect to weight loss as attending group exercise and diet education sessions. Diet and exercise goals will be reinforced by fortnightly phone calls by their designated advisor in the B-AHEAD-2 team and each participant will receive twelve fortnightly mailings throughout the 4.5-6.0 month chemotherapy period.

Fortnightly calls will last approximately 20 minutes and will check compliance and any problems with the diets, physical activity and address individual problems. Women will then be mailed an individualised summary of key motivational, behavioural, diet and exercise issues. Individual goals and recommendations will be discussed during each call. Participants can start their allocated diet any time between their baseline appointment and two days prior to their first chemotherapy. Phone calls will start from the week before or after chemotherapy starts. Standard mailings cover aspects of IER or CER, weight management, diet, physical activity and chemotherapy (Appendix 5).

Patients will be provided with a handheld record for weight and haemoglobin levels. They will be encouraged to take this with them to each chemotherapy session. They will be reminded to ask the staff on the chemotherapy unit to document their weight and haemoglobin in their record each time they attend for their chemotherapy. This data will be collected from the patient by the designated advisor at each fortnightly phone call.

Some of the increased food intake and fatigue amongst adjuvant chemotherapy patients may in part be a response to anxiety. As with our B-AHEAD-1 trial, all participants will be closely monitored by the study team, and psychological issues will be dealt with appropriately by referral to relevant cancer support services. All participants will be offered

individual additional diet and exercise advice at the end of the study.

The participant and their general practitioner will be informed of blood results after the end of the study, as well as other individual findings (e.g. body composition and measurements, dietary intake and physical activity results).

3. Outcomes and measurements

Main trial endpoints are changes in outcomes assessed between baseline assessment (prior to starting chemotherapy) and 3 weeks after the end of chemotherapy (4.5-6.0 months later). Post chemotherapy assessments will be undertaken three weeks after the final chemotherapy cycle. This time point avoids any acute effects of chemotherapy on blood serum markers, but will assess women before they start further breast cancer treatments, i.e. radiotherapy or endocrine therapy (adjuvant patients) or breast surgery (neoadjuvant patients).

Relative changes in the following outcomes between IER and the CER comparison group over the period of chemotherapy treatment (4.5 – 6 months):

3.1 Primary endpoint

Change in body weight, body fat and fat free mass
From total body DXA (Hologic Discovery A) using standardised methodology (Appendix 1)

Secondary outcomes
Change in:

- Waist and hip circumference (average of 3 measurements)
- Fasting serum insulin, glucose and insulin sensitivity (HOMA) which has been linked to breast cancer risk and prognosis (36;37)
- Serum high sensitivity C–reactive protein (hs CRP) and fasting total, LDL and HDL cholesterol, triglycerides, which are linked to overall and cardiovascular prognosis (49)
- Breast cancer related adipokines: fasting plasma leptin (enzyme immunoassay) and adiponectin (38) and IGF-I enzyme-linked immunosorbent assay (ELISA) developed by R&D Systems (Minneapolis, MN, USA)
- Fitness - exercise test on treadmill (62) (Appendix 2)
- Systolic/diastolic blood pressure (average of 3 measurements) using a Dinamap, ProCare 100 (Appendix 3)
- Quality of life & fatigues scales (FACT-B, FACT-ES, FACT-F)

3.2 Effects of IER and CER on chemotherapy associated toxicity

This will be assessed from:
a. Clinical data throughout the course of chemotherapy
b. Pre and post intervention oxidative stress markers
c. Two novel chemotherapy toxicity blood markers

Clinical data

- Patients will be asked to report toxicity according to the Common Terminology Criteria for Adverse Events version 4 (CTCAE v4) scale (46) when attending each chemotherapy session. They will be asked in retrospect about symptoms experienced since their previous chemotherapy infusion and this will be documented on the standard form for that hospital. This form will be securely faxed or e-mailed to the B-AHEAD 2 study team at the end of the study. Symptoms from the final chemotherapy will be collected at the final appointment at The Nightingale Centre. Common side effects from the standard FEC-T regimen include nausea/vomiting, nail disorders, arthralgia and myalgia, peripheral neuropathy

(Appendix 4). Each phone review will include questions about the items on the CTCAE scale relevant to the diet/exercise advice (e.g. nausea, constipation) so that these can be acted upon and advice altered accordingly.

- We will also assess pre chemotherapy haemoglobin level at each cycle throughout the trial

Plasma and red blood cell (RBC) markers of oxidative stress

- These will be assessed in all women prior to starting chemotherapy and 3 weeks after the end of chemotherapy:
- **Plasma markers:** Fructosamine, advanced oxidation protein products (40), Glutathione, Thioredoxin, ORAC (total antioxidant capacity), Uric acid, Homocysteine.
- **Red blood cell markers:** Catalase, Glutathione peroxidise, superoxide dismutase (SOD)

3.3 Optional Sub-study 1: Blood markers of chemotherapy associated toxicity Cytokeratin 18 (CK18) and FMS Like Tyrosine Kinase 3 ligand (FLT 3 ligand)

Background

In addition to collecting subjective self reported toxicity data, we will also assess objective measure of toxicity with two novel biomarkers which assess epithelial cell toxicity and myelosuppression. Objective measures are important in this study, since self reported toxicity data may be influenced by the unblinded nature of a diet study and knowledge they are in the different diet groups.

We will assess epithelial toxicity using serum Cytokeratin 18 (CK18), a cytoskeletal protein extensively expressed in epithelial but not haematological cells (55). CK18 is released during cell death and can be detected in the circulation. Importantly changes in circulating levels of CK18 as early as 48 hours following therapy can predict subsequent severe chemotherapy-related events and the need for hospital admission (56) (57).

We will assess myelosuppression with plasma levels of the cytokine FMS Like Tyrosine Kinase 3 ligand (FLT 3 ligand) which is thought to be particularly important in the expansion of the stem cell compartment in response to bone marrow stress (58). There is increasing evidence that circulating levels of FLT 3 ligand can reflect the state of the stem cell compartment even alongside normal peripheral counts. Serum FLT3 ligand is inversely correlated with colony forming cells (CFCs) in mice with bone marrow aplasia in response to both radiotherapy (59) and busulphan treatments(60). In patients with lymphoma receiving chemotherapy increases in FLT 3 ligand during the first cycle of chemotherapy predicted subsequent neutropenic sepsis and out-performed neutrophil nadir count (61).

Aim of sub study 1

Primary aim

To determine the effect of IER or CER on levels of the validated blood-borne toxicity biomarkers serum CK18 (epithelial toxicity) and plasma FLT 3 ligand (myelosuppression). This will be assessed after both the first and final chemotherapy cycle in 90 patients.

Secondary aim
The trial presents an invaluable opportunity to identify novel clinically useful blood borne biomarkers of chemotherapy toxicity which could be used to evaluate toxicity alongside dietary interventions. We therefore plan to collect an additional plasma sample at this time which will be used to perform exploratory analysis with proteomics and metabolomics within the biomarker laboratory at the Northern Centre for Cancer Care at The Freeman Hospital, Newcastle-upon-Tyne.

Timing of measurements
Plasma and serum samples will be taken at Cycle 1 (Day 1 or with prechemotherapy bloods usually up to two days before), and Cycle 1, Day 8 to assess acute toxicity following this treatment, and at the final cycle (Day 1 or with prechemotherapy bloods usually up to two days before), and Day 8 to assess acute toxicity following this final chemotherapy treatment.
All participants will be invited to enter this sub study, which is aiming to recruit 45 women in both dieting groups. The sub study involves an additional plasma and serum sample to be collected on or before Day 1 of the first and final cycles and also 2 additional trial visits for venepuncture 8 days after these chemotherapy treatments. The total additional volume of blood samples taken on the sub-study will be 36ml. Unlike the metabolic markers in sub study 2, toxicity markers are unlikely to be acutely affected by the 2 day restricted diet, so the 2 day diet does not have to be synchronised with these blood collections. Women will be asked to refrain from exercise for 4 hours prior to assessment, as exercise can exert acute effects on these blood tests.

Statistics
This sub study is exploratory, however previous work has demonstrated that patients who developed severe toxicity have an average rise of 45% in CK18 at day 8 compared to 12% in patients with no toxicity (S.D. 35%). Patients who developed severe toxicity also have an average increase of 350% in FLT 3 ligand compared to 140% in patients without toxicity (SD 160%) (61). Therefore if 35 patients from each arm donate samples we will be able to detect with 80% power at 5% significance level a 20% absolute reduction in CK18 elevation and a corresponding 100% decrease in FLT 3 ligand elevation between the two diet groups. Allowing for a 20% attrition we will recruit 45 women in both groups.

3.4 Adherence to the diet and exercise interventions:

Dietary adherence to IER and CER will be assessed from 7-day diet diaries (47). These will be completed:

a) 7 days prior to baseline assessment (prior to starting chemotherapy).
b) For one week prior to the final post chemotherapy assessment and
c) For 3 weeks mid treatment (immediately after the fourth chemotherapy cycle [first Docetaxel treatment for the majority of participants]) to assess adherence to the prescribed diet regimens throughout an entire chemotherapy cycle.

Diaries include self reported 7 day hunger and appetite visual analogue scales. (48) Energy, macronutrient and micronutrient intake will be analysed on WISP V3 (Tinuviel Software, UK).

Adherence to the physical activity component of the intervention will also be assessed during these weeks: a) objectively using an accelerometer (Actigraph USA) and b) three weeks of International Physical Activity Questionnaire (IPAQ) long version (41)

Adherence to the 2 day IER each week will be assessed by asking women to record their own compliance on a special trial diary sheet. Compliance will also be checked and recorded as part of the fortnightly call from their B-AHEAD-2 advisor.

Pre-menopausal women in both diet groups (~50% of cohort) will be asked to complete a diary sheet of their menstrual cycle throughout the study. This will inform whether women are experiencing chemotherapy associated ammenorhea, which is a factor in chemotherapy associated weight gain.

3.5 Behavioural and psychological factors which motivate or reduce adherence

We will explore these factors within the two diet and exercise groups using a questionnaire which comprises a series of short validated motivational, health belief, and self-efficacy scales. These scales will be used to understand the processes of behaviour change and adherence amongst women receiving chemotherapy, and will be used to identify if we can tailor future interventions to overcome identified barriers (42).
The scales included in the questionnaire are:

- Self efficacy and locus of control, i.e. whether women feel that their weight and breast cancer prognosis is in their control, adapted from Larsen et al (49)

- Habits and automaticity of diet and exercise behaviours, since habits are thought to contribute to maintenance of behaviour change (50)

- Dutch eating behaviour questionnaire which describes eating behaviour as either restrained or cued by external or emotional factors (51)

- Self efficacy for dieting during chemotherapy adapted from a self efficacy of dieting questionnaire which describes their confidence for dieting under certain conditions. Clark et al 1991 (52)

- Satisfaction of their assessed progress given the effort they have focussed on achieving their diet and exercise targets. The literature predicts that failure for prior expectations of outcomes of behaviour to be met could prompt disengagement, and so failure to maintain initial behaviour gains over the longer-term (53).

3.6 Assessment appointments

Assessments will be conducted by trial team at the Genesis Breast Cancer Prevention Centre, UHSM and will take approximately 2 hours. Women will be asked to fast for 12 hours, and refrain from exercise, alcohol, or fish oil for 24hrs prior to trial appointments. They will receive a complimentary light breakfast at the Genesis Centre immediately after blood collection.

Cardiovascular markers and insulin will be measured by clinical biochemistry, UHSM. Breast risk and oxidative stress markers by Professor Mark Mattson and his team at the NIH Baltimore, USA. Details of serum and plasma collection, handling and storage are described in Appendix 7. Samples will be stored at -70ºC at UHSM. All assays will be batched and performed on stored serum upon completion of the study to reduce inter-batch variation.

The schedule of assessments and venepuncture are summarised in Appendix 7.

3.7 Optional Sub-study 2: Examining the effects of the restricted and normal intake phases of the intermittent diet on insulin, glucose, IGF-I and oxidative stress biomarkers

Purpose
The benefits of IER are partly linked to chronic beneficial effects, but also acute metabolic effects elicited during the 2 days of restriction. Data from small groups of women in our earlier studies (10 patients) show that 3 months of IER reduces insulin by 25%, with a further 25% acute decrease during the 2 days of restriction (17). A true evaluation of IER thus requires assessment during both the restricted and normal intake phases.

Recent animal data suggests reduced chemotherapy toxicity and increased effectiveness are mediated by reductions in glucose, insulin and IGF-I during spells of restriction. Women in the IER group will be invited to an optional sub study to examine the acute effects of our intermittent diet on insulin, glucose the IGF-I axis and oxidative stress markers.

Method
All post intervention assessments will be made for the IER group during their normal intake days; 5 days after restricted days. Women will be invited to provide an optional additional fasting sample immediately after their 2 days of IER to allow us to assess the acute effects of 2 days of IER on serum markers. These samples will be obtained on the morning of their final chemotherapy treatment to minimise venepuncture. Alternatively women could attend the Nightingale Centre to have this blood sample taken up to a few weeks before or after final chemotherapy so long as they were still adhering to the diet.

Thirty percent of women in our previous cohorts have been willing to provide additional blood samples, we therefore anticipate obtaining 20-25 additional samples. Evaluating the effects of the diet during restricted and normal feeding phases will provide a better assessment of potential benefits of IER and its mechanisms of action.

3.8 Optional Sub-study 3: Qualitative study examining women's experiences of a diet and exercise intervention during chemotherapy

Qualitative research from our previous B-AHEAD study will inform the development of an interview schedule to be used in a series of in-depth individual interviews with women from the IER and CER groups. We plan to interview around 20 women or until data saturation with a maximum of 40 interviews. The interviews will explore participant centred viewpoints of strategies or interventions which may improve adherence to weight control programmes, including participant views on factors which either facilitated or limited adherence to IER or CER and exercise and self motivated efforts to lose weight.

In order to understand behavioural maintenance with this population, we will repeat the interviews after 6 months in women who opt to enter Sub study 4, the 6 month post chemotherapy follow up study. The interviews will explore the same factors as the initial interviews and adding new insight (from the patient's perspective) into how weight loss or maintenance of healthy weight can be sustained for the 6 months following the last

consultation with the B-AHEAD 2 dietitian. A second function of the interviews is to assess what information would support women in maintaining weight loss after support from the dietetics staff has been removed. As part of the B-AHEAD 2 maintenance phase, women will be provided with a booklet of information and top-tips for prevention of weight gain. The booklet will be used as a basis for women's ideas on how to improve patient information for weight loss maintenance. On the feedback from participants, we will redesign the booklet for use in future research.

We will also carry out semi-structured individual interviews with the B-AHEAD 2 research staff at the Nightingale Centre and Genesis Prevention Centre who have been involved in the fortnightly phone calls. Consent will be gained verbally and will be found at the beginning of the interview transcripts. The aim of this is to explore their perspectives on the weight loss and exercise advice they give and to highlight any barriers they feel to giving women weight loss advice during chemotherapy.

The interviews will be analysed using thematic analysis (54). Thematic analysis has a freedom of epistemology meaning that the qualitative data from interviews can account for both individual and group consensus, so that both convergent and divergent experiences and opinions across the corpus of the data can be taken into account. Participant comments will be transcribed verbatim and key points will be coded. Similar codes are grouped to form concepts which in turn form categories that will help to give us a greater understanding of the experience of following a diet and exercise programme for weight management during chemotherapy. The in-depth individual interviews will allow us to uncover individual factors concerning adherence to weight control and exercise interventions.

3.9 Optional Sub-study 4: Can an intensive health care supported weight control intervention delivered during chemotherapy lead to long term behaviour change and weight maintenance?

Aim: This sub study has two main aims:

1. To assess whether patients can self manage their weight in the six months immediately after completing chemotherapy and completing the highly supported B-AHEAD 2 intervention when they are no longer receiving support from health care professionals.
2. To collect preliminary data on issues faced by patients in this transition period and the tools and support which would help patients to self manage their weight and diet and exercise behaviours.

Sub study 4 will inform the development of weight maintenance interventions at this important and poorly understood transition time for patients.

Background: The main B-AHEAD 2 study will inform us of changes in weight, diet and exercise during the 4.5 – 6 month period of chemotherapy. We have deliberately targeted women at the time of diagnosis as we believe that this is a teachable moment when patients are more likely to want to join a diet and exercise programme and be motivated to change their diet and exercise behaviours (70). Our interventions will support patients during chemotherapy since this is a particularly challenging time. However, the potential reductions in risk of recurrence and other health problems will only occur if weight control and diet and exercise behaviours persist after the end of chemotherapy and are adopted long term. We need to ensure that patients do not perceive a healthy diet and exercise just

as something they did whilst undergoing chemotherapy. They need to view weight control as a long term strategy for reducing risk of recurrence and improving overall health.
Sub study 4 will gather information about what happens to weight once chemotherapy and the intensive diet and exercise intervention has finished. The six months immediately post chemotherapy can present a challenging time when weight can increase (71). This sub study will provide invaluable preliminary data of the potential long term behaviour change from initiating a diet and exercise programmed at the time of diagnosis, and what ongoing additional measures may be required to achieve this aim.

Tools to promote long term behaviour change
The B-AHEAD 2 diet and exercise interventions are intensive health care supported interventions to support women receiving chemotherapy. They are designed to maximize long term sustained behaviour change and promote self management of weight once the intensive health care professional input has ceased.
All participants in the main B-AHEAD 2 study will receive revised diet and exercise targets at their post chemotherapy visit and specific advice for continued weight loss or for weight maintenance depending on their progress and initial weight loss goals. They will be given the option of continuing with their current diet allocation (intermittent or continuous) or given the option of changing to the alternative diet if they prefer.
All participants in the main B-AHEAD 2 study will receive our specially designed moving on information booklet which includes advice for sustaining behaviour change using well known behavioural techniques including; self monitoring of weight, diet and physical activity, seeking personal support, getting back on track, vigilance for portion sizes and maintaining exercise levels (72) and forming habits (73). Participants following the intermittent diet (either two days per week for weight loss or one day per week for weight maintenance) will be provided with a calendar to self monitor adherence to restricted days.

Recruitment to sub study 4
For participants who have finished the study before this amendment has been approved, their designated study advisor will have discussed Sub study 4 with them while they were on the study and if they have shown an interest they will contact the participant by phone / e-mail / letter once this amendment is approved and provide the PIS at that time. They will then contact the participant 48 hours later and arrange consent (probably by post) if they wish to join. If the participant does not express an interest in continuing, no further contact will be made.

For participants on the study when the amendment is approved, Substudy 4 will be discussed during one of their fortnightly phone call and a PIS sent out with the final appointment letter. They can then consent at their final appointment if they wish to join.

For those joining after the amendment has been approved, they will receive the updated PIS including details of Substudy 4. A PIS will be sent out with the final appointment letter and they can consent at their final appointment if they wish to join.

Methods
Women who enter sub study 4 will be asked to follow their chosen intermittent or continuous diet for the six month extended trial period. We will reassess weight 12 months after starting chemotherapy i.e. 5–7 months after the post chemotherapy B-AHEAD 2 assessment appointment. Participants will be offered the opportunity to attend the

Nightingale Centre for a face to face half-hour appointment with a B-AHEAD 2 researcher (weight on bioimpedance scales, waist and hip measurements, discussion about diet and exercise progress and targets). If patients do not want to come for an additional appointment we will offer them a final review phone call. This call will ask patients to self report their current weight and the opportunity to discuss diet and exercise progress and targets. We would like them to weigh themselves on as accurate scales as possible. For this reason we will encourage them to weigh themselves at a Boots store (scales in Boots stores are regularly calibrated) or on regularly calibrated scales at another location for example a gym. If none of these options are possible for the participants, they will be able to weigh themselves on their home scales. We will report weight change at 12 months using weighed data, and self reported data separately as well as a combined figure of weighed and self reported data.

Likely uptake to sub study 4 and potential bias

We are classing this longer term follow up as a sub study because we wish it to be separate to the main study. We do not wish to put women off joining the main study which may happen if it is introduced to them as a year-long study rather than 4.5-6 months. Participation in Sub study 4 will be entirely voluntary and does not have to involve further visits to the Nightingale Centre. Large numbers of participants recruited to the study so far have requested a further review appointment after the main study has finished improving their chances of longer term success. We are therefore confident that the majority of women will wish to be included in this sub study and re attend for weighing thus providing a representative sample of participants and an indication of long term changes in our study population.

We acknowledge that this follow up data is unlikely to be complete and may be biased as less successful women may not wish to return for reassessment. Nevertheless we are keen to collect this data since the B-AHEAD 2 women are an important and unique cohort who have been in a weight control programme during treatment. They can provide some albeit possibly not complete data on what happens in the 6 months after chemotherapy and this intervention has ceased. The preliminary longer term data collected in this sub study will provide a basis for future trials designed to address the needs of patients at this time.

Collecting preliminary data on the key issues and support needs for patients in the transition after chemotherapy

Our moving on booklet will include a range of self-management tools used to promote weight maintenance in the non cancer setting and top-tips for prevention of weight gain from successful breast cancer patients in our previous trials. We anticipate this booklet will help but we will undertake in depth interviews (sub study 3) at the end of the 5-7 month trial extension to seek additional insight (from the patient's perspective) into how weight loss or maintenance of healthy weight can be sustained following chemotherapy. The moving on booklet will be used as a basis for women's ideas on how to improve patient information for weight loss maintenance. On the feedback from participants, we will redesign the booklet for use in future research. We will also explore their needs for peer to peer and on line support.

3.10. Sub-study 5: Circulating Micro-RNAs

Circulating micro-RNAs (miRNA) have been suggested to be robust and sensitive biomarkers. They have not only been used in patients with cancer to help measure disease burden, but have shown promise as sensitive detectors of tissue damage from

drugs. They have been recognised by regulatory authorities such as the FDA as valuable tools in the assessment of tissue specific damage. For example the liver-specific miRNA miR-122 is a sensitive biomarker of liver damage following paracetamol overdose.

The B-AHEAD 2 Study offers an opportunity in a well described cohort to answer the following questions

1) What is the impact of chemotherapy on circulating miRNA profiles?
2) Are circulating miRNAs more sensitive or specific to detect normal tissue damage following chemotherapy than the validated protein biomarkers assessed in this study (CK18 and FLT3 Ligand)
3) What is the impact of dietary restriction on circulating miRNAs

Approach

Residual serum samples from patients enrolled onto biomarker sub-study 1 will be used. Samples will be run on the Taqman Low Density Array Human panel A (Thermo Fisher Scientific corporation, California, USA) which robustly measures the levels of 384 human miRNAs.

We will compare in pooled serum samples:

1) Day 1 vs day 8 profiles
2) Day 8 profiles in patients with high self-reported toxicity vs not
3) Day 8 profiles in patients on intermittent dietary restriction vs those the Mediterranean diet

MiRNAs that are associated with toxicity in the pooled samples will then be analysed in individual patient samples using individual Taqman assays.

Samples will be analysed within the Good Clinical Practice Laboratory at the Northern Institute for Cancer Research in Newcastle University under the supervision of co-applicant Alastair Greystoke. No patient identifiable information or clinical data will be used in this analysis.

4. Statistics

4.1 Sample size

66 women in each group provides 90% power to detect differences in the change in body fat from baseline to 4.5-6.0 months between the IER and CER groups of ≥2.0kg (based on a simple t-test with a SD of change in body fat in our B-AHEAD-1 control group of 3.5 kg). Allowing for 20% attrition we will need to recruit 170 women. A detectable difference of 2.0 kg is achievable and clinically significant. A reduced weight of 2.7 kg was linked to 24% reduced recurrence in the WINS dietary trial (6). We estimate these numbers would allow us to have 80% power to detect a decrease in the prevalence of specific toxicities from 30% down to 10%.

4.2 Statistical methods

The primary analysis will be an intention to treat analysis to compare body fat measurements between the groups at 4.5-6.0 months using a last observation carried forward analysis of covariance (ANCOVA) adjusted for baseline levels. Secondary analysis

will compare change in body fat at 4.5-6.0 months in the 2 treatment groups stratified by adherence to the dietary regimen and changes in amounts of exercise to determine if there is an effect modification. Serum insulin and lipid assessments will not be made at set times in the menstrual cycle however day of menstrual cycle will included in the analyses for pre-menopausal women. Women on any diabetic medication will be excluded from HOMA analysis.

5. Timeline and Milestones

April 2012 – Feb 2013	Set up trial; obtain local hospital ethics approvals (10 months)
Feb 2013 – June 2014	Recruit to study (16 months).
Feb 2013 – Nov 2014	Delivering diet and exercise intervention (21 months)
July 2013 – July 2014	Qualitative study (12 months)
Dec 2014 – June 2015	Analysis write up, dissemination (6 months).
Total Length of project	8 months

6. Trial Governance

6.1 Trial Management Group

The Trial Management Group under the chairmanship of the Chief Investigator (MH) manages the trial on a day to day basis.

6.2 Trial Steering Committee

The Trial Steering Committee includes independent experts and monitors and supervises the progress of the trial towards its overall objectives including adherence to the protocol and patient safety.

Meetings are scheduled 6, 12, 24, and 30 months. Additional meetings will be schedules should the need arise.

The steering committee includes the lead investigator and co-applicants:

Dr Sacha Howell	Medical oncologist
Prof Anthony Howell	Medical oncologist
Prof Nigel Bundred	Breast surgeon
Dr Louise Donnelly	Qualitative researcher
Dr Lee Graves	Senior lecturer / exercise specialist
Dr Jane Wolstenholme	Senior health economist
Prof Judith Adams	Professor of radiology
Julie Morris	Senior statistician
Jeannie Willan	Patient representative

6.3 Trial Advisors

Prof Jane Wardle	Health psychology/behavior change
Lesley Thompson	Senior breast care nurse
Miss Janet Walls	Consultant breast surgeon
Mr Lester Barr	Consultant breast surgeon
Ms Helen Sumner	Clinical biochemistry
Ms Fiona Derbyshire	Research budget manager for breast services UHSM
Sian Hanison	Deputy Manager, research & development UHSM

6.4 Data Monitoring Committee

We have recruited a Data Monitoring Committee (DMC):, Daniel Rea, Medical Oncologist (University of Birmingham), Dr Adam Brentnall, (Biostatistics at Queen Mary University of London), and Dr Anna Campbell, Lecturer (Institute of Sport and Exercise, University of Dundee).

The DMC will be responsible for safeguarding the interests of B-AHEAD 2 participants, assessing the safety of the interventions during the trial and for monitoring the overall progress and conduct of the clinical trial. The DMC will approve a Charter (stating frequency of meeting and terms of reference, etc) early on in the trial before the first planned meeting six months after randomisation of the first participant.

6.5 Data Handling and Record Keeping

All data will be kept strictly confidential according to Good Clinical Practice (GCP) Guidelines. All data will be stored by the University Hospital of South Manchester NHS Foundation Trust in a secure fashion for 20 years in accordance with the ICH GCP. Weight data will be stored at local hospitals on a trial case report form during the trial. These forms will be transferred to the trial team at UHSM when patients complete the trial. The 'source data' for the study are the questionnaires, weight record sheets etc which are all stored in the participants' individual 'Participant Trial Files' kept in the Genesis research office in the secure area of The Nightingale Centre. Prior to statistical analysis, all data will be entered from the Participant Trial Files to secure databases that will be anonymous. We have developed an SOP for dealing with participant trial and medical records at UHSM. At the other 10 recruiting centres we require local PIs to be responsible for ensuring that their team meets local policies for documentation in both medical and trial records.

6.6 Clinical Risk Assessment

Acting on low weight, BMI and haemoglobin results
The study team will collect weight and haemoglobin levels from participants at their fortnightly phone calls. If a participant has not recorded this information or if the study team have concerns about the validity of this information they will contact the relevant hospital team in order to create an accurate and complete record. The study team will calculate percentage weight change and alert the participant's medical oncologist and chemotherapy nurse if weight loss or gain exceeds 10% as this may require a reassessment of chemotherapy dose. BMI will be calculated and study dietitians will give advice on increasing calories if BMI goes below 19 kg/m^2. If haemoglobin goes below 9 g/dl the study team will advise the participant to refrain from moderate or vigorous activity until they have been informed of a subsequent result above this level.

Exercise
The interventions encourage patients to increase their level of moderate cardiovascular and resistance exercise which may present a minimal risk of cardiovascular morbidity, fractures, and complications in the affected arm, e.g. cording or lymphoedema. Baseline and final assessments include resting blood pressure and heart rate, and a exercise test on treadmill. These assessments are repeated at the end of the trial. Any cardiovascular, musculo-skeletal or arm problems identified during the trial will be referred to appropriate care pathways for assessment and treatment.

Our exercise interventions are tailored for breast cancer patients. All participants will be encouraged to gradually increase the amounts and intensity of exercise. As stated in section 2.6, women will be encouraged to build up to 2.5 hours (5 x 30 minutes) of moderate activity/week (at 60–80% maximum heart rate) according to published guidelines for patients receiving adjuvant chemotherapy (45). This advice will be individualised and tailored according to current activity levels, participant preferences, abilities, co-morbidities and energy levels.

For women receiving Herceptin the B-AHEAD study team will inform the participants' Herceptin nurse team that the participant is on the study. The Herceptin nurse team will then inform the B-AHEAD team of echocardiogram results (usually before first Herceptin

generally started at chemotherapy cycles 4-6, and at 5th Herceptin, i.e. 15 weeks later if they are still on the study at time) if ejection fraction (EF) is < 50%. In such cases, exercise advice will be altered thus:

- If EF <40% or < 50% with breathlessness we will advise participants to refrain from all moderate and vigorous exercise until reviewed by cardiology. The B-AHEAD 2 study team will seek outcome of cardiology review and follow that advice.
- If EF 40-50% with no breathlessness we will advise participants to refrain from vigorous exercise but moderate exercise will still be encouraged.

Participants receiving Herceptin will be asked about symptoms of breathlessness at each fortnightly phone review after starting Herceptin and advice will be altered accordingly. Should symptoms of breathlessness develop, participants will be advised to inform their chemotherapy team.

The fitness tests will be undertaken at the exercise facility at UHSM which has resuscitation equipment and is covered by the main hospital crash team. In accordance with the UHSM trust resuscitation policy a member of trained nursing staff would be available to assist the trial exercise specialist in the unlikely event of an adverse event during a fitness test.

Arm function

The exercise intervention will focus on cardiovascular and resistance exercise and we will reinforce standard arm rehabilitation post breast surgery by ensuring all patients have received standard exercise leaflets. Our team has experience of recognising post breast surgery arm problems and will refer women to the breast physiotherapy services.

Serum blood tests

Fasting glucose will be assessed in all participants at the start and end of the trial. Lipid analyses for both time points will be conducted at the end of the trial. Blood test results will be fed back to the patient's general practitioner for information / appropriate assessment and treatment.

Blood pressure and phlebotomy will be undertaken on the non-operated arm to minimise the risk of lymphoedema. Where possible women will have their trial blood samples collected at the time of routine chemotherapy blood tests to minimise venepuncture.

DXA scans

Total body DXA scans estimate total bone density. We will seek guidance from Pam Coates, Lead Radiographer, Bone Densitometry at UHSM on required action for patients reported to have T score of < -2.0.

Other Identified problems

The ongoing communication with trial participants during treatment means other psychosocial or medical issues will be raised and communicated to the trial team. The trial team will refer patients to appropriate services for further assessment, treatment or psychosocial support.

Generic Risk Assessment

Hazards to patients, study and organisation have been perfomed for the B-AHEAD-2 trial and have been considered low risk.

Predicted/possible deviations from the protocol and adverse events

The following is a list of possible/predicted deviations from the protocol and adverse events that would not require reporting to the sponsor or ethics committee:

Deviation / adverse event	**Action**
The participant does not receive chemotherapy once randomised	Participant is withdrawn from the study
The participant does not receive the full, expected course of chemotherapy	Number of cycles given is documented in participant's study file. Whether participant is encouraged to stay on study will be agreed on an individual basis by discussions with the participant, and the nursing and oncology teams if appropriate.
Chemotherapy regime is not given according to initial schedule, e.g. if one or more cycles are delayed for medical reasons	Dates of chemotherapy cycles are documented in participant's study file. Participant remains on study. Final appointment is still after final chemo and may be later than initially estimated.
Blood samples cannot be taken	See Blood Sampling SOP (Appendix 8). Participant is encouraged to stay on study.
Participant does not (fully) complete required food diaries	Participant is encouraged to remain on study. Food diary checked through with participant and more detail added if possible. Participant is encouraged to complete next food diary fully, if applicable.
Participant does not wear accelerometer (for full time)	Participant will be encouraged to remain on study and wear accelerometer as much as possible during the monitoring period.
Participant cannot adhere to or does not wish to adhere to allocated diet	Participant will be encouraged to remain on study as they are providing very useful information to the study team. They will be encouraged to stick to their allocated diet as best as they can. Researcher will document reported adherence and reasons for not following allocated diet in participant's study file.
One or more chemotherapy treatments are delayed and toxicity scales cannot be completed for last 3 weeks.	Scales to be completed at next chemotherapy treatment and participant encouraged to give answers based on how they were feeling in the three weeks after last chemotherapy.
Participant would prefer advice over phone rather than prolong study visit	Both diet and exercise advice can be given over the phone instead of during the baseline appointment at the Nightingale Centre and Genesis Prevention Centre as planned. Following a such a phone consultation, written targets will be posted to participant.
Participant had bilateral lymph node surgery so Blood Sampling SOP cannot be followed	Blood sampling not attempted, participant informed of reasons and outcome documented in their study file. Participant encouraged to stay on study as still providing other valuable information.
The full complement of blood sample bottles cannot be filled	See Blood Sampling SOP (Appendix 8). As many as possible are filled. Outcome documented in

	participant's study file.
Participant cannot attend for final assessment at the Genesis Centre 3 weeks after final chemotherapy	Final assessment is to be arranged as close to 3 weeks after final chemotherapy as possible.
Pregnancy during study	In the unlikely event of a pregnancy occurring during the study, the participant will be withdrawn from the study. Outcome documented in participant's study file. Pregnancies will not be tracked as part of the study.
Hospital clinician / GP prescribes additional medication (e.g. for constipation) during study period	Medication, date started and duration to be documented in participant's study file at start and end of study. Also document if researcher has given additional diet/lifestyle advice (e.g. for constipation).
Participant has hospital admission as a result of chemotherapy (e.g. infection, neutropenia) during study period	Length of hospital admission to be documented in participant's study file. Diet and exercise advice will be reviewed following a hospital admission to ensure it is correct.

All protocol deviations and adverse events listed above and other minor deviations not listed will be documented in participant's study file stating the event and action. Serious protocol deviations and serious adverse events not listed above and specifically related to the diet / exercise intervention will be notified to the Chief Investigator and Sponsor and an appropriate course of action will be determined and documented. Complications or side effects commonly associated with cancer and/or chemotherapy, e.g. septicaemia, neutropenia, deep vein thrombosis and hospitalisation due to infection will not be reported. If reporting is required this will follow the flow chart in Appendix 9.

PIs at recruiting centres other than UHSM will be responsible for their own reporting of serious adverse effects according to their Trust protocols, and send a copy to CIs research team at UHSM.

Appendix 1

DXA scan methodology and analysis

Ordering DXA Scans

Designated B-AHEAD 2 Study staff can order the total body DXA scans on the UHSM electronic system. Such staff must have undergone UHSM IRMER training and be aware of main contraindications for DXA scans which are:

- suspected pregnancy
- recent (<5 days) oral administration of a contrast agent
- recent (<2 days) nuclear medicine scan.

Ordered scan are then verified by a radiologist before the DXA scan takes place.

Procedures for Performing Whole Body DXA Scans: Preparing the Patient

Before the subject lies down on the scanning table they will be questioned and examined for any metal objects that could be in the scanning field. Objects include earrings, glasses, wrist watches, coins, rings, other jewellery, buckles, metal zips, metal studs, under wired bras and breast prostheses. The subject will remove their shoes and any of the above metal items and it may be necessary to remove skirts, slacks etc. Light clothing without metal studs, zips or buckles will not affect the results. In some cases it will be necessary to provide a gown to wear during the scan. If rings cannot be removed they will be left on and a note made so that they are left on during subsequent scans. All the objects removed from the patient will remain in the DXA room and a check will be made with the subject at the end of scanning that everything has been returned.

Subject Positioning for Whole Body DXA Scanning

The patient will be asked to lie down on the scanning table. The radiographer will ensure they are lying centrally to the table and positioned appropriately.

Any variables will be noted, e.g. inclusion of a pillow, so that they can be repeated in subsequent scans.

The radiographer will ensure that the patient's entire body is within the edges of the scanning field marked on the mattress as far as possible. The hands should be placed palms down with the fingers together alongside the body and there should be a gap between the hands and the hips. If the patient is too large the hands should be positioned for the best fit. For follow-up scans the radiographer will have a print out of the baseline scan available and make sure that the patients hands and feet are positioned as identically as possible.

The patient will be instructed to keep very still until the scan is finished and take care not to move their hands or feet.

DXA analysis

Our previous B-AHEAD-1 study has highlighted that a number of patients will have artefacts which will influence DXA measurements including: Breast tissue expanders (10%), silicone breast implants (8%), pins/plates, metal joint replacements (3%) and arm lymphoedema (2%)**.** These artefacts are excluded from the scan by using an accepted 'mirroring' technique e.g. if the left knee has a metal replacement then data from the right

leg is mirrored for the left leg. This will be undertaken by experienced DXA researchers under the supervision of Professor Judith Adams (University of Manchester).

Appendix 2

Exercise test on treadmill and other measures of fitness

Participants will undertake predicted functional aerobic capacity testing using an exercise test on a treadmill in the gym facility in the Genesis Centre. The test we will be using is a six minute walk test during which participants can choose their own speed (68). The test will be performed on a treadmill (69). This test will be performed at the baseline and final assessment appointments. This test has been used in patients with severe chronic heart failure and cancer patients receiving chemotherapy. Heart rate will be monitored during the test and the test will be aborted if heart rate reaches maximum expected level for age. Participants will be familiarised with the treadmill before the test, they will be able to request termination of the test at any time, and they will wear a safety device to allow them to quickly stop the treadmill during the assessment should the need arise.

Participants will undertake pulmonary function testing using a spirometer to measure lung volume. This non-invasive test involves two breaths into the spirometer and will be performed prior to chemotherapy and 3 weeks after final chemotherapy. Measurements will be taken at rest and after the fitness test (65, 66).

Participants will undertake muscular strength testing using a handgrip dynamometer. This non-invasive test involves squeezing the dynamometer as hard as possible with the hand 3 times in succession. This test will be performed prior to chemotherapy and 3 weeks after final chemotherapy. Measurements will be taken at rest and after the fitness test (65, 66).

Some patients will not be able to perform predicted functional aerobic capacity testing using an exercise test on a treadmill in the gym because they are contraindicated to exercise (see table) or they do not wish to participate.

Unstable angina- angina at rest, unpredictable episodes or diagnosed within the last month
Unstable or acute heart failure
Ventricular or aortic aneurysm
A CVD patient who has not had a negative ECG stress test or recent satisfactory attendance at a Cardiac Rehab programme
Recent deterioration of symptoms from any cardiovascular or respiratory condition
Unstable diabetes
Uncontrolled arrhythmias
Uncontrolled tachycardia- heart rate > 100 bpm at rest
Resting systolic BP>180 mmHg or resting diastolic BP>100 mmHg
Symptomatic hypotension- low blood pressure with fainting , dizziness
Febrile illness
Medications must be present: GTN spray or Ventolin or both

Appendix 3

Blood pressure and resting heart rate

Guidelines for assessing resting blood pressure and pulse

- Measured after at least 10 minutes rest
- Cuff to be placed on opposite side to breast surgery
- Patient should be relaxed and not talking during measurements
- Repeat 3 measurements

Actions if raised blood pressure/ pulse

White coat syndrome will increase systolic and pulse rate, but have less effect on diastolic BP.

Repeat measurements later during appointment. Ask patient if they monitor their own blood pressure at home or have had a diagnosis of white coat syndrome.

Do not undertake fitness test and refer to GP if consistent levels are found after 3 repeat readings :

Systolic BP > 160 mmHg
Diastolic BP > 100 mmHg
Resting Heart Rate > 100 bpm

Patient to be fast tracked to be seen by GP if consistent levels are found after 3 repeat readings (phone call/fax to GP Surgery) if:

Systolic BP > 200 mmHg
or Diastolic BP >120 mmHg
but patient is not symptomatic (no breathlessness and/or no headache)
Resting Heart Rate > 120 bpm

Patient to attend A&E if consistent levels are found after 3 repeat readings:

Systolic BP > 200 mmHg
or Diastolic BP >120 mmHg
and is symptomatic (breathlessness and/or headache)
Resting Heart Rate < 50 bpm without medication
Resting Heart Rate >140 bpm

Appendix 5

Fortnightly trial mailings for the IER and CER groups.

Mailing	Topic
1	Ways to get motivated for diet and exercise change • Listing pros and cons (decisional balance) • Increasing your confidence • Goal setting: short, intermediate and long term • Sharing tips to get and stay motivated • Finding support. • Overcoming barriers
2	Energy balance: energy restriction, physical activity & healthy eating • Possible benefits of energy restriction • Weight gain and energy balance • Body fat and health and where it's stored • Energy density of food
3	Healthy eating • What is a Mediterranean diet? • 5-a-day • Supplements and vitamins • Foods for your immune system (from B-AHEAD booklet) • Fibre including ready reckoner done for book
4	Coping with breast cancer and chemotherapy • Diet and energy related side effects • Fatigue • Getting enough sleep • Planning
5	Healthy cooking • Healthy cooking methods • Simple recipes • Healthy snacking, packed lunches
6	Mood and hunger, food cravings • Dealing with cravings • Treating yourself in ways other than food • Gaining control over eating
7	Being active: A way of life • Health benefits of exercise • How much and how hard, safe exercise • Incorporating physical activity into everyday life • Activity options in the local community
8	Dairy / soya / live yogs / bone health / salt / calcium chart / bone pain
9	Tips for shopping and eating out • Healthy options for eating out • Shopping • Reading and understanding food labels
10	Drinks and sauces • Alcohol • Caffeine • Fizzy drinks • Sweeteners • Fructose • Sauce info
11	Problem solving • Taking charge of negative thoughts • Body image and self esteem • Hand massage
12	Diet myths

Appendix 6

Schedule of trial blood samples

Assays	Baseline	Chemo 1 day 1	Chemo 1 day 8	Final chemo day 1	Final chemo day 8	Post chemo
Main study (N= 170) Serum Plasma Glucose Full blood count	 2 2 1 1					 2 2 1 1
Sub-study 1 Toxicity markers (N= 70) Serum Plasma		 1 1	 1 1	 1 1	 1 1	
Sub-study 2 Effects of IER days N = 25 Serum Plasma Glucose Full blood count				 1 1 1 1		

Sample collection

Baseline and post chemo fasting blood samples will be collected during their baseline or post intervention trial assessments at UHSM. Women with difficult or limited venous access or portacath will have these fasting samples taken at the Christie chemotherapy unit, and spun, aliquoted and processed at Christie Hospital then transported to biochemistry at UHSM. Both sets of samples will be stored in The Nightingale Centre, UHSM.

Cardiovascular markers and insulin will be measured by Clinical Biochemistry at UHSM.

Insulin will be measured by a commercially available chemoluminescence immunoassay and glucose by a Hexokinase/glucose-6-phosphate dehydrogenase method, triglyceride using Glycerol Phosphate Oxidase assay, total cholesterol using Enzymatic assay, and HDL cholesterol using Accelerator Selective Detergent to established protocols using commercialy available assays (All Abbot Laboratories, Wiesbaden Germany). LDL cholesterol will be calculated using the formula of Friedewald et al.:

LDL = TOTAL CHOLESTEROL - HDL – TRIGLCERIDE /5.0 (mg/dL)

All assays will be batched and performed on stored serum (–70ºC) upon completion of the study to reduce inter-batch variation.

Lipid samples serum SST (orange top blood tube)
Samples should be centrifuged and the serum separated from the cells. Serum can then be stored at 2-8°C for 5 days or 1 month at -15 to -25°C before storing at -70°C.

Insulin serum SST (orange top blood tube)
Samples should be centrifuged as soon as possible after collection to separate the serum from the cells. Serum can then be stored for 24 hrs at 2-8ºC or 1 month at -15 to -25ºC before storing at -70°C

Glucose fluoride (grey top blood tube)
Analyse as soon as possible or store at 4°C. Freezing and long term storage of these samples is not recommended.

Breast cancer related biomarkers (green top blood tube) and full blood
High sensitivity C–reactive protein (hs CRP), breast cancer related adipokines: fasting plasma leptin (enzyme immunoassay) adiponectin, total IGF-I and plasma and red blood cell (RBC) markers of oxidative stress will be analysed by our collaborators Mark Mattson and colleagues in the National Institute of Health.
Plasma (from Heparin tubes) will be pipette transferred into Cobas specific sample tubes. For the whole blood (from K3EDTA tubes) samples, a cell lysis buffer is added and cell fragments centrifuged (1000 G) into a pellet; the supernatant will be pipette transferred into Cobas specific sample tubes for analyzing SOD, Catalase, and Glutathione peroxidase.

CRP, leptin and adiponectin require 1 ml of plasma and IGF-I 100ul of serum.

Oxidative stress markers require one 800 uL aliquot of plasma (from Heparin tube), and one 800 uL aliquot of whole blood (from K3EDTA tube).

All use of donated plasma and serum will have to be approved by both the institution and the local Ethics committee prior to utilisation of samples. Patients and treating physicians will not have access to the results of any specific assays performed as part of the translational research programme. However it is anticipated that the results of this sub study will be presented to the general scientific community in the form of presentations at international meetings and publications in peer-reviewed journals.

Biohazards and safety requirements to be met with the handling of all human blood samples; the procurement, handling and storage of blood specimens will be undertaken by personnel who have received training in the risks associated and safety requirements. Standard operating procedures are in place to comply with good clinical practice and good laboratory practice.

Collection, storage and transport of toxicity marker samples (substudy 1)
Toxicity marker blood samples can be taken at any of the recruiting hospitals where they will be stored until the end of the study then transported to the CRUK Manchester Institute at The Christie NHS Foundation Trust. Samples collected at The Christie will be stored in the CRUK Manchester Institute until the end of the study. All samples will be stored and collated here according to standard operating procedures in the Christie Hospital then

transferred to Dr Alastair Greystoke at the Northern Centre for Cancer Care at the Freeman Hospital, Newcastle-upon-Tyne.
Samples will be anonymised by assignment of a study code and logged to a database prior to storage and /or delivery to the laboratories where the research will be performed. A copy of the sampling log that will not contain any identifiable information will accompany samples. The custodian of the samples will be:

Dr Sacha Howell
CRUK Manchester InstituteChristie Hospital NHS Trust
Wilmslow Road
Manchester
M20 4BX

Collection, storage and transport of substudy 2 samples
Substudy 2 samples can be taken at any of the recruiting hospitals. The recruiting hospital will be responsible for analysing the glucose sample according to their standard operating procedures. This result will be transferred to the B-AHEAD 2 Study team at UHSM in accordance with the Data Protection Act 1998. The other samples will be processed and stored at the recruiting hospital under suitable conditions until the end of the study when they will be transported to The Nightingale Centre and Genesis Prevention Centre at UHSM.

Responsibility for transfer of samples
The B-AHEAD 2 Study team at UHSM will be responsible for monitoring transfer and receipt of biological specimens. Tracking forms will be sent by centres to the B-AHEAD 2 Study team at UHSM to monitor the transfer of all biological samples. All data will be handled, computerised and stored in accordance with the Data Protection Act 1998. Any transported samples should be kept frozen during the transport.

Appendix 7

Schedule of assessments and venepuncture for trial participants

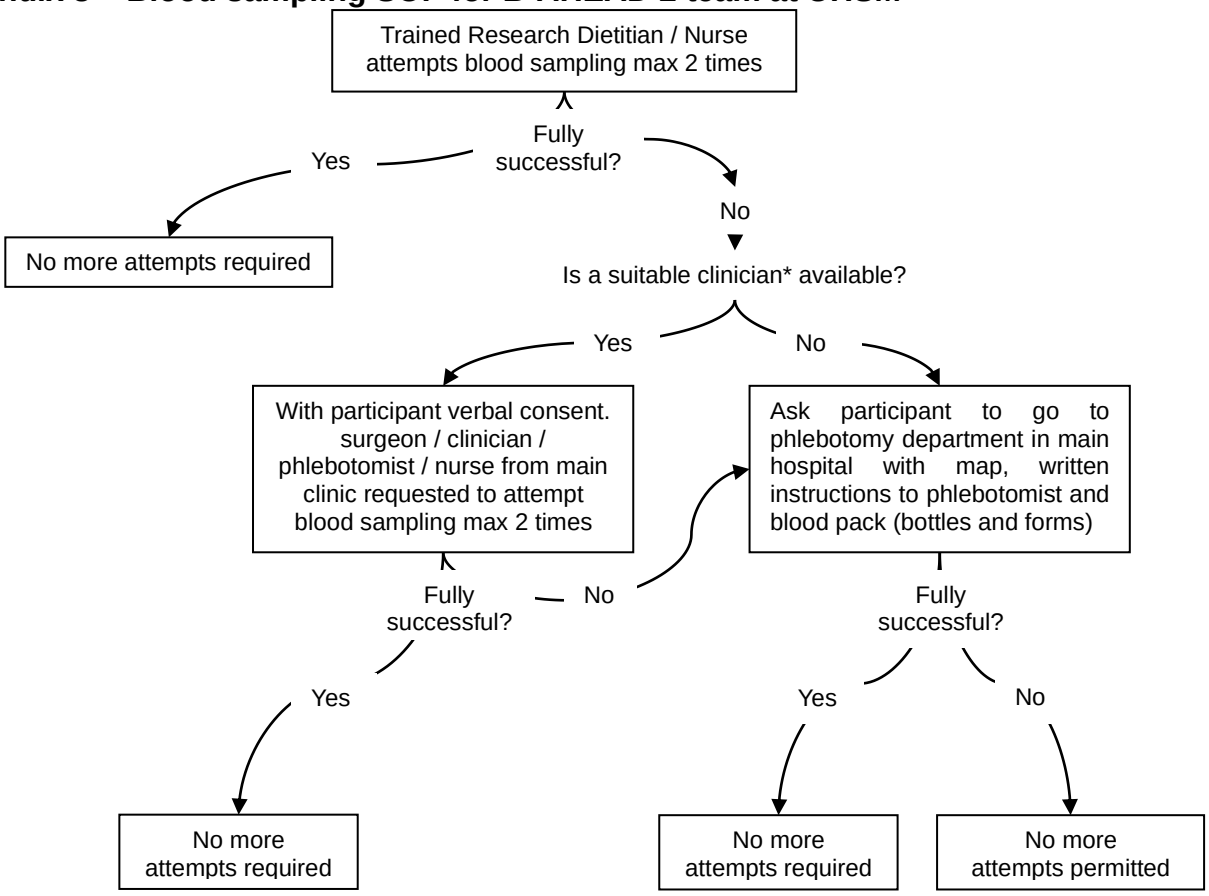
B= baseline PC= post chemotherapy

Main study

Outcomes	**Measure**	**Timings**
Weight, height, waist and hip	Calibrated scales, stadiometer and tape measure	B PC
Body fat and fat free mass	Total body DXA, impedence scales	B PC
Blood pressure		B PC
Insulin, glucose, Homa	Blood test	B PC
IGF-I and adipokines	Blood test	B PC
Lipids, CRP	Blood test	B PC
Oxidative stress markers	Blood test	B PC
Fitness		B PC
Quality of life	Questionnaire	B PC

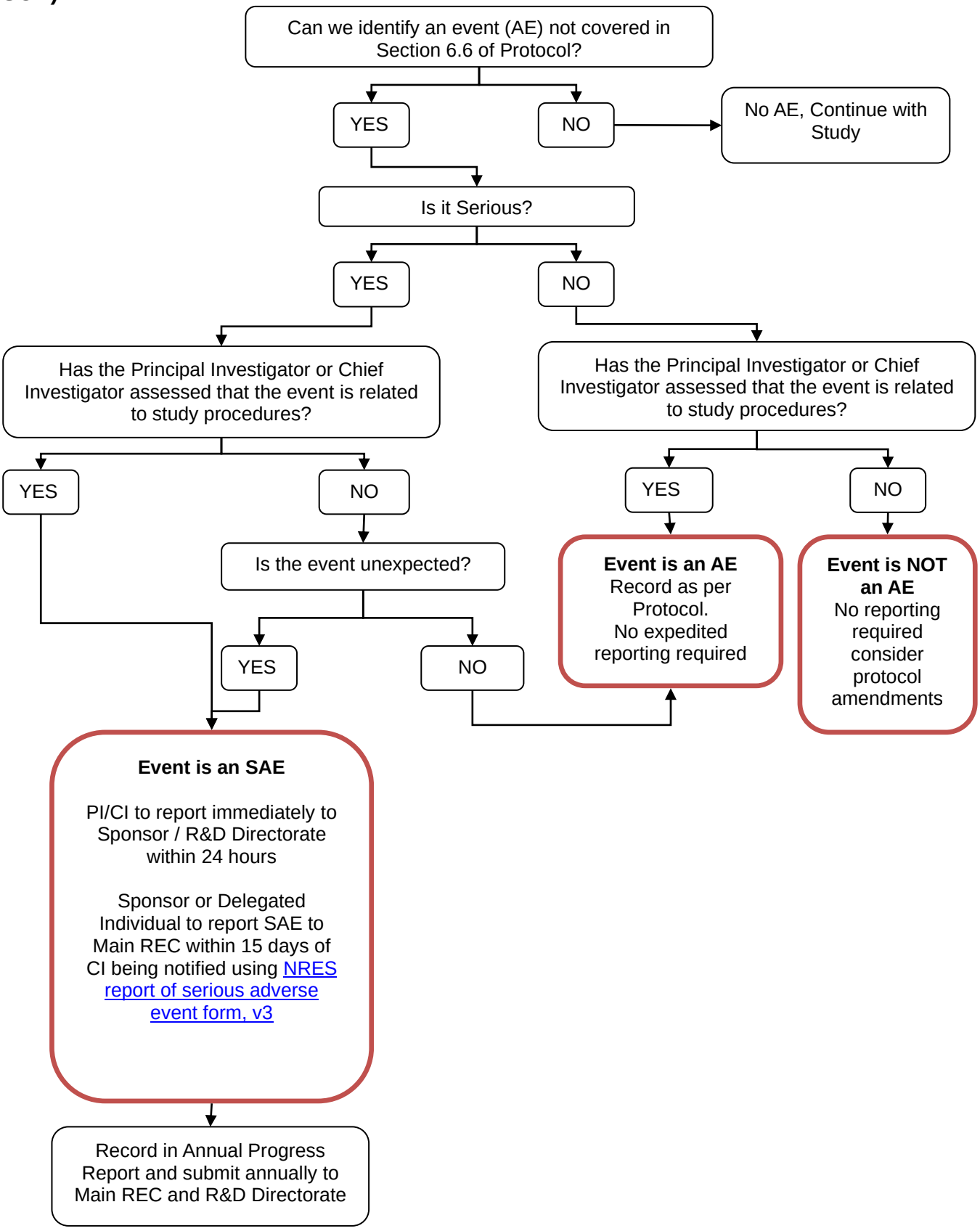
Motivation / self efficacy	Questionnaire	B PC
Chemotherapy toxicity	Questionnaire	In retrospect at next chemotherapy treatment
Oxidative stress markers	Blood test	B PC
Diet	7 day food diary	B PC & for 3 weeks of 4th chemotherapy cycle
Physical activity	Questionnaire Actigraph activity monitor	B PC & for 3 weeks of 4th chemotherapy cycle
Sub study 1: Blood markers of toxicity 45 IER and 45 comparison group	Blood test	4 additional samples on day 1 and 8 of chemotherapy: Cycle 1, day 1 and 8 Final cycle, day 1 and 8
Sub study 2: 25 women in IER group	Blood test	1 additional fasting blood sample at time of final chemotherapy blood test

Appendix 8 – Blood sampling SOP for B-AHEAD 2 team at UHSM



Notes:
- All blood from arm opposite to lymph node surgery.
- *suitable clinician could be a surgeon, doctor, nurse or phlebotomist with good experience of taking blood samples.
- Participants will have a maximum of 5 attempts at blood collection.

Appendix 9 – Adverse Event Decision Making and Procedure (UHSM R&D SOP)



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