Full title:

B-AHEAD 3

Breast - Activity and Healthy Eating After Diagnosis – 3

A randomised phase II trial of intermittent energy restriction and resistance exercise in women receiving chemotherapy for advanced breast cancer.

Short title: B-AHEAD 3; Breast - Activity and Healthy Eating After Diagnosis 3

B-AHEAD-3 Protocol	**Version 7**	**2nd October 2018**

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

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Dr Michelle Harvie - 23rd September 2014

1. Introduction

1.1 Rationale

Excess adiposity and reduced lean body mass has been linked to reduced survival and quality of life in women with advanced breast cancer (ABC). Preclinical models have shown that energy restriction and weight control protect normal cells from the effects of chemotherapy whilst increasing the sensitivity of malignant cells to oxidative stress, apoptosis and DNA damage – the so-called 'differential stress response'. Whilst patients with reduced lean body mass have increased chemotherapy toxicity and poorer outcome. The B-AHEAD 3 trial will randomise women initiating first, second or third line treatment for ABC to energy restriction and resistance exercise vs normal diet and resistance exercise. B-AHEAD 3 will test whether an energy restricted diet adds to the antitumour effect and ameliorates toxicity of chemotherapy compared to an exercise only intervention in the clinical setting.

2. Background

2.1 Weight loss, energy restriction and outcome amongst patients with ABC

Being overweight or gaining weight during treatment increases relapse and reduces survival in women with early breast cancer (1, 2), whilst weight loss / energy restriction may improve survival (3). Excess weight has also been linked to a poorer survival amongst patients with ABC, in some (4) (5-7), although not all studies (8), and to reduced quality of life (9). In a previous study of serial measures of weight, adiposity and energy balance amongst ABC patients receiving chemotherapy we demonstrated potentially detrimental gains in adiposity associated with increased energy intake during chemotherapy (10).

In animal models, energy restriction reduces both tumour growth and tumour progression (11, 12) (13). This reduction is mediated by well characterised metabolic and signalling changes which occur during energy restriction affecting both the tumour cells and tumour stroma (reviewed in Ford et al 2013 (14)). Energy restriction reduces serum concentrations of multiple host factors including insulin, insulin-like growth factor-1, leptin, steroid hormones, cytokines, vascular regulators, inflammatory mediators and increases adiponectin. In addition reductions are also seen in cellular and structural components of the tumour microenvironment, including hypoxia inducible factor-1 (HIF-1) (15), haem oxygenase-1 (HO-1), vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), and reductive oxygen species and hydrogen peroxide (16). These secreted and structural host factors are extrinsic to, but interact with, the intrinsic molecular characteristics of breast cancer cells (including breast cancer stem cells). A recent animal study reported that energy restriction reduced epithelial-to-mesenchymal transition and reduced tumour progression in aggressive murine claudin-low and basal-like mammary tumour models (17). Similarly energy restriction reduced the metastatic potential of triple-negative breast cancer by down regulating the production of extra cellular matrix and metastasis associated proteins (18). These studies suggest that the benefits of energy restriction are likely to be realised across the different histological subtypes of breast cancer (12) (18) (19).

2.2 Possible mechanisms mediating the link between weight, energy restriction and outcome amongst patients with ABC

The adverse effects of excess weight and weight gain on prognosis in breast cancer patients are thought to be largely mediated by reduced insulin sensitivity, impaired glucose tolerance and hyperinsulinemia (20). Reduced insulin sensitivity and impaired glucose tolerance have been reported in 50% of patients with metastatic breast cancer (MBC) in two recent cohorts where it was linked to poorer response (7) (21). In women receiving paclitaxel chemotherapy for MBC, Stebbing et al reported clinical benefit rates of 40.5% vs 65.8% in women with and without impaired glucose tolerance respectively (7). In a multivariate analysis the odds ratio (OR) of disease progression per unit increase in fasting glucose was 5.47 (95% CI 2.05–14.60). Similarly, Gennari et al reported a median progression free survival (PFS) of 8 months (IQR 2-17) in MBC patients with insulin resitance (HOMA index >2.5) compared with 14 months (IQR 8-18) in patients with normal insulin sensitivity (HOMA index ≤2.5) (n = 87 standard). The multivariate hazard ratio of disease progresion was 2.28 (95% CI 1.06–4.89) in patients with insulin resistance (21). Insulin resistance is common amongst these patients and can be further increased during chemotherapy because of weight gain, direct cellular effects of chemotherapy and the use of corticosteroids as anti-emetics and to prevent reactions to the taxanes (20, 22).

Energy restriction is thought to act partly down stream of insulin through inhibition of the mTOR signal transduction pathway. The likely benefits of energy restriction in patients with MBC are suggested by beneficial effects of mTOR inhibitors such as everolimus (Ev) which has been shown to improve PFS when given in combination with endocrine therapy (23, 24) and chemotherapy (23). However, drugs such as Ev are associated with a significant increase in toxicity. Our hypothesis is that weight loss and energy restriction in advanced disease will phenocopy mTOR inhibitors in extending PFS but, in contrast to pharmacological mTOR inhibition, will reduce toxicity and improve quality of life. It is also worth noting that energy restriction mimetics such as metformin are also being tested in MBC (25-27).

2.3 Weight, energy restriction and toxicity of and response to chemotherapy

Obesity may specifically reduce the efficacy of chemotherapy. Obese patients with breast cancer consistently have a poorer response to neoadjuvant chemotherapy, even with adequate dosing (28-31). Obesity leads to resistance to gemcitabine chemotherapy in an animal model of metastasis. Impaired response was linked to impaired blood flow, altered pharmacokinetics of tissue and tumour distribution and altered expression of genes related to uptake and metabolism of chemotherapeutic agents (32). Reduced response to neoadjuvant chemotherapy has recently been reported in patients with increased hepatic stearoyl co A desaturase (SCD) activity (the rate limiting enzyme of fatty acid biosynthesis), which occurs in obese insulin resistant subjects. Specifically SCD activity is increased in association with high circulating insulin and leptin, increased intake of carbohydrate and saturated fat and a low intake of n-3 fats (33).

In preclinical models, energy restriction protects normal cells from the effects of chemotherapy and radiotherapy, whilst increasing sensitivity of malignant cells via increased oxidative stress, apoptosis and DNA damage – the so-called 'differential stress response' (34-37). A recent paper reported that 2 days of fasting pre chemotherapy promoted fat oxidation and hematopoietic stem cell augmentation and stem cell regeneration and protection of lymphocytes from chemotoxicity (38). These data indicate that energy restriction may reduce chemotherapy associated toxicity and increase the effectiveness of treatment. The National Institute Health trials register Clinicaltrials.gov (USA) currently has six ongoing small scale trials of fasting and chemotherapy toxicity (two in the Netherlands, one in Germany and three in the USA), and one USA study assessing radiotherapy toxicity (39) in addition to a further eight low carbohydrate diet trials (all in the USA) amongst patients with advanced cancer patients with breast and other cancers (accessed on 13th August 2014)(40).

2.4 Rationale for testing an intermittent energy restricted diet in advanced metastatic breast cancer patients

The main aim of dietary intervention is to reduce energy intake. Our recent review of dietary intervention studies in animals (manuscript in preparation) indicates that energy restriction, mainly achieved by reduced carbohydrate intake, is associated with reduced tumour growth and progression in mice with orthotopically transplanted metastatic tumours (11, 12, 41), or with carcinogen induced mammary tumour models (42, 43).

In the trial outlined here we plan to use intermittent energy restriction (IER). Our previous studies suggest this may be the most efficacious way to achieve energy restriction. Previous randomised trials in women without breast cancer indicate that IER is associated with greater weight loss, preservation of fat free mass and reduction of insulin serum concentrations compared with standard continuous energy restriction (CER). The IER diet we have developed involves two consecutive days of severe energy restriction and five days of normal healthy eating. (44). We have initiated a second trial specifically to test this IER amongst women receiving adjuvant or neoadjuvant chemotherapy (B-AHEAD 2) for early breast cancer. Our preliminary data indicate that the IER taken for just 2 days per week is more effective for weight control during chemotherapy than standard daily dieting, since the 2 restricted days can be administered between cycles of chemotherapy (45).

Overall fat intake is reduced on IER, mainly through reducing saturated and polyunsaturated n-6 fats, whilst maintaining intake of monounsaturated fat and promoting n- 3 fats. Previous studies indicate that reducing intake of fat, mainly n-6 fats without reducing calories reduced growth and metastases of breast tumours transplanted into athymic nude mice (46) and Wistar rats (47), but not in two further studies amongst Wistar (48) and Fisher rats (49). Phase 2 studies have reported supplementation with the n- 3 fat docosahexaenoic acid (DHA, 1.8 g/day) improves the efficacy of chemotherapy amongst patients with MBC, possibly due to altered cell membrane structure and increased chemotherapy uptake and enhancement of the oxidative stress generated by anthracylines due to peroxidation of DHA (50, 51). The optimum protein intake for energy restricted women with ABC receiving chemotherapy needs careful consideration. Patients need to receive adequate protein to maintain lean body mass. Maintained lean body mass (not BMI or fat stores) is essential to limit chemotherapy toxicity. Recent studies indicate that chemotherapy toxicity is inversely related to lean body mass rather than any other measurable parameter (52). There are few data to guide optimal protein intakes to maintain lean body mass in non-cachexic cancer patients. A recent review recommends 1.2 g per kg body weight (53), which is comparable to recommendations for weight loss patients in the non-cancer setting and also the amount in the IER diet (54).

Higher protein intake maintains lean body mass as it suppresses gene expression in the ubiquitin–proteasome pathway in skeletal muscles to prevent atrophy and promotes muscle protein synthesis via the mTOR signalling pathway (55). The potential for higher protein intakes, specifically branch chain amino acids to stimulate mTOR and growth of tumours in cancer patients is a potential concern. This has been shown in two animal studies in a carcinogen induced mammary tumour (56) and a human ER +ve, PR and HER -ve xenograft model (WHIM16) (57). In the latter study there was a two fold increase in tumour growth with an isocaloric diet which provided 21% compared to 7% of energy as protein linked to stimulation of the IGF/AKT/mTOR pathway and epigenetic effects (57, 57).

With the exception of these data the balance of evidence from the majority of animal and human studies indicates that higher protein intakes will not promote tumour growth amongst breast cancer patients. Reduced tumour growth and metastases with energy restriction has been achieved with maintained protein intake in the animal studies described above (11, 12, 41) (42, 43). Reduced relapse events amongst early breast cancer patients in the Women's Intervention Nutrition Study were achieved with a 10% energy restriction and a 50% reduction in fat intake and maintained protein intake (1g/kg body

weight) (OR 0.76 [95% CI = 0.60 to 0.98]) (3). Higher protein intakes are consistently linked with improved outcome amongst breast cancer patients (11, 12, 41), perhaps because they enable preservation of lean body mass. Trials of nutrition support have mainly found protein and branch chain amino acids stimulate muscle, liver and albumin synthesis and have minimal effects on tumour protein synthesis. Hence protein intake appears to favours host anabolism over tumour growth in cancer patients (53).

Our IER diet will therefore aim to maintain protein intake at 1 – 1.2 g per kg per day on restricted and unrestricted days. We anticipate the intermittent diet group will reduce energy intake by 30% overall and based on our previous data amongst patients with ABC the control group are likely to increase intake by 10% as is usually seen during treatment with chemotherapy (58). Thus overall our intervention group will have a 40% lower energy intake than our control group.

2.5 The importance of maintaining lean body mass in patients with ABC and rationale for the resistance exercise intervention

Recent data from Canada using CT scans to evaluate body compartments indicate 25% of patients with ABC (20% of the overweight and obese patients) have reduced lean body mass (sarcopenia) at the initiation of chemotherapy, and that chemotherapy toxicity and poorer outcome is specifically related to reduced lean body mass and not fat mass or BMI (52, 59). Likewise Aslani et al found reduced body nitrogen to be a key predictor of neutropenia with CMF chemotherapy amongst patients with early and ABC (60)

We have published serial measurements of body composition in an observational study of patients with MBC receiving chemotherapy (who did not receive diet or exercise advice) and reported a mean (95 % CI) loss of lean body mass of 1.9 kg (-4.9 to 1.1) during a 5 month period of chemotherapy (10). Some patients with MBC may be susceptible to loss of lean body mass which could compromise their tolerance to treatment and outcome. Since loss of lean body mass could be exacerbated when following an energy restricted diet our energy restricted intervention includes four key elements to preserve lean body mass:

1. We are using IER which appears to preferentially cause loss of fat and retain lean body mass (44). Preliminary data from the first eight women who have completed treatment with chemotherapy and IER has shown a median loss of fat 2.4 kg and gain in lean body mass of 0.7 kg (45).

2. The diet provides the recommended 1.0 - 1.2 g protein per kg per day (as outlined above).

3. The IER group will be asked to include resistance exercise three times per week. A series of recent studies demonstrate that resistance exercise is achievable, safe and can maintain or increase lean body mass and muscle function amongst patients with MBC (61-66).

4. The timing of protein intake may be important for maintaining muscle mass. It is estimated that 30g of protein is required per meal to evoke an anabolic threshold in muscle (67). We will encourage participants to spread their protein intake in three meals during the day and to include >30 g protein per main meal wherever possible on both the two restricted days and the five unrestricted days.

2.6 Rationale for investigating protein blood-based biomarkers of response to the diet and exercise interventions

Innovative mass spectrometry (MS) technologies can now be used to identify blood protein biomarkers (proteomics) that correlate with cancer patient's response to therapies. We are planning to use novel SWATH MS technology (68) to assess the proteomic plasma signature of trial participants both prior to initiation of chemotherapy and how this changes in the early stages of the diet and exercise interventions (at the start of cycle 3 or 4).

SWATH MS uses a novel technology which assesses all detectable protein components in a biological sample using a validated isobaric tagging relative quantification approach which uses 2 dimensional liquid chromatography coupled to mass spectrometry (69-72). This will allow us to assess amounts of known host proteins of interest (such as IGF-1, HIF-1 leptin, adiponectin, steroid hormones, cytokines, vascular regulators, and inflammation related molecules) as well as novel biomarkers. Novel biomarkers discovered will be validated with immunoassays (ELISA and the capillary-based NanoPro assay).

SWATH MS allows the rapid generation, in a single measurement, of a complete permanent recording of all the detectable protein components in a biological sample. SWATH maps stand as a permanent digital proteomic record, they can be interrogated over and over as novel hypotheses emerge, so samples only need to be run on the mass spectrometer once and the spectral libraries for analysis can be applied *in silico*.

We will interrogate the SWATH MS maps, comparing both baseline and dynamic changes in maps across the two treatment arms. In addition we will examine the differences in baseline maps between subsequent responders and non-responders within the diet arm. This will hopefully enable us to identify a biomarker signature predictive of both enhanced weight loss and increased chemotherapy efficacy, to be prospectively tested in future dietary intervention studies.

3. Future studies

This is a randomised phase II screening trial. (73). The design has been developed to save large numbers of patients being given an ineffective therapy, but if effective, would form the basis of a later formal phase III trial. This approach is approved by the Cancer Research UK trials organisation (CTAAC). If the results from this diet and exercise weight loss intervention are positive we will look to perform a phase III study, possibly randomising similar women to IER and resistance exercise vs metformin, if the results of the current metformin studies are also positive. A combination (IER + resistance exercise and metformin) arm will also be considered.

4. Potential risks and benefits for participating patients.

4.1 Benefits:

The intervention group will receive individual tailored advice and support to undertake an energy restricted diet and resistance exercise programme, whilst the control group will receive individual tailored advice and support to undertake a resistance exercise programme whilst continuing on their normal diet. Previous studies have reported benefits of resistance exercise in women with MBC. We consistently receive positive feedback of psychological and physical benefits from patients treated with chemotherapy participating in our diet and exercise trials. We predict that both groups will benefit from the enhanced advice and support they receive from their participation of the trial.

4.2 Risks

The additional burden and time commitment of trying to adhere to a diet and /or exercise plan during chemotherapy for ABC and potential disappointment if participants do not achieve their weight loss / exercise goal.

5. Aim

The aim of B-AHEAD 3 is to determine whether a combined IER diet and resistance exercise programme (intervention group) enhances the efficacy and ameliorates the toxicity from chemotherapy compared to the resistance exercise programme alone (control group) in women with ABC.

We will assess the effectiveness of the intervention vs control on the following outcomes:

5.1 Primary objective

Progression free survival (PFS), i.e. the time from randomisation to disease progression or death between the intervention and control group using standard RECIST criteria (Response Evaluation Criteria in Solid Tumors 1.1).

5.2 Secondary objectives

1. Self-reported toxicity according to the Common Terminology Criteria for Adverse Events version 4 (CTCAE v4)
2. Taxane neuropathy assessed with the VibraTip (McCallan Medical Ltd, Northamptonshire)
3. Abdominal muscle and fat cross sectional areas at the L3 level in CT scans.
4. Quality of life & fatigue using the FACT-B, FACT-ES (74), FACT-F (75) and FACT-BP (78) for bone pain, hospital anxiety and depression scale (76) in all patients and also:
   - FACT-taxane scale for patients receiving taxanes (77)
5. Muscle strength in the lower and upper limb. Lower limb strength from five repetition sit to stand test (FRSTST) (79). Upper limb strength assessed with hand grip dynamometry (80)
6. Fidelity of the interventions. Self -reported dietary intake (7 day food diary or diet history over the phone) and resistance exercise
7. Changes in concomitant medication
8. Change in blood protein biomarkers (proteomics) (Sub study 1)
9. Change in insulin resistance with HOMA from fasting insulin and glucose (81) (Sub study 1)
10. Time to Treatment Failure (TTF), i.e. the time from randomisation to disease progression, death or discontinuation of therapy due to toxicity
11. Objective response and clinical benefit rate (objective response plus stable disease for ≥6 months) between treatment groups
12. Overall survival

5.3 Exploratory objectives

1a. Is there a different proteomic signal at baseline between responders and non-responders?
1b. Is there a difference in insulin resistance at baseline between responders and non-responders?
2a. Assessment of change in PFS in women successfully losing weight (≥5% weight loss) vs. those who do not (<5% weight loss)

2b. Assessment of change in PFS in those with significant reductions in HOMA score vs those with no reductions.
2c. Are there differences in the change in proteomic signature between responders and non-responders?

6. Methodology

6.1 Study design

This study will compare PFS amongst 134 overweight women (BMI ≥24 kg/m^2) commencing chemotherapy who are randomised to:

1. Intervention group: Advised to follow an intermittent energy restricted diet and undertake resistance exercise.

2. Control group: Advised to undertake resistance exercise only (and to follow their normal diet).

6.2 Eligibility

- Inclusion criteria:
    - Women with histologically confirmed breast cancer
    - Patients with ABC, i.e. locally advanced disease that is not amenable to curative surgical resection or with metastatic disease
    - HER2 positive or negative
    - ER and/or PR positive or negative
    - If ER positive there is no restriction on the number of lines of previous endocrine therapy for ABC
    - Performance status 0 or 1
    - Predicted life expectancy ≥ 3 months
    - BMI≥24 kg/m^2
    - Expressing a wish to lose weight
    - Not already entered or planned to enter a trial of an investigational medicinal product (IMP) for this line of therapy
    - Age >18 (can be pre or post menopausal)
    - Measurable or non-measurable disease by RECIST v1.1
    - Patients with brain or leptomeningeal metastases are eligible as long as all sites have been treated with radiotherapy (+/- surgery) with evidence of disease control at least 8 weeks after the last dose
    - Women in whom further endocrine therapy is planned after chemotherapy are eligible. The treating clinician must state what endocrine therapy is planned before chemotherapy is initiated.
    - Women with thyroid dysfunction are eligible provided they are euthyroid and on a stable dose of thyroxine for the last 6 months

- Exclusion criteria:
    - Physical or psychiatric conditions which may reduce compliance to and the safety of diet or resistance exercise, e.g.
        - Serious digestive and/or absorptive problems, including active inflammatory bowel disease.
        - Psychiatric disorders or conditions, e.g. history of eating disorders, untreated major depression, psychosis, substance abuse, severe personality disorder.

    - Bone metastases at risk of pathological fracture or that would limit resistance exercise through pain in all three areas of the body that are covered by the resistance exercises (upper limbs, trunk and lower limbs). Metastases may be ok if other areas of the body can safely be exercised.
- Medications affecting adiposity or muscle mass and function and energy intake e.g. continuous daily steroids for longer than 4 weeks (short term steroids with chemotherapy are acceptable)
- Diabetics on insulin or sulphonylureas (glibenclamide, gliclazide, glimepiride, glipizide, tolbutamide) as they could experience hypoglycaemia on restricted days of the intermittent diet (diabetics treated with diet alone or with any other medication including metformin are eligible)
- Greater than Day 15 of this course of chemotherapy.
- Visceral metastases that, in the opinion of the treating clinician, would result in death within 3 months if no response was achieved with this line of chemotherapy
- Symptomatic or uncontrolled brain or leptomeningeal metastases

6.3 Recruitment

Patients due to commence chemotherapy for advanced breast cancer will be invited to the study by their oncology team and National Cancer Research Network (NCRN) nurses in 11 Greater Manchester and Cheshire Cancer network breast units (Christie, Wigan, Macclesfield, Bolton, Tameside, North Manchester General Hospital, Oldham, Leighton, Stockport, Salford and Blackburn) at the start of the study and more sites in the UK will be added depending on recruitment.

All research nurses in participating centres will be fully informed about the study and recruitment procedure. Eligible patients will be identified by NCRN research nurses either at the metastatic breast cancer multidisciplinary team meeting or by screening clinic notes. Eligible women will be given a detailed patient information sheet and given the opportunity to ask questions concerning the trial. If it is not possible to see women in clinic, they will be telephoned by the research nurse or other member of the B-AHEAD 3 research team. The purpose of this phone call will be to briefly introduce the study and get consent to post or e-mail an information sheet to the participant if they are interested. We will seek permission for the research nurse or other member of the research team to contact them at least 24 hours after receipt of the information sheet to determine their interest in entering the trial. Ideally, interested women will be asked by the research nurse to reattend to sign a consent form. At that appointment they will also be asked whether they consent to their details being securely transferred on the trial proforma by fax or nhs.net e-mail to the B-AHEAD 3 research team at MFT. This verbal consent will be documented. If it is in the participant's interest, consent could be taken during the same visit to the recruiting centre. This would allow the participant to avoid an extra journey to hospital solely for consent (for example if they didn't have any other visits planned before chemotherapy started, or to allow a participant to take part in the study if they were interested but the time frame was tight and chemotherapy would potentially need to be delayed to allow them to reattend their local hospital/the Nightingale Centre for consent). Consent can also be attained at the baseline appointment at The Nightingale Centre, if the participant has given verbal consent for their details to be transferred to the study team at MFT.

Once the proforma has been received, the B-AHEAD 3 research team at MFT will then contact the patient to arrange a baseline appointment. The baseline appointment should ideally occur at least 2 days before commencing the planned course of chemotherapy. However potential participants will be informed that they can join the study at any time up to Day 15 of their chemotherapy cycle.

Using the initial approach we have successfully recruited 47 - 48% of eligible chemotherapy patients from the Greater Manchester NIHR Clinical Research Network into our previous randomised diet and

exercise B-AHEAD 1 and B-AHEAD 2 studies in the same hospitals outlined above. Assuming a conservative 35% uptake amongst advanced breast cancer patients we anticipate recruitment should be completed within 15 months.

Ideally baseline assessments will take place at The Nightingale Centre. It is recognised that between consent and undergoing the baseline assessments at The Nightingale Centre, participants have time to change their mind about joining the study so we are not concerned that not having this consent visit would make women join the study before they have had time to consider it.

As recruitment has been slower than anticipated, participants can now have their baseline assessments and blood samples in their recruiting hospital. Blood samples will only be taken if the centre has -80°C storage facilities or if the samples can be transferred to MFT or another centre with -80°C storage facilities within an acceptable time frame.
If baseline assessments are taking place at the recruiting hospital, there must be at least 24 hours between the participant receiving the PIS and the assessments taking place in order to allow the participant time to consider the study.

Posters and leaflets to raise awareness of the study will be displayed in patient areas.

6.4 Procedures for during and end of the study

The energy restriction and exercise interventions will continue until the first disease progression after commencement of study chemotherapy, even if chemotherapy had been stopped due to toxicity. Approximately 30% of patients have an objective response, whilst 20% have stable disease. The average chemotherapy period of treatment is projected to be 5.4 months. The longest treatment period is predicted to be two years. We plan to censor patients in the study once the final recruited patient has been in the trial for 12 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 or local hot line for advice as to the management of toxicity, as well as their breast care nurse and the research nurses.

As the *a priori* aim of the study is for participants on the diet arm to lose weight, a loss of greater than 10% body weight may be seen more frequently on the intervention arm than the control group. In this case, if grade ≥2 toxicity has been observed within the last 2 cycles before the weight loss reached 10% or more, a dose reduction should be performed by recalculating the body surface area with the most recent weight. If no grade ≥2 toxicity is observed then no dose recalculation should be performed. If therapy is continued in this a manner and grade ≥2 toxicity develops subsequently then the recalculation should be performed on the most recent weight, not the one first observed to be <10% from baseline.

A change of chemotherapy regimen is not permitted prior to disease progression. This includes a switch from paclitaxel to 'maintenance' capecitabine which is specifically prohibited. A patient who has an allergic reaction to the first or second dose of weekly paclitaxel, who has not been treated previously with capecitabine, may change to capecitabine if, in the opinion of the treating clinician, this is an appropriate therapeutic option. HER2 targeted agents may be stopped during the study and the patient continue with chemotherapy. The date of stopping the HER2 targeted agent will be recorded.

6.5 Randomisation

Stratification

Women will be randomised to each of the two study arms over the phone* or during their baseline B-AHEAD 3 appointment at MFT using a computer minimisation programme, stratifying for five factors:
1. First and second line chemotherapy vs third or greater line chemotherapy

2. Paclitaxel/docetaxel containing vs capecitabine containing vs other

3. Projected median BMI of our cohort, i.e. BMI ≥ 30 kg/m^2 and BMI < 30 kg/m^2

4. Whether ER + or ER – ve

5. Number of previous lines of endocrine therapy for metastatic disease (0 or 1 vs 2 or more)

6. Combination therapy vs single agent

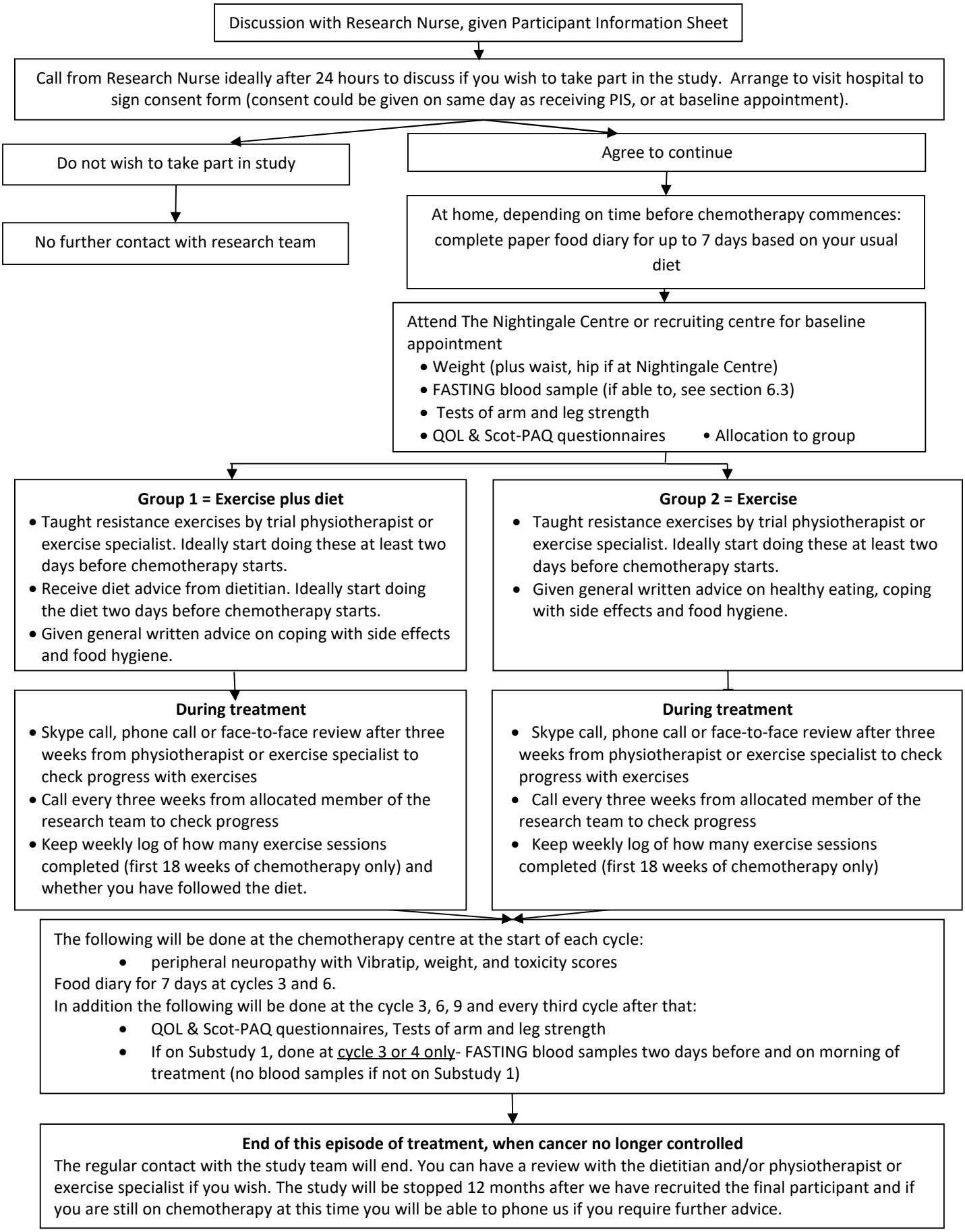
7. HER2 +ve or HER2 -ve

* If the baseline bloods and assessments have taken place at the recruiting hospital patients can be randomised over the phone.

This minimisation programme will be created by the trial statistician (Julie Morris) at MFT. The programme will be located on a department computer in the Prevent Breast Cancer Research Unit at MFT and used by the trial administrator to determine group allocation.

All staff assessing trial endpoints will be blinded to the participant study arm i.e. radiology staff assessing tumour response and change in fat and muscle area in CT scans, research nurses assessing toxicity, and researchers analysing the quality of life questionnaires.

6.6 Figure 1: Study Flowchart



7. Procedures

7.1 Intervention group: Intermittent diet & resistance exercise

7.1.1 Intermittent energy restriction

Women will be asked to follow the intermittent diet for 2 days/week throughout the trial ideally beginning 2 days immediately prior to the first chemotherapy cycle or before the 2nd cycle if recruited within 2 weeks of starting chemo. The 2 restricted days must be consecutive and should be the 2 days immediately prior to the first dose of chemotherapy at each cycle. 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 intake (<50 g per day) and has 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 and monounsaturated fat (MUFA), five portions of vegetables and one portion of fruit, limited vegetarian protein foods and low fat dairy foods which contain some carbohydrate i.e. Quorn, hummus, and at least 2 litres of low energy fluids (43). The diet typically self-limits to 70–90 g protein and <60g fat/day and is limited in saturated (82) and PUFA fat (51), which have possible links with breast cancer. We will encourage a high fluid intake which is important as this population may suffer chemotherapy-induced constipation.

Women will be asked to follow a healthy Mediterranean type diet for the remaining five days of the week. Energy intake on these days will be tailored according to their estimated energy requirements to 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 at least five portions of vegetables and two portions of fruit per day and is limited in alcohol (<10 units per week).

7.1.2 Home based resistance exercise programme

The programme has been designed by our specialist breast cancer physiotherapists (Karen Livingstone, Claire Edwards) adapted from Cormie et al (65). The resistance exercise programme includes three sessions per week. It includes a brief warm up, and 5-10 standard exercises for muscle groups in the lower limbs, upper limbs and trunk and a cool down. The trial physiotherapist will adapt this standard resistance programme for patients at baseline according to their metastatic site, co-morbidities and current activity and energy levels (Appendix 1). The programme is designed to be progressive with 3 progressive stages. The speed of progression will be advised according to feedback on function and energy levels from participants and will occur after 4 – 8 weeks. Progression will be achieved with the same type of exercises by increasing the number of reps and sets and increasing resistance when exercising by using simple home light weights (i.e. holding a 500 ml bottle of water = 500g).

Participants will be shown the correct technique for undertaking the resistance exercises via a face to face or telephone baseline appointment with the trial physiotherapist, including the correct speed to undertake the exercises. Some exercises may be advised to be undertaken when seated or lying down depending on the site of any bone metastasis. Women with lymphoedema will be asked to wear their sleeve when undertaking upper body exercise. Participants will be provided with demonstration on line videos and written leaflets of their recommended exercise programme to use at home using an on line physiotherapy tool (Physiotec, Canada www.physiotec.ca). Medical oncology teams will inform the B-AHEAD 3 team of any change in health status and metastases which may require adaptations to the programme as the trial progresses.

The trial is specifically testing the benefits of an energy restricted diet and resistance exercise programme, and is not testing the effects of cardiovascular exercise. Large increases in moderate (60–75% maximum heart rate) or vigorous (75–90% maximum heart rate) cardiovascular exercise in trial participants could be a confounder which could have a positive influence on weight loss and possibly outcome (83). We acknowledge that some patients may wish to undertake cardiovascular exercise. Patients will be given general guidance to include 150 minutes of moderate (not vigorous) activity per week as per standard health advice for cancer patients receiving chemotherapy and for general health (84). We do not however anticipate that large increases in moderate exercise are likely to be achieved by our trial participants. Previous attempts to increase cardiovascular exercise amongst metastatic cancer patients have only managed to promote modest increases in light (50 – 60% maximum heart rate), but not moderate cardiovascular exercise (62, 63). A recent home based walking programme only achieved an additional mean (95% CI) of 77 (1.9 to 152) minutes of low intensity walking at 1.7 mph (2.7 km/h) on level ground per week (65). Light exercise, e.g. low intensity walking, light housework, gardening has been associated with modest improvements in well-being and fatigue, and will be encouraged alongside the resistance exercise programme. We will assess the actual amount of moderate or vigorous cardiovascular and resistance exercises undertaken in the previous 7 days at baseline, cycle 3 and then every 3 cycles after that in the trial using the Scot-PAQ questionnaire. This will be compared between the groups.

7.2 Comparison group: Resistance exercise programme only

This group will receive instruction to follow the resistance exercise programme described above. This group will be also be given a standard leaflet which advises them to follow a Mediterranean diet, and provides basic advice on healthy diet for breast cancer patients. They will not receive specific guidance or support to reduce their energy intake. We have found a standard healthy eating leaflet without tailored diet advice and support has minimal effects on dietary intake in breast cancer patients in our previous B-AHEAD 1 study, with a reported reduction of 50 kcal per day. Hence we anticipate that this group will follow their normal diet.

7.3 Initial diet and exercise advice and ongoing support and monitoring in the intervention and control groups

7.3.1 Initial advice sessions

The intervention group will have a face to face or phone* dietary consultation with one of the trial dietitians to receive instruction on how to follow the intermittent diet (45 minutes). Each patient will receive guidance on appropriate food choices, portion sizes, menus, recipes and appropriate behavioural techniques to enhance dietary adherence. Foods eaten whilst following the diet will be self-selected by the patients and not provided by the study team. They will also receive a leaflet which contains basic advice on healthy diet for breast cancer patients, discourages patients from taking inappropriate self-prescribed nutritional supplements (we typically find 20% of patients self-prescribe inappropriate high dose antioxidant vitamin supplements), guidance on food hygiene and advice for dealing with chemotherapy related side effects which can affect dietary intake such as nausea, dry and sore mouth, constipation or diarrhoea.

This group will also receive face to face or phone instruction of how to follow the resistance exercise programme from the trial physiotherapist or exercise specialist (45minutes).

The Control group will receive face to face or phone advice of how to follow the resistance exercise programme from the trial physiotherapist or exercise specialist (45minutes) as outlined above. They will also receive a leaflet which provides basic advice on healthy diet for breast cancer patients,

discourages patients from taking inappropriate self-prescribed nutritional supplements and guidance on food hygiene and advice for dealing with chemotherapy related side effects which can affect dietary intake such as nausea, dry and sore mouth, constipation or diarrhoea.

* If baseline bloods and assessments take place in the recruiting hospital and if they are allocated to Group 1 (diet and exercise), patients will receive stop gap diet advice on the 2 day diet from a MFT dietitian over the phone with the aim of attending MFT for exercise advice and more detailed diet advice before the 2nd chemotherapy cycle. If they are allocated to group 2 the aim is for them to attend MFT for exercise advice before the 2nd chemotherapy cycle.

If the patient is unable to attend the Nightingale Centre for baseline appointment or at any time before the 2nd chemotherapy cycle, diet and exercise advice can be delivered over the phone provided the B-AHEAD 3 team is happy that this is appropriate for the individual patient.

7.3.2 Ongoing support and monitoring

Intervention group

Participants will be asked to start their allocated diet and resistance exercise programme at least two days prior to their first or second chemotherapy. Individual diet and goals and recommendations will be reinforced by three weekly phone calls by their allocated dietitian following a standard script which will check understanding of the diet, explore adherence in the past 3 weeks and trouble shoot.

The intervention group will also have phone or Skype (or similar application) reviews with the trial physiotherapist/cancer exercise specialist around every three weeks to assess their progress with resistance exercise and check that they are using the correct technique which was demonstrated to them at baseline.

The research dietitian will subsequently assess adherence and any trouble shooting the participant may have with resistance exercise using a standard script which covers a number of key issues, e.g. adherence in the past 3 weeks, current fatigue, bone pain, balance, change in medications including pain relief.

Progressions of exercise within the exercise programme will be progressions of the same exercises using additional resistance and reps which will be instructed by the research physiotherapist using the Physiotec tool, phone call, Skype or face to face consultation at MFT as appropriate.

The three weekly calls from the dietitian will last approximately 20 – 30 minutes and will check compliance and any problems with the diet and the resistance exercise programme and address individual problems. Women will then be mailed an individualised summary of key motivational, behavioural issues for adhering to the diet and resistance exercise programme. The dietitian will also post the intervention group standard diet specific mailings every 3 weeks as appropriate for that individual which cover a range of topics to increase adherence to the energy restricted diet (Appendix 4). Mailings will focus specifically on promoting adherence to the energy restricted diet only and will not refer to adherence to exercise or other psychosocial parameters. We will aim to start three weekly review phone calls one week after joining the study and will continue until they leave the study. If an alternative contact plan is agreed with the participant, for example if they request less frequent calls, this will be documented.

Control group

Participants will be asked to start their resistance exercise programme at least two days prior to their first or second chemotherapy. The control group will have phone or Skype (or similar application)

reviews (depending on patient preference) around every three weeks to assess their progress with resistance exercise and to check that they are using the correct technique which was demonstrated to them at baseline. If neither are possible, these consultations can be delivered over the phone if the physiotherapist is happy that this is appropriate for the individual patient. These calls will last 20-30 minutes and will **not** discuss diet. If an alternative contact plan is agreed with the participant, for example if they request less frequent calls, this will be documented. This group will not receive any additional trial mailings.

Both groups
All patients will have a face to face assessment around every 6 months on the study to ensure the exercises are still safe and relevant. The physiotherapist / exercise specialist may request a further Skype/phone/face to face assessment if they are concerned by any changes in the patient's clinical situation or if any concerns have been raised over the phone.

7.3.3 Monitoring weight

All patients will have their weight monitored at each chemotherapy cycle in their chemotherapy unit. This will allow us to make any necessary modifications to their dietary prescription if patients are losing too much weight (see section 16.1 'Acting on low weight and BMI'). A change in weight by - 10% alongside a grade ≥2 toxicity (as per section 6.4) will be communicated to the patient's medical oncologist so they can consider whether they wish to adjust the chemotherapy dose, which are dosed according to body weight and body surface area (see section 6.4 'Procedures for during and end of the study'). Patients in Group 1 will be provided with a diary to record their weight and waist.

7.3.4 Monitoring after discontinuation of chemotherapy due to excess toxicity or at the end of planned treatment

Participants will be offered three options at this point as they will no longer be having visits to their chemotherapy hospital at each cycle:

1. Continue with B AHEAD 3 phone calls from the research dietitian/ physiotherapist and attend for study assessments at chemotherapy centre around every 8-12 weeks (these appointments will hopefully coincide with visits to the centre for CT scans or clinic appointments)
2. Don't attend for any more study assessments with research nurses, but continue with phone calls from B-AHEAD 3 research dietitian/ physiotherapist until CT scans show tumour progression
3. Don't attend for any more study assessments with research nurses, no more phone calls from B-AHEAD 3 study team CT scans will continue to be monitored until tumour progression

7.3.5 Procedure for progression of bone metastases

In the case of progression of bone metastases that stop all safe resistance exercises in view of study team, participants will be advised to discontinue the study exercises. Group 1 participants will be advised to continue with the diet and all regular review calls from study dietitian and study assessments at each cycle. Group 2 participants will no longer have review calls (unless the participant would like them to continue) as no study interventions (diet, exercise) will require review but study assessments at each cycle will continue.

7.4 Procedure for patients leaving the trial

Patients will leave the study at their request, or at the point that they experience disease progression (either during treatment, or after discontinuation of chemotherapy due to excess toxicity). We will ensure that there is excellent communication between oncology teams, research nurses and the B-AHEAD 3 research team to ensure patients who are progressing and leaving the study are not contacted for diet or exercise review or sent mailings by the B-AHEAD 3 team. We will however offer participants in both groups moving on diet and exercise advice from the trial dietitian and physiotherapist when they leave the study if they wish. Patients who were in the exercise only control group will not be offered information on the intermittent diet as this is our test diet and we do not have enough evidence that it is beneficial, which is the aim of the study. Patients who are still on chemotherapy at study closure will be encouraged to continue with their current regimen and be provided with "a moving forward on your own" booklet with information to help them maintain their current regimen. They will be informed that they can ring the trial dietitians if they experience any problems following the diet and exercise plans at any point in the next 12 months.

At the end of the trial we will ask the control group whether they had followed any particular diet during their chemotherapy treatment.

The participant is invited to contact the research team if they would like to receive a copy of the results, due in 2022.

Participants will be asked by their research nurse for their verbal consent for the study team to continue to review their medical records and CT scans after they leave the study.

8. Outcome measures

8.1 The effectiveness of the intervention

8.1.1 Primary objective

To assess for difference in progression free survival (PFS) between the intervention and control group according to standard RECIST 1.1 (Response Evaluation Criteria in Solid Tumors) guidelines. CT scans will be performed as per local protocols in the recruiting centres. This is usually every 3 or 4 cycles of therapy or around 9 - 12 weekly during treatment even if chemotherapy cycles are delayed. If chemotherapy is stopped, e.g. due to excess toxicities, CT scans will revert to standard of care in the participant's chemotherapy centre, which is usually around every 12 weeks. The MFT radiologists will not have sight of the local CT report at the time of RECIST reporting.

Amendment 7: Clarification of whether the primary endpoint of PFS is decided locally or centrally
A blinded analysis of CT scans will be performed retrospectively and both local and central assessment of PFS calculated. Central (blinded) assessment will be used as the primary endpoint. As this is blind and impartial, this option is superior.

8.1.2 Secondary objectives

We will assess the effect of the intervention vs. control on chemotherapy toxicity at day 1 of each chemotherapy cycle. If cycles are delayed these assessments will be pushed back to coincide with day 1 of each chemotherapy cycle.

1. Self-reported toxicity. Subjective measure for chemotherapy patients

Toxicity will be graded by the attending physician or research nurse according to the Common Terminology Criteria for Adverse Events version 4 (CTCAE v4) scale. (85). The key toxicities to be assessed are:

- Peripheral neuropathy
- Hand and foot syndrome (Palmar Plantar Erythrodysaesthesia [PPE])
- Diarrhoea
- Nausea
- Vomiting
- Neutropenia
- Fatigue

2. Taxane neuropathy.

Taxane neuropathy will be assessed by an automatic vibration assessment tool (VibraTip,McCallan Medical Ltd, Northamptonshire) as used for detecting diabetic neuropathy in diabetic populations. This tool has been shown to be more sensitive than a tuning fork (86) and comparable to a light touch test in diabetic populations (87). We plan to use the VibraTip rather than the light touch test in this study. The vibratip uses a standard vibration rather than pressure applied by an individual and hence provides a reproducible stimulus between practitioners and patient which is important in this trial as the assessment will be undertaken by different research nurses in the recruiting centres. This will be measured in patients receiving taxanes and other chemotherapy regimens used in the trial.

3. Change in fat and lean body mass.

Assessed with CT scan around baseline, and then at intervals defined by local protocol in recruiting centres (usually every three or four cycles or around every 9 – 12 weeks) thereafter even if chemotherapy cycles are delayed.
It is important that we ensure the safety of the intermittent diet in these advanced breast cancer patients particularly to ensure they do not lose lean body (largely muscle) mass as outlined above (52). Abdominal muscle and fat areas have been used in previous studies to predict toxicity and prognosis in advanced breast cancer patients receiving chemotherapy (52). We do not anticipate loss of lean body mass with the IER and resistance exercise intervention but need to document this. We plan to assess changes in abdominal muscle and fat in the cross sectional area at the L3 region in CT scans which are routinely conducted to assess their response to chemotherapy. These scans will be undertaken at intervals as defined above. If chemotherapy is stopped, e.g. due to excess toxicities, CT scans will revert to standard of care in the participant's chemotherapy centre, which is usually around every 12 weeks.

Thus assessing CT muscle and fat does not require any additional scanning time hence reducing the cost of the trial and the burden to patients.

CT measures of lean and fat mass will be obtained from the CT scans by our trial radiologists. Muscle and fat cross-sectional areas will be measured at the level of the L3 transverse processes using image analysis software such as Analyze 11.0 (AnalyzeDirect, Inc. Cheshire UK). Abdominal muscles at L3 include the psoas, paraspinal muscles (erector spinae, quadratus lumborum) and the abdominal wall muscles (transversus abdominus, external andinternal obliques, rectus abdominus). Abdominal muscle mass has been used to estimate total lean body mass in advanced cancer patients using the equation described by Mourtzakis et al (LBM (kg) = 0.30 x [skeletal muscle at L3 using CT (cm2)] + 6.06) (88). This method will be insensitive to any changes in lean body mass which may be occurring in the upper and lower limbs, hence we are also including functional assessments of upper and lower muscle strength in the trial (79).

Oedema is a well-recognised side-effect of treatment with taxanes, and this may therefore interfere with body composition analysis using the CT method described above. In order to measure and correct for this attenuation values of subcutaneous fat and also the psoas muscles on the serial CT scans will be recorded using standardised regions of interest.

The following 2 assessments will be assessed at baseline, at the start of cycle 3, and then every 3 cycles thereafter whilst patients remain in the trial i[1].
If cycles are delayed these assessments will be pushed back to coincide with day 1 of each chemotherapy cycle.

4. Quality of life fatigue, anxiety and depression

   These will be estimated using the FACT-B, FACT-ES (74), FACT-F (75), FACT-BP for bone pain (78), and HAD scales (76) in all patients. The FACT-Taxane scale for patients receiving taxanes. Questionnaires will be completed by patients when they attend their chemotherapy unit (77).

5. Muscle strength.

   Lower limb strength will be assessed from five repetition sit to stand test (FRSTST), normalised by weight (79) (Appendix 3). Arm muscular strength will be assessed using hand grip dynamometer. Both tests will be assessed at baseline, and at cycle 3 and every three cycles thereafter during the study by the B-AHEAD 3 study team or research nurses in the recruiting centres, who have been trained to undertake assessments according to the trial standard operating procedure (Appendix 3).

6. Fidelity of the interventions. Adherence to the diet and exercise interventions

- The intervention group will be asked to record compliance to the IER diet each week during the study using a specially designed self-monitoring diary. They will be asked to record whether they have managed to undertake two, one or no restricted days that week. Compliance to the intervention will be checked and recorded as part of the three weekly calls from their B-AHEAD 3 advisor.
- Both groups will be asked to record compliance with the resistance exercise in a special trial diary and the number of sessions achieved each week for the first 18 weeks of chemotherapy only. This diary will ask patients to record pain pre and post as assessed by Cormie et al (65). The diet and exercise diaries will monitor fidelity with the interventions and also act as a behavioural tool to increase compliance and a record of any problems encountered when they try to follow an exercise programme.
- We will record receipt of the three weekly phone calls from the trial dietitian and physiotherapist / cancer exercise specialist in the intervention group and from the physiotherapist / cancer exercise specialist in the exercise only group, which are part of the diet and exercise interventions.
- Dietary intake in both the intervention and control groups will be assessed using 7 day paper food diaries or using a diet history of the past 7 days assessed by a B-AHEAD 3 research dietitian over the phone (89). Intake of energy, macro and micronutrients in both groups will be determined from paper diaries using Wisp nutrition analysis software (Tinuviel Software Anglesey, Wales).
- Seven day recall of physical activity (resistance and cardiovascular exercise) will be assessed with the Scottish Physical Activity Questionnaire (Scot-PAQ) (90), which records the amount of time spent undertaking moderate, vigorous activity or resistance exercise in the previous week.

7. Changes in concomitant medication

Changes in concomitant medication such as antiemetics will be recorded using a standard proforma at day 1 of each chemotherapy cycle. If cycles are delayed these assessments will be pushed back to coincide with day 1 of each chemotherapy cycle. This will allow us to assess any differences in prescription of drugs between the intervention and control group.

8. Protein blood-based biomarkers and insulin resistance

   We will be assessing baseline proteomic signal and insulin resistance in **all** intervention and control participants to assess whether this can predict response and non-response to chemotherapy.

9. We will be assessing change in protein blood-based biomarkers and insulin resistance and proteomic with HOMA from fasting insulin and glucose between baseline and cycle 3 or 4 in a **sub set** of patients and whether this predicts response to chemotherapy (sub study 1) (81)

10. Time to Treatment Failure (TTF), i.e. the time from randomisation to disease progression, death or discontinuation of therapy due to toxicity.

11. Objective response and clinical benefit rate (objective response plus stable disease for ≥6 months) between treatment groups

12. Overall survival analysis

Consent will be obtained for the medical notes of participants to be checked on a 6 monthly basis after completion of trial therapy to evaluate the overall survival secondary endpoint.

8.1.3 Sub-study 1

Change in insulin resistance and protein blood-based biomarkers

We plan to assess the effects of the intervention vs. control on insulin resistance which we hypothesise to be an important mediating variable which could predict success of the IER and resistance exercise intervention. We will assess the effects of the interventions on insulin resistance using HOMA (Homeostasis model assessment) from fasting insulin and glucose measurements (81).

We are assessing whether baseline proteomic signal can predict response and non-response to chemotherapy in **all trial participants** (see above). Patients in this sub-study will have repeat blood tests at cycle 3 or 4 so we can assess the effect of the intervention on the proteomic plasma signature to obtain a comprehensive assessment of any changes in known host proteins of interest, e.g. IGF-1, HIF-1 leptin, adiponectin, steroid hormones, cytokines, vascular regulators, and inflammation related molecules as well as novel biomarkers.

The benefits of IER are due to both acute metabolic effects which occur during the 2 days of restriction and more chronic beneficial effects throughout the subsequent non fasting days. Typically 3 months of IER reduces insulin by 25%, with a further acute 25% decrease during the 2 days of restriction (44, 92). A true evaluation of the metabolic effects of IER thus requires assessment of during both the fasting and non-fasting days.

We therefore plan to assess HOMA insulin resistance and protein blood-based biomarkers at:

1. Baseline (before starting chemotherapy and the diet/and or resistance exercise)

2. On the day of the third or fourth chemotherapy cycle just after the 2 fasting days in the IER group†.

3. Two days before the third or fourth chemotherapy cycle, i.e. five days after completion of fasting days in the IER group to assess the durability of the effects of IER on the five normal eating days†.

† We will take two fasting blood samples in the exercise only control group. Timing these samples around diet days is not vital in this group so the timing of these samples can be flexible. For premenopausal women we will record the day of menstrual cycle as this can have a small effect on measures of insulin resistance (93).

Exploratory objectives

1a. Is there a different proteomic signal at baseline between responders and non-responders?

1b. Is there a difference in insulin resistance at baseline between responders and non-responders?

2a. Assessment of change in PFS in women successfully losing weight (≥5% weight loss) vs. those who do not (<5% weight loss)

2b. Assessment of change in PFS in those with significant reductions in HOMA score vs those with no reductions.

2c. Are there differences in the change in proteomic signature between responders and non-responders?

8.2 Additional measures

8.2.1 Patient demographics

The following demographic health characteristics and body size of the two groups will be collected at baseline:

- General information: age, ethnicity, language spoken and religion (e.g. fasting and Ramadan etc), marital status, number of children living at home, employment status.
- Index of multiple deprivation score (based on post code) (91), highest level of education
- Details of breast cancer diagnosis and previous treatment
- Family history of breast cancer
- Personal weight history: timing of weight gain before or after diagnosis of breast cancer, ever advised to lose weight by a health care professional, ever referred to a weight management service by a health care professional, and recent attempts to lose weight
- Weight, height and BMI assessed in the recruiting centres or at MFT
- Body fat (bioelectrical impedance, Tanita 180), waist, hip measurements in subjects who attend MFT for their baseline appointment

8.2.2 Menstrual cycle

Pre-menopausal women in both diet groups (~10-20% of the 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 can be a factor in chemotherapy associated weight gain.

8.2.3 Sub-study 2: Qualitative evaluation of the weight loss / lifestyle programme in the energy restriction and resistance exercise only groups.

We are proposing to include a small in depth qualitative study amongst a sub set of participants to assess the acceptability of the intervention and IER diet amongst women with metastatic breast cancer. This sub study will be submitted as a future application to ethics.

9. Criteria for discontinuation of participants in the trial

The energy restriction and exercise interventions will extend until the first disease progression after commencement of study chemotherapy, even if chemotherapy had been stopped due to toxicity or completion of planned treatment (unless excluded by progression of bone metastases, see section 7.3.5). The average chemotherapy period of treatment is projected to be 5.4 months. The longest treatment period is predicted to be two years. We plan to censor patients in the study once the final recruited patient has been in the trial for 12 months.

Ongoing assessment of CT scans as per frequency described in section 8.1.1 (reported back within 21 days) will inform us if patients are experiencing reductions in lean body mass and developing sarcopenia. Sarcopenia is defined using the cut off point for lumbar skeletal muscle index of <38.5 cm^2/m^2 for women (96). In the unlikely event that this occur the B-AHEAD 3 dietitian and physiotherapist will review the patient's advice and progress and provide appropriate diet and exercise advice to prevent further reductions. If this advice requires patients to stop following the diet their data will still be used in the primary analysis which is on an Intent To Treat (ITT) basis.

10. Statistical Considerations

10.1 Sample size and power calculations

The sample size was calculated for a phase II screening design, based on a primary outcome of progression free survival, with a time-to-event hazard ratio of 0.65, and with 90% power and a one-sided significance of 20%. Assuming that the estimated PFS in the control arm is 5.4 months, and that we will have a 15 month accrual period and a 15 month follow-up, then a total of 98 events will be required overall. This corresponds to 114 patients required in all, allowing for a 15% drop out we will need to recruit 134 patients overall. The sample size is for a randomised phase II screening design where a randomised phase III is planned in the future. This approach is approved by the Cancer Research UK trials organisation (CTAAC) (73).

10.2 Method of analysis

Assessment of progression-free survival between the two groups will be carried out using Cox-proportional hazards regression with appropriate adjustment for competing risks if required.

A comparison of secondary outcomes (toxicity, neuropathy, changes in fat and lean body mass, QOL and muscle strength) between the two groups measured at multiple time points during the study period will be made using regression modelling which takes account of the correlated structure of the data. Generalised estimating equations will be used (with either a binary or normally distributed response as appropriate) with an auto-regressive correlation structure.

11. Finance

The trial is funded by a grant from Anticancer Fund (A Belgian private foundation) www.anticancerfund.org.

12. Publication and dissemination

We will aim to publish this feasibility study in a peer reviewed cancer journal. The findings of this feasibility study will inform the design of a planned Health Technology Assessment (HTA) application for a large scale RCT testing the benefits of energy restriction amongst metastatic breast cancer patients We will inform all study participants that they can request the results of the study.

13. Timelines and milestones

4 month set up locally and within participating centres in the Greater Manchester area and develop resources

15 month for recruitment

12 months for follow up

2 months write up and dissemination

14. Trial Governance

14.1 Trial Management Group

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

14.2 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 at 3, 6, 12, 24, and 30 months. Additional meetings will be scheduled should the need arise.

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

Dr Michelle Harvie	Research dietitian
Dr Sacha Howell	Consultant Medical oncologist
Prof Anthony Howell	Consultant Medical oncologist
Mrs Karen Livingstone	Senior physiotherapist

Dr Lee Graves	Senior lecturer / exercise specialist
Dr Anthony Maxwell	Consultant Radiologist
Dr Yit Lim	Consultant Radiologist
Dr David French	Qualitative researcher
Julie Morris	Senior statistician
Vicky Lau	Senior breast cancer research nurse
Sharon Foy	Senior breast cancer nurse

14.3 Trial Advisors

Prof David French	Health psychology/behavior change
Ms Karen Morris	Clinical biochemistry, MFT
Ms Fiona Derbyshire	Research budget manager for breast services, MFT
Ms Faye O'Keefe	Deputy Manager, Research & Development, MFT

14.4 Data Monitoring Committee

We have recruited a Data Monitoring Committee (DMC): Dr Carlo Palmieri (Medical Oncologist, Clatterbridge Centre for Oncology NHS Foundation Trust), Dr Adam Brentnall, (Biostatistics at Queen Mary University of London), Dr Jacky Gracey (Lecturer, School of Health Sciences, University of Ulster)

The DMC will be responsible for safeguarding the interests of B-AHEAD 3 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.

15. 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. Source data for the trial are pre chemo weights, CTCAE v 4 toxicity, Vibratip, quality of life data, treatment schedules and details of hospital admissions which will be stored at local hospitals on a trial case report form during the trial. These semi-anonymised forms (trial ID and patient initials) will be transferred to the B-AHEAD 3 researchers at MFT during the trial using secure methods i.e. nhs.net or fax. The B-AHEAD 3 researchers will also have source data; food diaries, physical activity questionnaires, muscle function tests, log of review calls from dietitian and physiotherapist and demographic information collected at baseline appointment. These data are stored in the participants' individual 'Participant Trial Files' kept in the B-AHEAD 3 research offices in the secure area of The Prevent Breast Cancer Research Unit. 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 MFT. In the other 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.

The CT scans used as part of routine care in the recruiting oncology centres will be transferred to The Nightingale Centre at MFT via the Image Exchange Portal. This is a secure method for transferring scan images that is widely used within the NHS.

16. Clinical Risk Assessment

16.1 Acting on low weight and BMI

The study team will collect weight from participants at their three weekly 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.

Patients on the intermittent diet who achieve their healthy weight aim during the trial and are trying to maintain their weight should be encouraged to include 2 restricted days each week, whilst increasing amounts of the healthy Mediterranean diet (i.e. carbohydrate, protein and fat allowances) on the other 5 days.

16.2 Maintaining lean body mass

It is important that we ensure the safety of intermittent diet and resistance exercise interventions in these women with recurrent breast cancer, particularly to ensure they do not lose lean body (largely muscle) mass and that the interventions do not have adverse effects and increase toxicity from chemotherapy.

We only plan to include patients with ABC in the trial who are overweight or obese (BMI≥24 kg/m^2) (~ 65% of our metastatic population). Such patients tend to have higher levels of adipose stores. Patients with raised adipose stores tend to lose body fat and preserve lean body mass. Unlike many advanced cancers at other sites (eg. lung) only a very small minority of advanced breast cancer patients have cachexia, and they will not be recruited to the study.

We are mindful however that some of our cohort may have reduced lean body mass at baseline despite being overweight with increased adiposity. Previously published data reported 20% of overweight patients receiving second line chemotherapy for metastatic breast cancer have low lean body mass (sarcopenia) (52). The trial includes ongoing assessment of CT scans at the frequency described in section 8.1.1 which are reported back to the trial team within 21 days. These CT scans will inform us if patients are reducing lean body mass and developing sarcopenia. In the unlikely event that this occurs the B-AHEAD 3 dietitian and physiotherapist will review the patient's advice and progress and provide appropriate diet and exercise advice to prevent further reductions. If this advice requires patients to stop following the diet patients would be censored as leaving the study.

16.3 Exercise

The interventions encourage patients to increase their level of resistance exercise, and light / moderate cardiovascular exercise in a carefully designed staged programme which presents a minimal risk of fractures, cardiovascular morbidity and other complications from breast cancer, e.g. cording or lymphoedema. The programme will be advised and supervised by experienced physiotherapists / cancer exercise specialist based on a rigorous assessment of their medical history and site of metastases, and current activity and energy levels and reviewed 3 weekly (Appendix 1). Any cardiovascular, musculoskeletal or arm problems identified during the trial will be referred to appropriate care pathways for assessment and treatment.

16.4 Serum blood tests

Fasting glucose and insulin will be assessed where possible in all participants attending baseline appointment either at MFT or their recruiting centre, and repeated at the start of cycle 3 or 4 for patients on sub-study 1 only. Glucose results will be fed back to the patient's oncologist during the study if outside of the normal range. Other blood test results will not be available until after the end of the study so there are no plans to feed these back to the medical team. Baseline and sub study blood samples will only be taken in centres which have -80°C storage facilities or if the samples can be transferred to MFT or another centre with -80°C storage facilities within an acceptable time frame.

Phlebotomy will be undertaken on the non-operated arm wherever possible to minimise the risk of lymphoedema. The study is designed so participants will have their trial blood samples collected at the time of routine chemotherapy blood tests wherever possible to minimise venepuncture.

16.5 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. We will be assessing anxiety and depression every three cycles during the trial using the hospital anxiety and depression scale. Patients who score > 11 are likely to have clinical depression or anxiety and will be referred for further support with their permission (e.g. referred to their breast care nurse, who may subsequently refer to psychological services in the hospital or in the community). Scores of 8-10 are borderline and referral will be made where considered necessary.

Any potential toxicity or adverse effects of IER will be assessed and acted upon three weekly by the research dietetic and / or clinical team.

We will have a data monitoring committee to independently assess recruitment, drop out and any adverse effects of the intervention.

16.6 Generic Risk Assessment

Hazards to patients, study and organisation have been perfomed for the B-AHEAD 3 trial and have been considered low risk.

17. 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
Chemotherapy regimen 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.
Research blood samples cannot be taken	See Blood Sampling SOP (Appendix 6). 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. A diet history can be taken by BAHEAD 3 dietitians to describe the normal dietary intake.
Participant cannot adhere to or does not wish to adhere to allocated diet and/or exercise plan	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/exercise plan as best as they can. Researcher will document reported adherence and reasons for not following allocated diet/exercise plan in participant's study file. Diet/exercise plan will be adapted to patient's requirements if possible and adaptations will be recorded.
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	Diet advice can be given over the phone instead of during the baseline appointment at the Prevent Breast Cancer Research Unit as planned. Exercise advice can be given using Skype (or similar application). If Skype is not possible, exercise advice can be delivered over the phone if the physiotherapist is happy that this is appropriate for the individual patient. Following such a consultation, written targets will be posted to participant.
Participant does not have access to Skype for the 3 week resistance exercise review and cannot / is not willing to come in for a face-to-face assessment.	In depth phone consultation with BAHEAD 3 physiotherapist / exercise specialist. If still concerned may need to arrange face to face review.

The full complement of blood sample bottles cannot be filled	See Blood Sampling SOP (Appendix 6). As many as possible are filled. Outcome documented in participant's study file.
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 nausea) during study period	Medication, date started and duration to be documented in participant's study file. This information will also be collected at each cycle by the research nurses. Also document if the patient has given additional diet/lifestyle advice (e.g. for nausea).
Unable to call participant every three weeks, or partipant requests calls at a different frequency	If consistently struggling to get a participant on the phone, ask them the next time you get them the frequency that they'd like to be called. If a participant requests less frequent calls the study team will document this and aim to adhere to their request.

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.

Reporting of key adverse events (see CRF) will follow the flow chart in Appendix 8 and data for all SAEs will reviewed by the DMC.

As there are many common side effects associated with delivering chemotherapy to women with advanced breast cancer, unrelated to the delivery of a diet and exercise programme, certain SAEs do not require reporting. These are:

- Admission for treatment of febrile neutropenia
- Non-neutropenic infection (whether admitted or not)
- Grade 3 or 4 palmar plantar erythrodysaesthesia (PPE)
- Grade 3 or 4 peripheral neuropathy.

All such events should, however, be recorded in the CRF.

PIs at recruiting centres other than MFT will be responsible for reporting of serious adverse events according to their Trust protocols, and for alerting MFT R&D (as study sponsor) and the Chief Investigators to all SAEs that are not excluded from reporting.

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Appendix 1: Resistance exercise programme

Information to be provided to the trial physiotherapist prior to the baseline appointment so programme can be planned prior their visit:

- Information to be provided by the referring oncology team:
  - What is the patient's performance status? Either 0 or 1.
  - Date of latest CT scan and what the scan shows, e.g. hepatomegaly, pleural effusion, ascites.
  - Recent scans and reports which show site of metastases.
  - Location of any metastases, including bone metastases.
  - Do the metastases hinder the patients movement or mobility?
  - Pain in bone metastases?
  - What treatment has previously been given for metastases eg. XRT, denusamab, bisphosphonates, steroids, etc.
  - When was this treatment completed?
  - What general medication is the patient receiving now?
  - Any previous breast surgery or radiotherapy?
  - Does the patient have a Hickman line or peripherally inserted central catheter (PICC) line in situ?
  - Any co-morbidities and other relevant past medical history affecting the patient's ability to exercise, e.g. presence of abdominal ascites, breathlessness, balance impairment, osteoarthritis, arthropathy, risk of fracture due to osteoporosis, cardiac e.g. arrhythmias lymphoedema etc
  - Any other comments.
- Information to be provided by the patient:
  - Current level of activity and mobility i.e. how far can they walk.
  - What limits them, e.g. fatigue, breathlessness, arthritis, pain etc.
  - What is their upper body strength and any limitations, e.g. contraindications to exercise.
  - Any areas of weakness they might be concerned about.

<u>Assessments undertaken at baseline appointment to tailor programme (physiotherapist at MFT or research nurse in recruiting centre with input from MFT physiotherapist).</u>

- Fatigue – FACT F, FACT – BP, FACT- Taxane
- Pain scale at rest and after activity using a 10 point visual analogue score
- Tinetti assessment tool for balance (94) (Appendix 2)
- Range of movement for upper limb, lower limb and trunk
- Five times sit to stand test (FTSTST) for lower limb strength
- Hand grip strength for upper limb strength
- Pain vas before and after undertaking basic resistance exercise programme

<u>Examples of resisted exercises for the upper limbs</u>

The number of repetitions or weight can be altered to suit the patients ability/performance status:

- Double arm press-ups against the wall in standing
- Single arms press-ups against the wall in standing
- Biceps arm curls with weight in standing or sitting
- Biceps arm curls followed by shoulder flexion with weight in standing or sitting
- Shoulder abduction to the ceiling with elbow in extension with weight
- Shoulder abduction with the elbow flexed with or without weight
- Shoulder circumduction backwards with or without weight
- Shoulder circumduction forwards
- Shoulder presses (ie using pectoralis major/minor)adduction and abduction with elbows and shoulders flexed to 90 degrees

- Shoulder extension against the wall

Examples of resisted exercises for the lower limbs

- Sitting to standing without use of hands
- Half squats holding onto the back of a chair
- Step up and down off a step
- Flex the knee to the chest in standing
- Abduct straight leg in standing
- Etc

Examples of trunk exercises

- Side flexion to both sides with a weight in standing
- Trunk rotation with weight to both sides
- Stomach crunches in supine
- Stomach crunches diagonal on supine

Appendix 2 Tinetti assessment tool for balance

TINETTI BALANCE ASSESSMENT TOOL

Tinetti ME, Williams TF, Mayewski R, Fall Risk Index for elderly patients based on number of chronic disabilities. Am J Med 1986:80:429-434

PATIENT'S NAME _____ DOB _____

BALANCE SECTION

Patient is seated in hard, armless chair;

	Date			
Sitting Balance	Leans or slides in chair Steady, safe	= 0 = 1		
Rises from chair	Unable to without help Able, uses arms to help Able without use of arms	= 0 = 1 = 2		
Attempts to rise	Unable to without help Able, requires > 1 attempt Able to rise, 1 attempt	= 0 = 1 = 2		
Immediate standing Balance (first 5 seconds)	Unsteady (staggers, moves feet, trunk sway) Steady but uses walker or other support Steady without walker or other support	= 0 = 1 = 2		
Standing balance	Unsteady Steady but wide stance and uses support Narrow stance without support	= 0 = 1 = 2		
Nudged	Begins to fall Staggers, grabs, catches self Steady	= 0 = 1 = 2		
Eyes closed	Unsteady Steady	= 0 = 1		
Turning 360 degrees	Discontinuous steps Continuous	= 0 = 1		
	Unsteady (grabs, staggers) Steady	= 0 = 1		
Sitting down	Unsafe (misjudged distance, falls into chair) Uses arms or not a smooth motion Safe, smooth motion	= 0 = 1 = 2		
	Balance score		/16	/16

PTO

GAIT SECTION

Patient stands with therapist, walks across room (+/- aids), first at usual pace, then at rapid pace.

	Date		
Indication of gait (Immediately after told to 'go'.)	Any hesitancy or multiple attempts = 0 No hesitancy = 1		
Step length and height	Step to = 0 Step through R = 1 Step through L = 1		
Foot clearance	Foot drop = 0 L foot clears floor = 1 R foot clears floor = 1		
Step symmetry	Right and left step length not equal = 0 Right and left step length appear equal = 1		
Step continuity	Stopping or discontinuity between steps = 0 Steps appear continuous = 1		
Path	Marked deviation = 0 Mild/moderate deviation or uses w. aid = 1 Straight without w. aid = 2		
Trunk	Marked sway or uses w. aid = 0 No sway but flex. knees or back or uses arms for stability = 1 No sway, flex., use of arms or w. aid = 2		
Walking time	Heels apart = 0 = 0 Heels almost touching while walking = 1		
	Gait score	/12	/12
	Balance score carried forward	/16	/16
	Total Score = Balance + Gait score	/28	/28

Risk Indicators:

Tinetti Tool Score	**Risk of Falls**
≤18	**High**
19-23	**Moderate**
≥24	**Low**

Appendix 3: Strength tests

3a: Sit to stand test

Test Administration:

- Place the chair against a wall or otherwise stabilise for safety
- Read the following script to the participant:
    - *This is a test of how quickly you can stand up and sit down five times.*
    - *You need to sit in the centre of the seat with your feet shoulder width apart and flat on the floor.*
    - *You need to cross your arms at your wrists and hold them close to your chest.*
    - *You need to stand up completely, and make firm contact with the chair when you sit down*
    - *Do not use your hands to push from the chair*
    - *Do not to touch the back of the chair during the test*
    - *Try not to talk during the test as this may make you breathless*
    - *I will now demonstrate the five repetitions [assume correct position and complete the full five repetitions].*
    - *Do you think you are able to do that today? [document consent]*
    - *I would now like you to do two practice repetitions – please stand up and sit down twice [patient does their practice]*
    - *Now we'll do the test so please stand up and sit down five times as quickly as you can when I say 'Go'*
- Patient starts from sitting.
- Start the stopwatch at *'Go'* and stop it when the buttocks touch the chair after the 5th repetition
- Record time on CRF
- Record any deviations from SOP, e.g. inability to complete 5 repetitions without assistance or use of arm support, any modifications should be documented

Norms for Community Dwelling Adults: (79)

Descriptive statistics for time (sec) for 5 sit-to-stand repetitions

Measurement (n)	Mean ± SD	Minimum-Maximum
Trial 1: all ages (94)	7.8 ± 2.8	4.0 – 16.3
Trial 2: all ages (94)	7.5 ± 2.8	4.0 – 17.0
Mean: all ages (94)	7.6 ± 2.7	4.0 – 16.0
Mean: 19-49 years (39)	6.2 ± 1.3	4.1 – 11.5
Mean: 50-59 years (15)	7.1 ± 1.5	4.4 – 9.1
Mean: 60-69 years (18)	8.1 ± 3.1	4.0 – 15.1
Mean: 70-79 years (16)	10.0 ± 3.1	4.5 – 15.5
Mean: 80-89 years (6)	10.6 ± 3.4	7.8 – 16.0

3b: Hand grip dynamometer

American Society of Hand Therapists method (95).

- Patient should be in a seated position with shoulder adducted (arm against side) and elbow in a 90^0 flexion.
- Gentle, not a quick squeeze (that could cause needle to jump)
- Take the value in kilograms (on the outside of the dial).
- Take three readings for each arm (alternate arms so that each arm has a short rest between readings).

Appendix 4: Standard 3 weekly diet mailings which can be sent to the IER group.

Mailing	Topic
1	Ways to get motivated for changing your diet • 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 • Possible benefits of energy restriction • Weight gain and energy balance • Body fat and health and where fat is stored • Energy density of food
3	The Mediterranean diet • What is a Mediterranean diet? • 5-a-day • Supplements and vitamins • Fibre including ready reckoner done for book
4	Tips for following a diet during 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 • Stress mood and food (use some tips from bahead 2 problem solving sheet)
7	Tips for shopping and eating out

	• Healthy options for eating out • Shopping • Reading and understanding food labels
8	<u>How to make the 2 day diet work for you</u>
9	<u>Drinks and sauces</u> • Alcohol • Caffeine • Fizzy drinks • Sweeteners • Fructose • Sauce info
10	<u>Diet myths</u>

Appendix 5: Serum and plasma collection, handling and storage

Sample collection

Baseline blood samples will be collected at MFT or chemotherapy centres before initiating the diet and exercise programmes. The two sub-study 1 samples will be collected in the chemotherapy centres two days before chemotherapy (this may coincide with the time of normal pre-treatment blood tests for women on paclitaxel) and on the day of chemotherapy treatment. Women will be asked to fast for 12 hours and they will receive a complimentary light breakfast immediately after blood collection if at MFT. Samples will be spun, aliquoted and processed and stored at -80°C at MFT or in the chemotherapy centres.

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.

Insulin serum SST (BD Vacutainer: orange top)

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 2-8°C or 1 month at -15 to -25°C before storing at -80°C. Insulin samples will be stored at -80°C at the site of collection then transported for analysis to MFT. Insulin will be measured by clinical biochemistry at MFT using a commercially available chemoluminescence immunoassay. All assays will be batched and performed on stored serum (-80°C) upon completion of the study to reduce inter-batch variation.

Glucose fluoride (BD Vacutainer: grey top)

Glucose will be assessed using a Hexokinase/glucose-6-phosphate dehydrogenase method. This will be analysed in the recruiting centres or at MFT as soon as possible or stored at 4°C. Freezing and long term storage of these samples is not recommended.

Plasma collection and storage for proteomic analysis (BD Vacutainer: purple top)

Sample for proteomic analysis will be collected in EDTA tubes (10ml tube or 3 x 4ml tube or equivalent). After collection the tube should be inverted 8-10 times and spun within 30 minutes. Centrifugation will be for 10 minutes at 2000g with plasma removed from the upper layer. After preparation the sample

will be split into 2ml aliquots and frozen. Storage will be at -80°C at the site of collection with any transport under dry ice to ensure samples don't thaw. Proteomic analysis will be carried out by Dr Julie Brazatti in the Institute of Cancer Sciences at the University of Manchester after all samples have been collected.

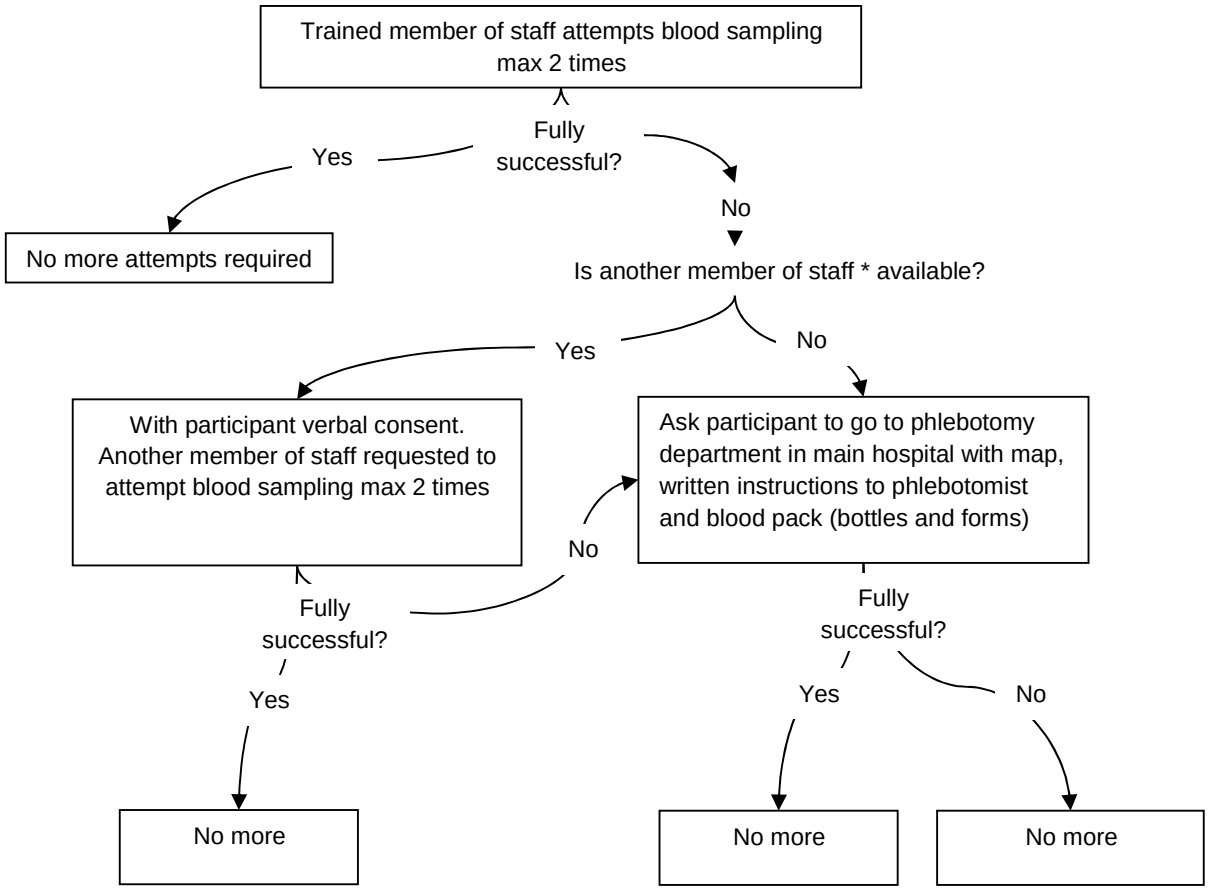
Collection, storage and transport of serum and plasma samples

If possible, blood samples will be taken at all of the recruiting hospitals where -80°C storage facilities exist. Samples will be stored at -80°C at the site of collection until transportation to The Nightingale Centre at MFT The plasma samples will then be collated and transported to Dr Julie Brazatti in the Institute of Cancer Sciences at the University of Manchester. 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.

Responsibility for transfer of samples

The B-AHEAD 3 Study team at MFT will be responsible for monitoring transfer and receipt of biological specimens. Tracking forms will be sent by centres to the B-AHEAD 3 Study team at MFT 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 6: Blood sampling SOP for B-AHEAD 3 staff at Manchester University NHS Foundation Trust



Notes:

- All blood ideally 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 7: Schedule of assessments in the B-AHEAD 3 trial

Intervention and control patients			
Assessment	**Timing**	**Location**	**Undertaken by**
CT scan for assessment of response and lean body mass	Usually every 3 cycles as per local guidelines	Local hospital	Organised by research nurse
Self-report CTCAE v4 (chemotherapy toxicity)	At each chemotherapy cycle	Chemotherapy units	NIHR research nurse
Taxane neuropathy with vibratip	At each chemotherapy cycle	Chemotherapy units	NIHR research nurse
Quality of life & fatigue, hospital anxiety depression score	Baseline, then every 3 cycles	Chemotherapy units	NIHR research nurse
Fasting insulin & glucose, protein blood-based biomarkers	Baseline (and twice around cycle 3 or 4 if on sub-study 1)	Chemotherapy units or Prevent Breast Cancer Research Unit according to patient preference	NIHR research nurse or B-AHEAD 3 researcher
Lower limb & upper limb strength from sit to stand and hand grip dynamometer tests	Baseline, then every 3 cycles	Prevent Breast Cancer Research Unit or chemotherapy units	NIHR research nurse or B-AHEAD 3 researcher
7 day food diary and physical activity questionnaire	Baseline, cycle 3 and cycle 6	Completed by patient at home & e-mailed / posted back to the B-AHEAD 3 researchers	Checked by B-AHEAD 3 researcher
Demographics, weight and medical history	Baseline	Prevent Breast Cancer Research Unit or over phone	B-AHEAD 3 researcher
Exercise tracker	Weekly throughout trial for the first 18 weeks of chemotherapy	Patient to complete at home	Patient to complete at home

Intervention group			
Advice	**Timing**	**Location**	**Undertaken by**
Diet advice for intervention group	Baseline	Prevent Breast Cancer Research Unit or over phone	B-AHEAD 3 dietitian
Resistance exercise advice	Baseline	Prevent Breast Cancer Research Unit or Skype (or similar application) or over phone or at local unit	B-AHEAD 3 physiotherapist / cancer exercise specialist / local physiotherapist
Review calls to discuss diet & exercise	3 weekly whilst in the trial	Patients called at home	B-AHEAD 3 dietitian
Review call / Skype to discuss resistance exercise	At 3 weeks	Patients called at home	B-AHEAD 3 physiotherapist / cancer exercise specialist

Control group			
Advice	**Timing**	**location**	**Undertaken by**
Resistance exercise advice	Baseline	Prevent Breast Cancer Research Unit or Skype (or similar application) or over phone or at local unit	B-AHEAD 3 physiotherapist / cancer exercise specialist / local physiotherapist
Review calls / Skype to discuss resistance exercise	3 weekly whilst in the trial	Patients called at home	B-AHEAD 3 physiotherapist / cancer exercise specialist

Appendix 8: Adverse Event Decision Making and Procedure