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BREAst Cancer Personalized NuTrition (BREACPNT) - dietary intervention in breast cancer survivors treated with endocrine therapy: Rational and study design

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ABSTRACT

Introduction: Breast cancer survivors treated with adjuvant endocrine therapy commonly experience weight gain, which has been associated with low adherence to therapy and worse breast cancer prognosis. We aim to assess whether a personalized postprandial glucose targeting diet will be beneficial for weight management as compared to the recommended Mediterranean diet in this patient population

Methods and Analysis: The BREAst Cancer Personalized NuTrition (BREACPNT) study is a phase-2 randomized trial in Hormone Receptor positive (HR+) breast cancer patients, treated with adjuvant endocrine therapy. The study objective is to assess whether dietary intervention intended to improve postprandial glycemic response to meals results in better weight and glycemic control in this population as compared to the standard recommended Mediterranean diet. Consenting participants will be assigned in a single blinded fashion to either of two dietary arms (Mediterranean diet or an algorithm-based personalized diet). They will be asked to provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for two weeks, at the initiation and termination of the intervention period. Microbiome composition data will be used to tailor personal dietary recommendations. After randomization and provision of dietary recommendations, participants will be asked to continuously log their diet and lifestyle activities on a designated smartphone application during the 6-month intervention period, during which they will be monthly monitored by a certified dietitian. Participants' clinical records will be followed twice yearly for five years for treatment adherence, disease free survival and recurrence.

Ethics and dissemination: The study has been approved by the ethics committee in the Sheba medical center (file 5725-18-SMC, Ramat Gan, Israel) and the Weizmann Institutional Review Board (file 693-2, Rehovot, Israel). The finding of the study will be published in a peer reviewed publication.

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Strengths and limitations of this study

- A trial testing the efficacy of Personalized Postprandial-glucose-response Targeting (PPT) diet, based on an innovative dietary approach for weight maintenance, as compared to standard diet, in breast cancer survivors.
- The personalized diet involves advanced technologies, including microbiome, continuous glucose monitoring, metabolomics features and full dietary records are used to allow better understanding of the interactions between dietary intake with metabolic and health parameters.
- A homogenous study population, that includes HR+, early stage breast cancer survivors treated with adjuvant endocrine therapy. Patients are randomized into the two study arms and stratified by stage, treatment type, menopausal status and BMI. Yet, the study patients are representative of women from the center of Israel.
- The study design includes daily use of smartphone application for logging dietary intake and lifestyle events. While this may improve patient' adherence to diet, it can lead to exclusion of patients who do not hold a smartphone or with no capability to work with a smartphone app on a daily basis.

INTRODUCTION

The majority (~75%) of breast cancer patients are diagnosed with hormone receptor-positive (HR+) tumors and are assigned adjuvant endocrine treatment (ET) for a period of at least 5 years, which was shown to improve survival. However, adjuvant ET is associated with distressing side-effects which may be long lasting and substantially impair patients' quality of life and adherence to treatment. These side effects include weight gain and body composition changes, which are common in breast cancer survivors and are experienced by many women during treatment and for years after diagnosis(1–3). Weight gain in this population is complex and is

associated with various factors such as tumor type, menopausal status(4), pre-diagnosis body mass index (BMI)(5) and neo-adjuvant/adjuvant treatment type including chemotherapy and ET(2,6). Importantly, weight gain after breast cancer diagnosis is associated with increased risk for metabolic syndrome and cardiac disease (7,8), and was reported as a risk factor for breast cancer recurrence and shorter survival(4,9,10). Therefore, weight management strategies including diet, regular physical activity and cognitive behavioral therapy are recommended for controlling weight gain in breast cancer patients. Previous studies showed that weight loss interventions, incorporating diet, exercise and psychosocial support, in overweight or obese breast cancer survivors appear to result in decreased body weight, BMI and waist circumference and improvement in overall quality of life(11). However, the optimal weight loss intervention method and the impact of weight loss on survival outcomes is unclear. Furthermore, the interaction between the microbiome of breast cancer patients and dietary intervention has not been assessed.

The comprehensive role of the gut microbiome in modulating immune and metabolic health is increasingly recognized. Dysbiosis, referring to the disruption in the balance of gut bacterial communities, is associated with many conditions(12). The gut microbiome homeostasis can be influenced by internal factors, such as genetic, age-related and hormonal-related, as well as by external factors, such as stress, lifestyle and antibiotics(13). In addition, the microbiome is directly affected by the individual diet which in turn affects the body's response to food (14,15). Particularly relevant to breast cancer, diet plays an important role in creating a microbiome environment involved in estrogen metabolism(16). High estrogen levels contribute to breast cancer risk in postmenopausal women(17). In a recent study, gut microbiome diversity was linked to weight gain(18) and microbiome alterations were found to contribute to post-dieting weight regain(19). In addition, it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol (20). In a previous study we showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food(21). Importantly, this study emphasized the involvement of functional microbial pathways and bacterial taxa in

host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on personal measurements, including blood tests, personal lifestyle and gut bacteria profiles. In a following study implementing a 6-month dietary intervention plan in individuals with prediabetes, the Personalized Postprandial-glucose-response Targeting (PPT) approach significantly improved glycemic control and reduced PPGRs as compared to the commonly recommended Mediterranean diet (22).

In this study we seek to evaluate the clinical efficacy of the PPT diet combined with caloric restriction, compared to the Mediterranean diet, in promoting weight maintenance or weight loss and glycemic control in HR+ early stage breast cancer survivors treated with adjuvant ET.

METHODS

Study design

This study is a two-arm, parallel group, single-blinded, randomized controlled trial in early stage HR+ breast cancer patients treated with adjuvant endocrine therapy. Eligible participants will undergo a 6-month nutrition intervention program which will include dietary recommendations, daily logging and monthly follow up meetings provided by a certified dietician. Upon trial entry and after profiling (described below) participants will be randomly and equally assigned to the Personalized Postprandial-glucose-response Targeting (PPT) dietary (arm A) or to the Mediterranean-style (MED) dietary (arm B) (as shown in **Figure 1**). All meetings will take place in the Breast Oncology Institute at the Sheba Medical Center. The primary objective of the study is to evaluate the efficacy of the PPT arm vs the MED arm in controlling body mass changes in the patient population during the intervention period (study endpoints are detailed in **BOX 1**)

BOX 1 – STUDY ENDPOINTS

Primary endpoint

Body weight changes defined as the net body weight gained/lost in the 6–month intervention period

Secondary endpoint

Glycemic response as measured by continuous glucose monitoring (CGM) device

Exploratory endpoint

- Five years Disease free survival (DFS)
- Microbiome and blood metabolites modulation during the diet interventions
- Adherence to algorithm-based personalized diets, compared to standard diets advised for weight control
- HR+ breast cancer patients' adherence to hormonal treatment
- Translational studies

Patient and public involvement

Patients are being involved in the recruitment effort by actively publishing the study recruitment information and sharing their own experience during the study, via social networks and breast cancer survivors' groups. At the end of the study patients will receive an analysis of their glucose responses to the foods that they ate, and get an access to different nutritional tools that will be available to them on a secure website or on a dedicated mobile application. Furthermore, At the end of the study all patients will be given an access to their personal tailored dietary recommendations, built for them by the study team based on their personal data, regardless of their assigned arm during the study.

Study population

This trial will enroll breast cancer survivors treated with ET and followed at the Breast Oncology Institute at the Sheba Medical Center. Eligibility criteria (inclusion and exclusion criteria) are detailed in **Table 1**. Potentially eligible participants will be identified and recruited to the study by the medical team during regular clinic visits or via database search and phone calls by the clinical study coordinator (SC). Information leaflets and a poster describing the study design and contact information will be available at the institute's reception and waiting area. Additionally, a video explaining the study and its aims will be shown on screens at the institute's reception and waiting area and will be sent to potential participants (https://youtu.be/kxrqONj3KGM). All participants will assign informed consent.

Table 1: Eligibility criteria

Inclusion Criteria	Exclusion Criteria
Female patients	Oral Antibiotics/antifungal use in the previous one month to profiling stage *
Age ≥ 18 and ≤ 80	Known Diagnosis of diabetes or the use of anti-diabetic and/or weight-loss medication
Diagnosis of stage 1-3 HR+ breast cancer, who underwent surgery	BMI<18.5
At least 60 days after last non-endocrine oncology treatments (i.e. definitive surgery, radiation or chemotherapy – whichever is last) if these were indicated.	Patients under another diet regime and/or a dietitian consultation/ clinical study
Adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists) taken for at least 30 days but no more than 24 months.	Pregnancy, breast feeding
Willing to operate a smartphone application	HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
	Known diagnosis of psychiatric disorders (Schizophrenia, Bipolar Disorder)
	Known diagnosis of IBD (inflammatory bowel diseases)
	Patients that underwent Bariatric surgery
	Known Alcohol or substance abuse

*Patients will be offered to join the study at a later point

Study Procedures and Intervention

Screening and profiling stage

Consenting patients will proceed to the screening and profiling stage. During this stage they will undergo the following procedures:

1. Meeting with the SC and completion of questionnaires detailing relevant medical background, nutritional habits and lifestyle activities. Questionnaires will be filled online using the REDCap (23) software (a secure web application for managing online surveys and clinical trials).
2. Participants will provide blood samples after a night fast (12 hours) for CBC and blood chemistry, including liver function, lipid profiling, Fasting Plasma Glucose (FPG) and HbA1c. LH, FSH and Estradiol will be measured only in pre-menopausal patients. Participants will provide urine sample for estradiol derivatives for future exploratory analyses.
3. Anthropometrics measurements, including weight, height, waist and hip circumference will be taken at this meeting.
4. Stool sample: Patients will receive a designated stool kit (Genotek OMR200) to collect stool at home. The SC will instruct them how to provide the stool samples and will ask to return this kit during the following week for further processing of the microbiome data. Microbiome sequenced data are essential for the algorithm predictions, thus stool sample is obligatory for participation in the study.
5. Continuous glucose monitoring (CGM) connection: Patients will be connected to a CGM (Abbott Freestyle LibrePro) for 2 weeks. The CGM kit includes a sensor affixed to the back of the arm that continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels.
6. Food diary: Patients will be instructed to download the study dedicated smartphone application ('personalized nutrition project') for food logging. They will log in real-time their food intake, physical activities, sleep duration and quality and special events. During the profiling period, patients will be asked to follow their regular dietary and lifestyle habits (see examples of

logging activities in **Figure 2A**). All participants will receive a registration code and their data will be anonymized.

7. Data collected during the profiling period, including microbiome, anthropometrics, blood parameters and questionnaires will be analyzed and used by the PPT algorithm to provide personal dietary recommendations for each participant.

Menus construction

Before randomization, menus will be constructed for each patient and will be adjusted for, patients' caloric target and clinical data. The menus construction flow is presented in **Figure 2B**.

Meal bank (list)

The menus provided to patients in this study are constructed from a meal bank that we previously generated(22), with over 2,000 meals representative of the Israeli typical diet and with a variety of different food combinations. We divided the meals in the meal bank into four meal types (breakfast, lunch, dinner and snacks) and labeled them according to meal categories (dairy; meat; fish etc.) in order to generate menus according to patients' personal preferences.

Caloric target calculation

In order to provide the patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as an average between:

1. Estimated Energy Requirements (EER) calculated with the use of the Mifflin equation for Resting Energy Expenditure (REE), using their weight, height, age and gender, and multiplied by Physical Activity (PA) factor, based on the level of PA that the person performs on a regular basis (24).

2. Energy expenditure assessed by Basal Metabolic Rate (BMR) value measured by body composition analyzer (Tanita). The result from this equation will be divided by 0.7 (as REE represents ~70% of total energy expenditure).
3. Average daily caloric intake obtained from the patients' dietary records during the profiling stage, to account for the subject's dietary habits prior to the intervention.

Furthermore, for individuals with BMI>25 a total of 500Kcal will be reduced from their calculated caloric target, but not less than 1200 calories/day, to allow weight loss according to common recommendations for weight loss (25,26).

Diets

Mediterranean-style (MED) diet

In this arm we included meals that were scored by four external dietitians according to the Mediterranean-style dietary recommendations. Meals were binary scored as recommended (=1) or not recommended (=0) and we applied scores 1 to 5 to all meals, depending on how many dietitians marked the meal as recommended or not. The diet is based on recommended foods such as vegetables, fruits, legumes, whole grain products, unsaturated fats such as olive oil and nuts, fish, poultry and low-fat dairy products. Consumption of red meat, high fat dairy products, processed foods and sweet pastries, was discouraged as part of the diet. Additionally, menus in this arm were designed with the following target for daily macronutrient composition: 45-65% of calories from carbohydrates; 15-20% from protein; and 20-35% from fat, with up to 10% from saturated fat. Menus include only meals that received scores 1 and 2. Participants will be encouraged to consult with the dietitian regarding meals that may not appear in the constructed menu.

Personalized Postprandial-glucose-response Targeting (PPT) diet

In this arm, dietary recommendations will be based on the algorithm predictions of the postprandial glucose responses (20), shown to improve glycemic control and metabolic health in healthy individuals or in individuals with prediabetes and

diabetes(22,27). Notably, these interventions were not caloric restricted as in the current study. Meals will be scored from 1-5 based on a unique scoring method that we developed and based on their predicted response, taking into consideration a list of features including questionnaires data, anthropometrics, blood parameters and microbiome abundances (see **Supplementary table 1** for the full list)**.** The algorithm is trained using PPGRs collected via CGM devices from previous and ongoing studies, and it is able to generate predictions without the patient's personal CGM-based features, already done in a pilot study within individuals diagnosed with Type-2 Diabetes (27).

Randomization

Following the profiling stage patients will be randomly assigned to one of two arms of the study. Approximately 100 subjects will be assigned to each arm using a blinded randomization algorithm and the following stratification factors: (1) menopausal status at study entry (post/pre) (2) received/not received chemotherapy prior to study entry (3) ET type (Tamoxifen/Aromatase inhibitor) (4) Breast Cancer stage at diagnosis and (5) BMI above/below 27. Patients and study team, excluding the dietitian, will be blinded to the study arm assigned.

Recommendation meeting

Upon menus construction, patients from both arms will be invited to a recommendations meeting (hereafter 'day-0') with the dietitians. Patients will receive general information regarding their menu and will be instructed to consume and log their meals according to it. In order to ensure accurate logging the dietitians will schedule an online follow up two weeks after 'day-0'. Anthropometrics measurements, including weight, hip and waist circumference, taken at this meeting will be used as baseline measurements.

Follow up Meetings

All patients will participate in monthly follow-up meetings with a dietitian (total of 6 meetings) in order to evaluate their compliance to the dietary recommendations they

received and provide additional advice if needed. Anthropometric measurements (weight, hip and waist circumference) will be taken at each time point. Furthermore, patients will be asked to fill a follow-up questionnaire and report any changes within their lifestyle and treatment. At the beginning of Month 4 of the intervention period, patients will be offered to be reconnected to CGM for two weeks (optional). At the monthly meeting before the end of intervention patients will receive a stool kit, to be returned at the end of intervention meeting.

End of intervention

At the end of the 6-month intervention period, patients will be invited to a meeting in which anthropometrics measurement will be taken, as well as urine, blood and stool samples. Additionally, participants will be connected to CGM for 2 weeks (mandatory) for the third time (Figure 1). When patients return CGM they will be unblinded to their assigned intervention arm by the study dietitian.

Long term follow up

At 12-month time point, patients will be invited to a follow up meeting and will be asked to fill follow-up questionnaires, including food frequency questionnaire. Anthropometric measurements and a 3-day food diary on the study app will be recorded. Patients will receive the menu of the other study arm and will be offered to follow either one of the diets. Long-term clinical follow-up information will be collected from the electronic medical records twice yearly for treatment adherence, recurrence and survival calculation purposes, for a period of up to five years post randomization.

Adherence to the study recommendations

The adherence to the prescribed diets during the intervention will be evaluated by the dietitian by a close monitoring of the patients' self-recorded dietary intake in the logging application, as well as by monthly electronic follow-up questionnaires that participants will be asked to fill out. In order to encourage dietary adherence and self-monitoring, we will generate a bi-weekly semi-automatic feedback reports that will

include composite grades on a scale of 0-100 (from worse to best) for diet composition, calorie intake and dietary fiber intake separately, for the entire two-week period (**Figure 2C**).

- *MED-diet composition grade:* indicates how well the participant sticks to the dietary recommendations based on the MED approach including Carbohydrate (as % of daily caloric intake), fats in general (as % of daily caloric intake) and specifically saturated fat intake below and above 10% of caloric intake. Dietary fiber intake per each of 1000 kCal per day will be also calculated as part of the score.
- *PPT-diet composition grade:* indicates how well the participant sticks to predictor-based meal scores. Each meal score was assigned with a grade as follows: meal score 1=grade 100; meal score 2=80; meal score 3=50; meal score 4=25; meal score 5=0. The grades are averaged calorie-wise (with food energy trimmed to be within (100,500) kcal interval)- Σ kcal(i) $\cdot$ grade(i). For example, if a participant eats three meals: 600 kcal of meal score 2, 1000 kcal of meal score 5 and 80 kcal of meal score 1, feedback grade would be: (500*80+500*0+100*100) / (500+500+100) = 45. If too few (100 by default) calories are logged (overall), we did not compute a score.
- *Calories grade:* indicates how well the participant sticks to the prescribed caloric target. When caloric intake deviates within 15% of caloric target (CT) the applied grade is 100; when caloric intake deviation exceeds 60% of CT the applied grade is 0; when caloric intake deviation is between 15% to 60%, a linear penalty is applied to the grade depending on the deviation.
- *Dietary fiber grade:* indicates if participants consumed the recommended amount of dietary fibers from the diet at the referred time. When fiber intake in grams per day reaches the recommended amount, or higher the applied grade is 100 and when it is below the recommended amount a linear penalty is applied to the grade.

In addition to grades, feedback reports also included a list of recommended meals and non-recommended meals (by meal score) to highlight the best and worst meals consumed on that time period (as logged by the participant). The best and worst meals

lists will be generated systematically and be reviewed by a dietitian from the study team.

STATISTICAL ONSIDERATION

Sample size determination

To estimate the required sample size, we performed power analysis while estimating effect size using the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. (28), the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis

All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variables, the Spearman correlation coefficient will be used. To compare parameters for continuous variables in two time points, the paired-samples t-test will be performed (or Wilcoxson test for non-normally distributed variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous/categorical variables in the number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer Translational Research laboratory at Sheba Medical Center. The blood and urine samples will be stored at -80°C and bacterial DNA samples will be stored at -20°C. The samples will be encoded with no identifying information. Identifying details and codes will be kept in an encrypted file stored at Sheba medical center. Encoded stool samples will be transferred to the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team. Samples will be stored for up to 10 years. All future use of stool and blood samples will be subjected to IRB approval.

Ethics and dissemination

The study has been approved by the Sheba medical center Institutional Review Board (IRB 5725-18) and the Weizmann institute of science Institutional Review Board. The findings of this study will be published in a peer reviewed publication. De-identified individual participant data and applicable supporting clinical trial documents will be available upon request for 12 months after publication.

CURENT STATUS

To date (February 2022), 120 participants have been recruited, out of them 60 completed the 6-month intervention period including 38 participants who completed the 12-month time point.

DISCUSSION

Dietary interventions are the first-line treatment for weight management within breast cancer survivors and have beneficial results. Yet, the ability to maintain these outcomes is questionable and require further research(11,29,30). In this trial, we aim to assess the effect of a PPT diet on weight maintenance as compared to Mediterranean-style diet in early stage HR+ breast cancer patients, taking ET.

Advantages of this study design include a comprehensive profiling of each participant, which allow us to better understand participants' metabolic baseline and to assess the

effect of the dietary changes. Additionally, the continuous food logging by the study patients using a designated smartphone app can provide us with insights on the patients compliance to the dietary recommendations in both arms. However, this may limit the study population to individuals who are able to work with smartphone application on a daily basis. Furthermore, the study participants are being closely followed by a dietitian from the study team who monitor their food intake and meet them on a monthly basis in order to increase compliance during the first 6-months. However, on the long term, without intensive monitoring, the feasibility of the PPT diet and the ability to follow the diet recommendations should be investigated. Notably, Ben-Yacov et al. (22), reported that prediabetes individuals following PPT diet were able to maintain the results during 12-month follow up as compared to those who followed the MED diet.

Lastly, as gut microbiome composition and pathways were recently associated with weight changes and metabolic health parameters, as well as with risk for breast cancer diagnosis and recurrence (31), the rich dataset will allow us to further explore whether gut microbiome composition and pathways have a predictive role in weight management, metabolic health parameters and glycemic control within early stage HR+ breast cancer patients.

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AUTHOR CONTRIBUTIONS

MR, MD and AW conceived the study and designed the intervention. MR and MD wrote the manuscript. AG is responsible for directing the computational aspects of the study. DK is responsible for the feedback reports and summary reports being sent to participants. MBG, DMS and YV coordinate participants' recruitment and management throughout the intervention and follow-up. AW developed the protocols and directed and performed the microbiome sample sequencing with the help of MLP.

ES and ENG conceived the study, designed the intervention and wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST STATEMENT

ENG reports Honoraria and Consulting fees from Pfizer, Novartis, Roche Eli-lilly AstraZeneca. ES is paid scientific consultants for DayTwo Inc. No pharmaceutical manufacturers or other companies from the industry contributed to the planning, design, or conduct of the trial. No other potential competing interest are relevant to this article were reported.

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FIGURES LEGEND

Figure 1. An illustration of the study design

Figure 2. Study application and menus construction. **(A)** The food logging application, examples of logging activities and information available on the application. **(B)** Menus construction flow. **(C)** An example of the bi-weekly feedback report that will be sent to participants.

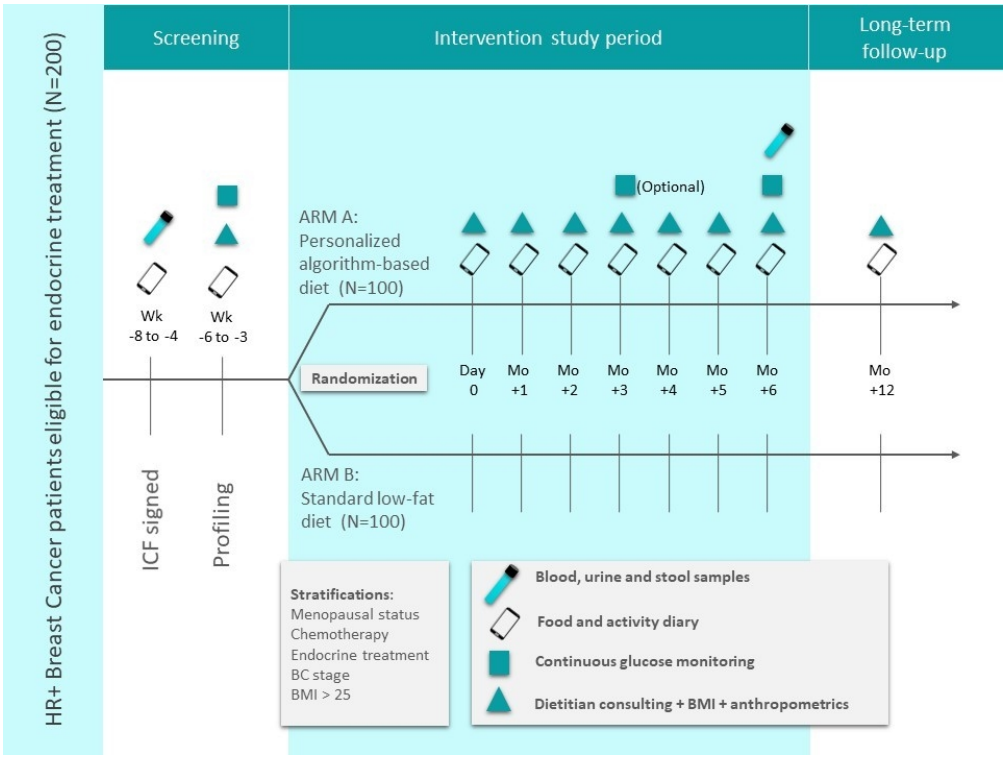

Figure 1. An illustration of the study design

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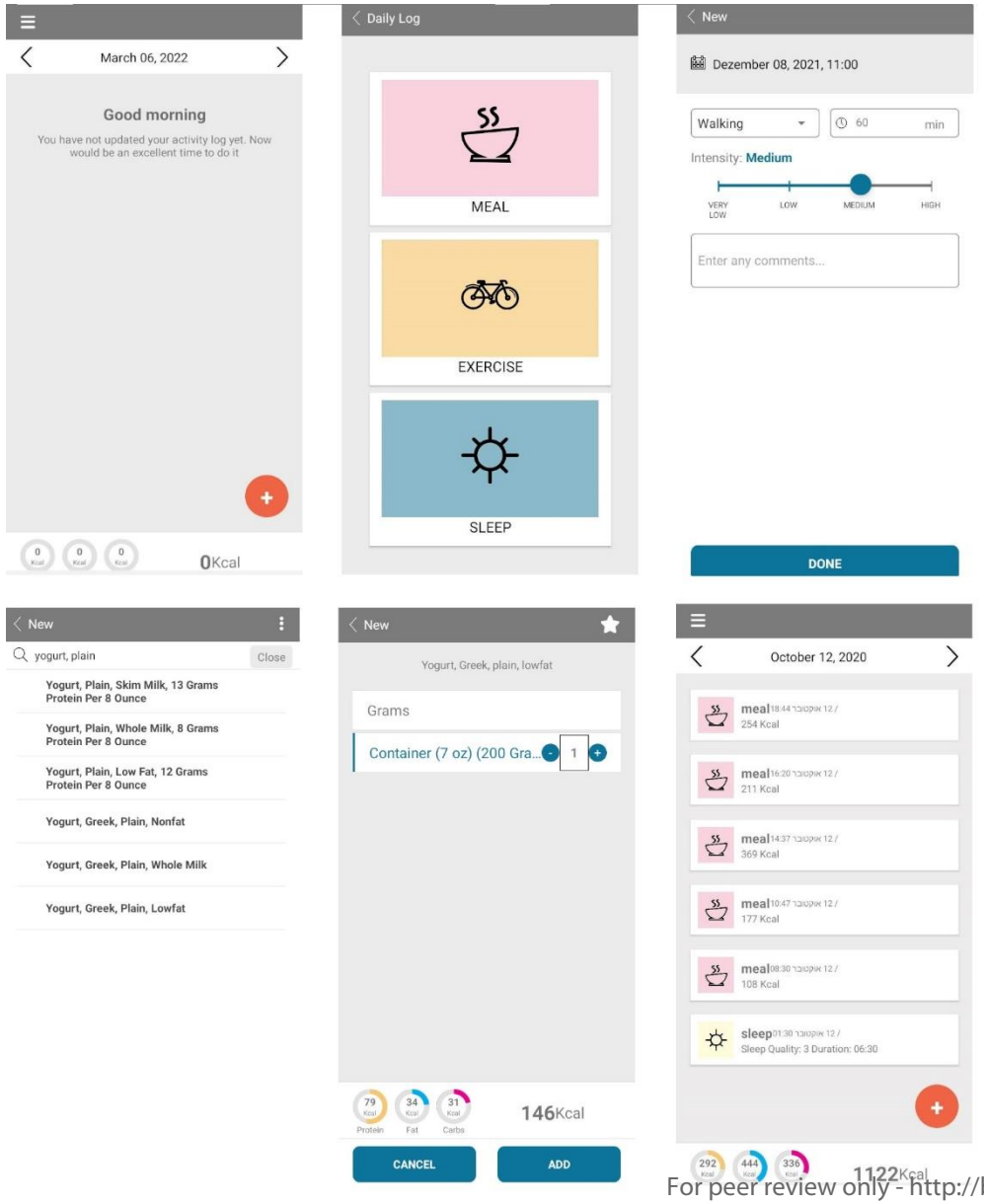
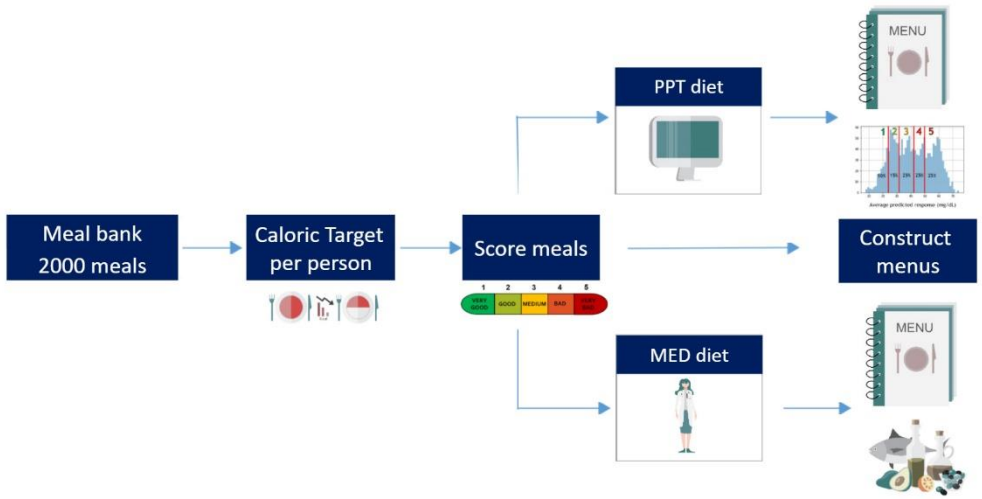
A



B



C

List of features - PPGR predictions
Blood tests
HbA1C%
Hemoglobin
FastingGlucose
Anthropometric (measured at profiling)
BMI
BodyFat
Waist
Weight
Hips
Dietary components of the meal
Alanine_g
Alcohol_g
Arginine_g
Caffeine_mg
Calcium_mg
Carbohydrate_g
Cholesterol_mg
Energy_kcal
Fructose_g
Galactose_g
Glucose_g
Isoleucine_g
Lactose_g
Leucine_g
Magnesium_mg
Maltose_g
Niacin_mg
Phenylalanine_g
Protein_g
Sodium_mg
Starch_g
Sucrose_g
SugarsTotal_g
Thiamin_mg
TotalDietaryFiber_g
TotalLipid_g
TotalMonounsaturatedFattyAcids_g
TotalPolyunsaturatedFattyAcids_g
TotalSaturatedFattyAcids_g
TotalTransFattyAcids_g
VitaminC_mg
VitaminD_IU
VitaminE_mg
Water_g
Zinc_mg
Health questionnaire
Age
Currently_smokes
Evening_Hunger
Ever_smoked
Gender

General_Hunger
Is_pregnant
Midday_Hunger
Morning_Hunger
Physical_activity_-_freq
Physical_activity_-_mins
Regular_defecation
Sleep_quality
Stress
Work_activity
Microbiome features
s_Acidaminococcus_unclassified
s_Adlercreutzia_equolifaciens
s_Akkermansia_muciniphila
s_Alistipes_finegoldii
s_Alistipes_indistinctus
s_Alistipes_onderdonkii
s_Alistipes_putredinis
s_Alistipes_senegalensis
s_Alistipes_shahii
s_Anaerostipes_hadrus
s_Anaerotruncus_unclassified
s_Bacteroidales_bacterium_ph8
s_Bacteroides_caccae
s_Bacteroides_cellulosilyticus
s_Bacteroides_clarus
s_Bacteroides_dorei
s_Bacteroides_eggerthii
s_Bacteroides_faecis
s_Bacteroides_finegoldii
s_Bacteroides_fragilis
s_Bacteroides_intestinalis
s_Bacteroides_massiliensis
s_Bacteroides_nordii
s_Bacteroides_ovatus
s_Bacteroides_plebeius
s_Bacteroides_salyersiae
s_Bacteroides_stercoris
s_Bacteroides_thetaiotaomicron
s_Bacteroides_uniformis
s_Bacteroides_vulgatus
s_Bacteroides_xylanisolvens
s_Barnesiella_intestinihominis
s_Bifidobacterium_adolescentis
s_Bifidobacterium_animalis
s_Bifidobacterium_bifidum
s_Bifidobacterium_catenulatum
s_Bifidobacterium_longum
s_Bifidobacterium_pseudocatenulatum
s_Bilophila_unclassified
s_Bilophila_wadsworthia
s_Burkholderiales_bacterium_1_1_47
s_Catenibacterium_mitsuokai

s_Clostridium_bartlettii
s_Clostridium_bolteae
s_Clostridium_leptum
s_Collinsella_aerofaciens
s_Coprobacter_fastidiosus
s_Coprococcus_catus
s_Coprococcus_comes
s_Coprococcus_sp_ART55_1
s_Desulfovibrio_desulfuricans
s_Desulfovibrio_piger
s_Dorea_formicigenerans
s_Dorea_longicatena
s_Eggerthella_unclassified
s_Erysipelotrichaceae_bacterium_6_1_45
s_Escherichia_coli
s_Escherichia_unclassified
s_Eubacterium_biforme
s_Eubacterium_eligens
s_Eubacterium_hallii
s_Eubacterium_ramulus
s_Eubacterium_rectale
s_Eubacterium_siraeum
s_Eubacterium_ventriosum
s_Faecalibacterium_prausnitzii
s_Flavonifractor_plautii
s_Gordonibacter_pamelaeae
s_Haemophilus_parainfluenzae
s_Holdemania_unclassified
s_Lachnospiraceae_bacterium_1_1_57FAA
s_Lachnospiraceae_bacterium_2_1_58FAA
s_Lachnospiraceae_bacterium_3_1_46FAA
s_Lachnospiraceae_bacterium_5_1_63FAA
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s_Lactococcus_lactis
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s_Methanobrevibacter_smithii
s_Odoribacter_splanchnicus
s_Oscillibacter_unclassified
s_Oxalobacter_formigenes
s_Parabacteroides_distasonis
s_Parabacteroides_goldsteinii
s_Parabacteroides_johnsonii
s_Parabacteroides_merdae
s_Parabacteroides_unclassified
s_Paraprevotella_clara
s_Paraprevotella_unclassified
s_Paraprevotella_xylaniphila
s_Parasutterella_excrementihominis
s_Peptostreptococcaceae_noname_unclassified
s_Phascolarctobacterium_succinatutens
s_Prevotella_copri

s_Roseburia_hominis
s_Roseburia_intestinalis
s_Roseburia_inulinivorans
s_Roseburia_unclassified
s_Ruminococcus_albus
s_Ruminococcus_bromii
s_Ruminococcus_callidus
s_Ruminococcus_gnavus
s_Ruminococcus_lactaris
s_Ruminococcus_obeum
s_Ruminococcus_sp_5_1_39BFAA
s_Ruminococcus_torques
s_Streptococcus_parasanguinis
s_Streptococcus_salivarius
s_Streptococcus_thermophilus
s_Subdoligranulum_unclassified
s_Sutterella_wadsworthensis
s_Veillonella_parvula

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a, We added the MOH identifier
Protocol version	#3	Date and version identifier	Protocol attached
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16-17
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	16
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4, figure 1
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1, page 7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6-7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	12-13; Figure 2c
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1, page 7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline,	14, Box 1

		final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1 ; 8-12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate	11

		document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires,	9-11

		laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Figure 2A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8,15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14-15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14-15
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15

Notes:

- 2b: n/a, We added the MOH identifier
- 8: 4, figure 1
- 11c: 11; figure 2c

- 12: 12-13, Box 1

- 13: Figure 1 ; 6-9 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 24. February 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

BREAst Cancer Personalized NuTrition (BREACPNT) - dietary intervention in breast cancer survivors treated with endocrine therapy: Rational and study design

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	NUTRITION & DIETETICS, Breast tumours < ONCOLOGY, Microbiology < NATURAL SCIENCE DISCIPLINES

BREAst Cancer Personalized NuTrition (BREACPNT) - dietary intervention in breast cancer survivors treated with endocrine therapy: Protocol for a randomized clinical trial

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Word count: 3984

ABSTRACT

Introduction: Breast cancer survivors treated with adjuvant endocrine therapy commonly experience weight gain, which has been associated with low adherence to therapy and worse breast cancer prognosis. We aim to assess whether a personalized postprandial glucose targeting diet will be beneficial for weight management as compared to the recommended Mediterranean diet in this patient population

Methods and Analysis: The BREAst Cancer Personalized NuTrition (BREACPNT) study is a phase-2 randomized trial in Hormone Receptor positive (HR+) breast cancer patients, treated with adjuvant endocrine therapy. The study objective is to assess whether dietary intervention intended to improve postprandial glycemic response to meals results in better weight and glycemic control in this population as compared to the standard recommended Mediterranean diet. Consenting participants will be assigned in a single blinded fashion to either of two dietary arms (Mediterranean diet or an algorithm-based personalized diet). They will be asked to provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for two weeks, at the initiation and termination of the intervention period. Microbiome composition data will be used to tailor personal dietary recommendations. After randomization and provision of dietary recommendations, participants will be asked to continuously log their diet and lifestyle activities on a designated smartphone application during the 6-month intervention period, during which they will be monthly monitored by a certified dietitian. Participants' clinical records will be followed twice yearly for five years for treatment adherence, disease free survival and recurrence.

Ethics and dissemination: The study has been approved by the ethics committee in the Sheba medical center (file 5725-18-SMC, Ramat Gan, Israel) and the Weizmann Institutional Review Board (file 693-2, Rehovot, Israel). The finding of the study will be published in a peer reviewed publication.

ClinicalTrials.gov Identifier: NCT04079270

MOH Identifier: 2019-03-28_006056

Strengths and limitations of this study

- A trial testing the efficacy of Personalized Postprandial-glucose-response Targeting (PPT) diet, based on an innovative dietary approach for weight maintenance, as compared to standard diet, in breast cancer survivors.
- The personalized diet involves advanced technologies, including microbiome, continuous glucose monitoring, metabolomics features and full dietary records are used to allow better understanding of the interactions between dietary intake with metabolic and health parameters.
- A homogenous study population, that includes HR+, early stage breast cancer survivors treated with adjuvant endocrine therapy. Patients are randomized into the two study arms and stratified by stage, treatment type, menopausal status and BMI. Yet, the study patients are representative of women from the center of Israel.
- The study design includes daily use of smartphone application for logging dietary intake and lifestyle events. While this may improve patient' adherence to diet, it can lead to exclusion of patients who do not hold a smartphone or with no capability to work with a smartphone app on a daily basis.

INTRODUCTION

The majority (~75%) of breast cancer patients are diagnosed with hormone receptor-positive (HR+) tumors and are assigned adjuvant endocrine treatment (ET) for a period of at least 5 years, which was shown to improve survival. However, adjuvant ET is associated with distressing side-effects which may be long lasting and substantially impair patients' quality of life and adherence to treatment. These side effects include weight gain and body composition changes, which are common in breast cancer survivors and are experienced by many women during treatment and for years after diagnosis(1–3). Weight gain in this population is complex and is

associated with various factors such as tumor type, menopausal status(4), pre-diagnosis body mass index (BMI)(5) and neo-adjuvant/adjuvant treatment type including chemotherapy and ET(2,6). Importantly, weight gain after breast cancer diagnosis is associated with increased risk for metabolic syndrome and cardiac disease (7,8), and was reported as a risk factor for breast cancer recurrence and shorter survival(4,9,10). Therefore, weight management strategies including diet, regular physical activity and cognitive behavioral therapy are recommended for controlling weight gain in breast cancer patients. Previous studies showed that weight loss interventions, incorporating diet, exercise and psychosocial support, in overweight or obese breast cancer survivors appear to result in decreased body weight, BMI and waist circumference and improvement in overall quality of life(11). We chose the Mediterranean (MED) diet as a control diet because it is commonly recommended in different countries including Israel(12) and was suggested to improve metabolic health in the general population as well as within breast cancer survivors(13–15). Still, the optimal weight loss intervention method and the impact of weight loss on survival outcomes is unclear. Furthermore, the interaction between the microbiome of breast cancer patients and dietary intervention has not been assessed.

The comprehensive role of the gut microbiome in modulating immune and metabolic health is increasingly recognized. Dysbiosis, referring to the disruption in the balance of gut bacterial communities, is associated with many conditions(16). The gut microbiome homeostasis can be influenced by internal factors, such as genetic, age-related and hormonal-related, as well as by external factors, such as stress, lifestyle and antibiotics(17). In addition, the microbiome is directly affected by the individual diet which in turn affects the body's response to food (18,19). Particularly relevant to breast cancer, diet plays an important role in creating a microbiome environment involved in estrogen metabolism(20). High estrogen levels contribute to breast cancer risk in postmenopausal women(21). In a recent study, gut microbiome diversity was linked to weight gain(22) and microbiome alterations were found to contribute to post-dieting weight regain(23). In addition, it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol (24). In a

previous study we showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food(25). Importantly, this study emphasized the involvement of functional microbial pathways and bacterial taxa in host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on personal measurements, including blood tests, personal lifestyle and gut bacteria profiles. In a following study implementing a 6-month dietary intervention plan in individuals with prediabetes, the Personalized Postprandial-glucose-response Targeting (PPT) approach significantly improved glycemic control and reduced PPGRs as compared to the commonly recommended Mediterranean diet (26).

In this study we seek to evaluate the clinical efficacy of the PPT diet combined with caloric restriction, compared to the Mediterranean diet, in promoting weight maintenance or weight loss and glycemic control in HR+ early stage breast cancer survivors treated with adjuvant ET.

METHODS

Study design

This study is a two-arm, parallel group, single-blinded, randomized controlled trial in early stage HR+ breast cancer patients treated with adjuvant endocrine therapy. Eligible participants will undergo a 6-month nutrition intervention program which will include dietary recommendations, daily logging and monthly follow up meetings provided by a certified dietician. Upon trial entry and after profiling (described below) participants will be randomly and equally assigned to the Personalized Postprandial-glucose-response Targeting (PPT) dietary (arm A) or to the Mediterranean-style (MED) dietary (arm B). All meetings will take place in the Breast Oncology Institute at the Sheba Medical Center. The primary objective of the study is to evaluate the efficacy of the PPT arm vs the MED arm in controlling body mass changes in the patient population during the intervention period (see summarized study endpoints in **BOX 1.** For complete SPIRIT checklist and the full protocol see Supplemental material no.1 and 2 respectively)

BOX 1 – STUDY ENDPOINTS

Primary endpoint

Body weight changes defined as the net body weight gained/lost in the 6–month intervention period

Secondary endpoint

Glycemic response as measured by the area under the glucose curve (AUC) during continuous glucose monitoring (CGM) period pre-intervention and during the intervention.

Exploratory endpoint

- Five years Disease free survival (DFS)
- Microbiome and blood metabolites modulation during the diet interventions- tested using the samples taken at profiling and 6month time points.
- Adherence to algorithm-based personalized diets, compared to standard diets advised for weight control – assessed by monthly compliance questionnaire.
- HR+ breast cancer patients' adherence to hormonal treatment
- Translational studies

Patient and public involvement

Patients are being involved in the recruitment effort by actively publishing the study recruitment information and sharing their own experience during the study, via social networks and breast cancer survivors' groups. At the end of the study patients will receive an analysis of their glucose responses to the foods that they ate, and get an access to different nutritional tools that will be available to them on a secure website or on a dedicated mobile application. Furthermore, At the end of the study all patients will be given an access to their personal tailored dietary recommendations, built for them by the study team based on their personal data, regardless of their assigned arm during the study.

Study population

This trial will enroll breast cancer survivors treated with ET and followed at the Breast Oncology Institute at the Sheba Medical Center. Eligibility criteria (inclusion and exclusion criteria) are detailed in **Table 1**. Potentially eligible participants will be identified and recruited to the study by the medical team during regular clinic visits or via database search and phone calls by the clinical study coordinator (SC). Information leaflets and a poster describing the study design and contact information will be available at the institute's reception and waiting area. Additionally, a video explaining the study and its aims will be shown on screens at the institute's reception and waiting area and will be sent to potential participants (https://youtu.be/kxrqONj3KGM). All participants will assign informed consent.

Table 1: Eligibility criteria

Inclusion Criteria	Exclusion Criteria
Female patients	Oral Antibiotics/antifungal use in the previous one month to profiling stage *
Age ≥ 18 and ≤ 80	Known Diagnosis of diabetes or the use of anti-diabetic and/or weight-loss medication
Diagnosis of stage 1-3 HR+ breast cancer, who underwent surgery	BMI<18.5
At least 60 days after last non-endocrine oncology treatments (i.e. definitive surgery, radiation or chemotherapy – whichever is last) if these were indicated.	Patients under another diet regime and/or a dietitian consultation/ clinical study
Adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists) taken for at least 30 days but no more than 24 months.	Pregnancy, breast feeding
Willing to operate a smartphone application	HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
	Known diagnosis of psychiatric disorders (Schizophrenia, Bipolar Disorder)
	Known diagnosis of IBD (inflammatory bowel diseases)
	Patients that underwent Bariatric surgery
	Known Alcohol or substance abuse

*Patients will be offered to join the study at a later point

Study Procedures and Intervention

ICF signed (wk -8 to -4)

Eligible participants will be invited to sign an inform consent at the oncologic clinic in Sheba medical center (as shown in **Figure 1**).

Profiling stage (wk -6 to -3)

Consenting patients will proceed to the profiling stage. During this stage they will undergo the following procedures:

1. Meeting with the SC and completion of questionnaires detailing relevant medical background, nutritional habits and lifestyle activities. Questionnaires will be filled online using the REDCap (27) software (a secure web application for managing online surveys and clinical trials).
2. Participants will provide blood samples after a night fast (12 hours) for complete blood count and blood chemistry, including liver function, lipid profiling, Fasting Plasma Glucose (FPG) and HbA1c. LH, FSH and Estradiol will be measured only in pre-menopausal patients. Participants will provide urine sample for estradiol derivatives for future exploratory analyses.
3. Anthropometrics measurements, including weight, height, waist and hip circumference will be taken at this meeting.
4. Stool sample: Patients will receive a designated stool kit (Genotek OMR200) to collect stool at home. The SC will instruct them how to provide the stool samples and will ask to return this kit during the following week for further processing of the microbiome data. Microbiome sequenced data are essential for the algorithm predictions, thus stool sample is obligatory for participation in the study.
5. Continuous glucose monitoring (CGM) connection: Patients will be connected to a CGM (Abbott Freestyle LibrePro) for 2 weeks. The CGM kit includes a sensor affixed to the back of the arm that continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels.

6. Food diary: Patients will be instructed to download the study dedicated smartphone application ('personalized nutrition project') for food logging. They will log in real-time their food intake, physical activities, sleep duration and quality and special events. During the profiling period, patients will be asked to follow their regular dietary and lifestyle habits (see examples of logging activities in **Figure 2A**). All participants will receive a registration code and their data will be anonymized.
7. Data collected during the profiling period, including microbiome, anthropometrics, blood parameters and questionnaires will be analyzed and used by the PPT algorithm to provide personal dietary recommendations for each participant.

Randomization

After completion of the profiling stage patients will be randomly assigned to one of two arms of the study by one programmer from the trial personnel who had no contact with participants. Approximately 100 subjects will be assigned to each arm using a blinded randomization algorithm and the following stratification factors: (1) menopausal status at study entry (post/pre) (2) received/not received chemotherapy prior to study entry (3) ET type (Tamoxifen/Aromatase inhibitor) (4) Breast Cancer stage at diagnosis and (5) BMI above/below 27. Notably, we only used the stratification factors to minimize differences between groups in the allocation process and did not analyze the data according to the stratification factors. Patients and part of the study team (oncologists and study coordinators), excluding the dietitian, will be blinded to the study arm assigned. At the end of intervention, dietary assignment was revealed, and participants were asked to continue following their respective diets for 6 additional months.

Recommendation meeting (Day 0)

Upon menus construction, patients from both arms will be invited to a recommendations meeting (hereafter 'day-0') with the dietitians. Patients will receive general information regarding their menu and will be instructed to consume and log

their meals according to it. In order to ensure accurate logging the dietitians will schedule an online follow up two weeks after 'day-0'. Anthropometrics measurements, including weight, hip and waist circumference, taken at this meeting will be used as baseline measurements.

Follow up Meetings (Mo +1 up to Mo +5)

All patients will participate in monthly follow-up meetings with a dietitian (total of 6 meetings) in order to evaluate their compliance to the dietary recommendations they received and provide additional advice if needed. Anthropometric measurements (weight, hip and waist circumference) will be taken at each time point. Furthermore, patients will be asked to fill a follow-up questionnaire and report any changes within their lifestyle and treatment. At the beginning of Month 4 of the intervention period, patients will be offered to be reconnected to CGM for two weeks (optional). At the monthly meeting before the end of intervention patients will receive a stool kit, to be returned at the end of intervention meeting.

End of intervention (Mo +6)

At the end of the 6-month intervention period, patients will be invited to a meeting in which anthropometrics measurement will be taken, as well as urine, blood and stool samples. Additionally, participants will be connected to CGM for 2 weeks (mandatory) for the third time (Figure 1). When patients return CGM they will be unblinded to their assigned intervention arm by the study dietitian.

Long term follow up (Mo +12)

At 12-month time point, patients will be invited to a follow up meeting and will be asked to fill follow-up questionnaires, including food frequency questionnaire. Anthropometric measurements and a 3-day food diary on the study app will be recorded. Patients will receive the menu of the other study arm and will be offered to follow either one of the diets. Long-term clinical follow-up information will be collected from the electronic medical records twice yearly for treatment adherence, recurrence and survival calculation purposes, for a period of up to five years post randomization.

Menus construction

Before randomization, menus will be constructed for each patient and will be adjusted for, patients' caloric target and clinical data. The menus construction flow is presented in **Figure 2B**.

Meal bank (list)

The menus provided to patients in this study are constructed from a meal bank that we previously generated(26), with over 2,000 meals representative of the Israeli typical diet and with a variety of different food combinations. We divided the meals in the meal bank into four meal types (breakfast, lunch, dinner and snacks) and labeled them according to meal categories (dairy; meat; fish etc.) in order to generate menus according to patients' personal preferences.

Caloric target calculation

In order to provide the patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as an average between:

1. Estimated Energy Requirements (EER) calculated with the use of the Mifflin equation for Resting Energy Expenditure (REE), using their weight, height, age and gender, and multiplied by Physical Activity (PA) factor, based on the level of PA that the person performs on a regular basis (28).

2. Energy expenditure assessed by Basal Metabolic Rate (BMR) value measured by body composition analyzer (Tanita). The result from this equation will be divided by 0.7 (as REE represents ~70% of total energy expenditure).
3. Average daily caloric intake obtained from the patients' dietary records during the profiling stage, to account for the subject's dietary habits prior to the intervention.

Furthermore, for individuals with BMI>25 a total of 500Kcal will be reduced from their calculated caloric target, but not less than 1200 calories/day, to allow weight loss according to common recommendations for weight loss (29,30).

Diets

Mediterranean-style (MED) diet

In this arm we included meals that were scored by four external dietitians according to the Mediterranean-style dietary recommendations. Meals were binary scored as recommended (=1) or not recommended (=0) and we applied scores 1 to 5 to all meals, depending on how many dietitians marked the meal as recommended or not. The diet is based on recommended foods such as vegetables, fruits, legumes, whole grain products, unsaturated fats such as olive oil and nuts, fish, poultry and low-fat dairy products. Consumption of red meat, high fat dairy products, processed foods and sweet pastries, was discouraged as part of the diet. Additionally, menus in this arm were designed with the following target for daily macronutrient composition: 45-65% of calories from carbohydrates; 15-20% from protein; and 20-35% from fat, with up to 10% from saturated fat. Menus include only meals that received scores 1 and 2. Participants will be encouraged to consult with the dietitian regarding meals that may not appear in the constructed menu.

Personalized Postprandial-glucose-response Targeting (PPT) diet

In this arm, dietary recommendations will be based on the algorithm predictions of the postprandial glucose responses(25), shown to improve glycemic control and metabolic health in healthy individuals or in individuals with prediabetes and diabetes(26,31). Notably, these interventions were not caloric restricted as in the current study. Among the features used to predict PPGR to meals were anthropometrics, blood tests (FPG, HbA1c% and Hemoglobin), lifestyle features derived from questionnaires, microbiome (abundances of species estimated by MetaPhlAn2 and meal features (macro- and micronutrient composition) were used (see Supplementary table 1 for the full list). Since no events around the meal were

used for prediction, trained predictor could predict response for any profiled participant to any given meal.

All logged meals will be scored from 1-5 based on a unique scoring method that we developed and tested in previous studies, and study participants will be asked to consume only meals with score 1 or 2. Importantly, the PPT diet, by definition, was not aimed to have a predetermined macronutrient distribution, In contrast to the Med-diet

Adherence to the study recommendations

The adherence to the prescribed diets during the intervention will be evaluated by the dietitian by a close monitoring of the patients' self-recorded dietary intake in the logging application, as well as by monthly electronic follow-up questionnaires that participants will be asked to fill out. In order to encourage dietary adherence and self-monitoring, we will generate a bi-weekly semi-automatic feedback reports that will include composite grades on a scale of 0-100 (from worse to best) for diet composition, calorie intake and dietary fiber intake separately, for the entire two-week period (**Figure 2C**).

- *MED-diet composition grade:* indicates how well the participant sticks to the dietary recommendations based on the MED approach including Carbohydrate (as % of daily caloric intake), fats in general (as % of daily caloric intake) and specifically saturated fat intake below and above 10% of caloric intake. Dietary fiber intake per each of 1000 kCal per day will be also calculated as part of the score.
- *PPT-diet composition grade:* indicates how well the participant sticks to predictor-based meal scores. Each meal score was assigned with a grade as follows: meal score 1=grade 100; meal score 2=80; meal score 3=50; meal score 4=25; meal score 5=0. The grades are averaged calorie-wise (with food energy trimmed to be within (100,500) kcal interval)- Σ kcal(i) $\cdot$ grade(i). For example, if a participant eats three meals: 600 kcal of meal score 2, 1000 kcal

of meal score 5 and 80 kcal of meal score 1, feedback grade would be: (500*80+500*0+100*100) / (500+500+100) = 45. If too few (100 by default) calories are logged (overall), we did not compute a score.

- *Calories grade:* indicates how well the participant sticks to the prescribed caloric target. When caloric intake deviates within 15% of caloric target (CT) the applied grade is 100; when caloric intake deviation exceeds 60% of CT the applied grade is 0; when caloric intake deviation is between 15% to 60%, a linear penalty is applied to the grade depending on the deviation.
- *Dietary fiber grade:* indicates if participants consumed the recommended amount of dietary fibers (set to 14 gram for every 1000 kcal/day for both arms) from the diet at the referred time When fiber intake in grams per day reaches the recommended amount, or higher the applied grade is 100 and when it is below the recommended amount a linear penalty is applied to the grade.

In addition to grades, feedback reports also included a list of recommended meals and non-recommended meals (by meal score) to highlight the best and worst meals consumed on that time period (as logged by the participant). The best and worst meals lists will be generated systematically and be reviewed by a dietitian from the study team.

STATISTICAL ONSIDERATION

Sample size determination

To estimate the required sample size we performed power analysis, using an unpaired t-test assuming normal distribution of the primary outcome (weight change), estimating the effect size based on the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. (32), the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis

All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variables, the Spearman correlation coefficient will be used. To compare parameters for continuous variables in two time points, the paired-samples t-test will be performed (or Wilcoxson test for non-normally distributed variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous/categorical variables in the number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer Translational Research laboratory at Sheba Medical Center. The blood and urine samples will be stored at -80°C and bacterial DNA samples will be stored at -20°C. The samples will be encoded with no identifying information. Identifying details and codes will be kept in an encrypted file stored at Sheba medical center. Encoded stool samples will be transferred to the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team. Samples will be stored for up to 10 years. All future use of stool and blood samples will be subjected to IRB approval.

Ethics and dissemination

The study has been approved by the Sheba medical center Institutional Review Board (IRB 5725-18) and the Weizmann institute of science Institutional Review Board. The findings of this study will be published in a peer reviewed publication. De-identified individual participant data and applicable supporting clinical trial documents will be available upon request for 12 months after publication.

CURENT STATUS

Enrollment and recruitment initiated on July 2019. To date (February 2022), 120 participants have been recruited, out of them 60 completed the 6-month intervention period including 38 participants who completed the 12-month time point.

DISCUSSION

Dietary interventions are the first-line treatment for weight management within breast cancer survivors and have beneficial results. Yet, the ability to maintain these outcomes is questionable and require further research(11,33,34). In this trial, we aim to assess the effect of a PPT diet on weight maintenance as compared to Mediterranean-style diet in early stage HR+ breast cancer patients, taking ET.

This study has several strengths and limitations. Advantages of this study design include a comprehensive profiling of each participant, which allow us to better understand participants' metabolic baseline and to assess the effect of the dietary changes. Additionally, the continuous food logging by the study patients using a designated smartphone app can provide us with insights on the patients' compliance to the dietary recommendations in both arms. However, this may limit the study population to individuals who are able to work with smartphone application on a daily basis. Furthermore, the study participants are being closely followed by a dietitian from the study team who monitor their food intake and meet them on a monthly basis in order to increase compliance during the first 6-months. However, on the long term, without intensive monitoring, the feasibility of the PPT diet and the ability to follow the diet recommendations should be investigated. Notably, Ben-Yacov et al. (26), reported that prediabetes individuals following PPT diet were able to maintain the

results during 12-month follow up as compared to those who followed the MED diet. Additionally, as a novel tool, the algorithm is not available for general use which makes it difficult to replicate the intervention. Nevertheless, we do publish the full list of features we use to generate the menus, based on personal and microbiome data (Supplementary table 1).

Lastly, microbiome composition and pathways were recently associated with weight changes and metabolic health parameters, as well as with risk for breast cancer diagnosis and recurrence (35). This may allow us to further explore whether gut microbiome composition and pathways have a predictive role in weight management, metabolic health parameters, glycemic control and even disease recurrence on the next 5 years after the intervention within breast cancer patients, although for disease recurrence differences the sample size may not be large enough.

Taken together, our rich dataset including deep phenotyping of each patient may allow us to deeply investigate associations between clinical and Omic data to DFS in early stage HR+ breast cancer patients and may pave the way to larger studies.

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AUTHOR CONTRIBUTIONS

MR, MD and AW conceived the study and designed the intervention. MR and MD wrote the manuscript. AG is responsible for directing the computational aspects of the study. DK is responsible for the feedback reports and summary reports being sent to participants. MBG, DMS and YV coordinate participants' recruitment and management throughout the intervention and follow-up. AW developed the protocols and directed and performed the microbiome sample sequencing with the help of MLP. ES and ENG conceived the study, designed the intervention and wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST STATEMENT

ENG reports Honoraria and Consulting fees from Pfizer, Novartis, Roche Eli-lilly AstraZeneca. ES is paid scientific consultants for DayTwo Inc. No pharmaceutical manufacturers or other companies from the industry contributed to the planning, design, or conduct of the trial. No other potential competing interest are relevant to this article were reported.

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FIGURES LEGEND

Figure 1. An illustration of the study design

Figure 2. Study application and menus construction. **(A)** The food logging application, examples of logging activities and information available on the application. **(B)** Menus construction flow. **(C)** An example of the bi-weekly feedback report that will be sent to participants.

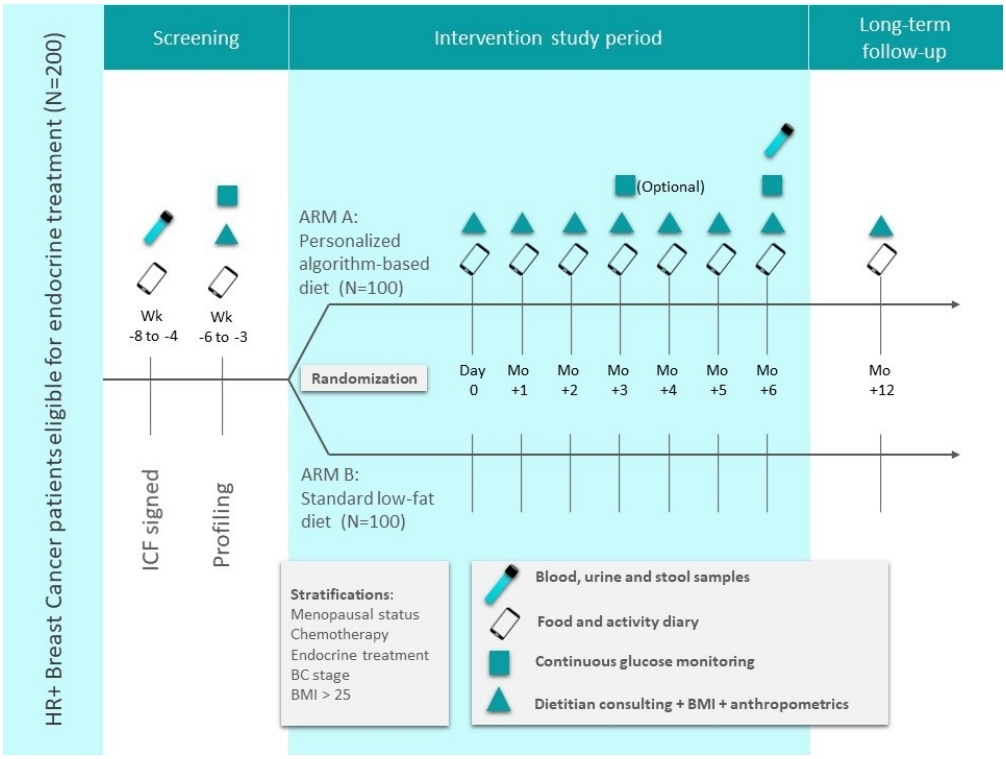

Figure 1. An illustration of the study design

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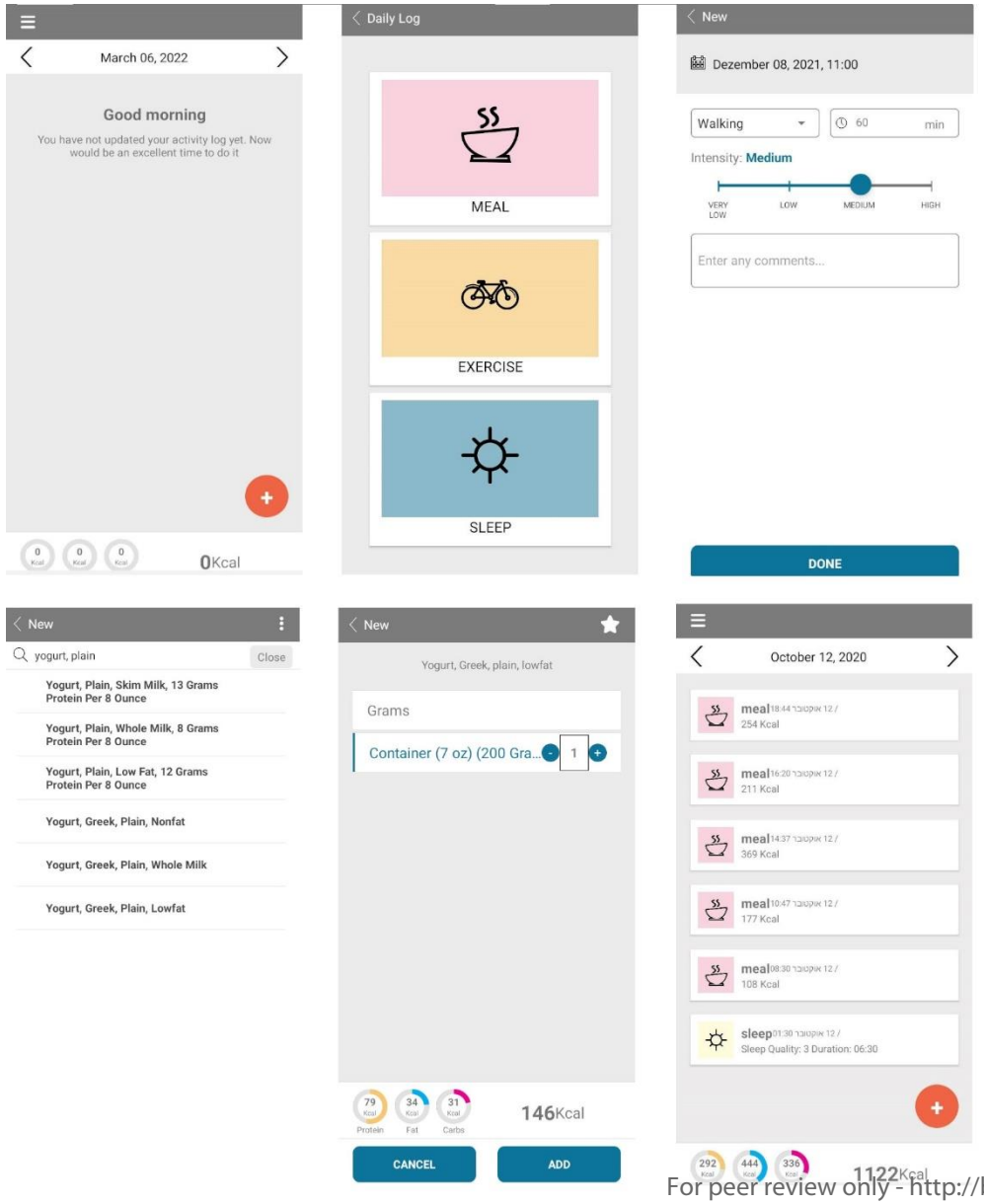

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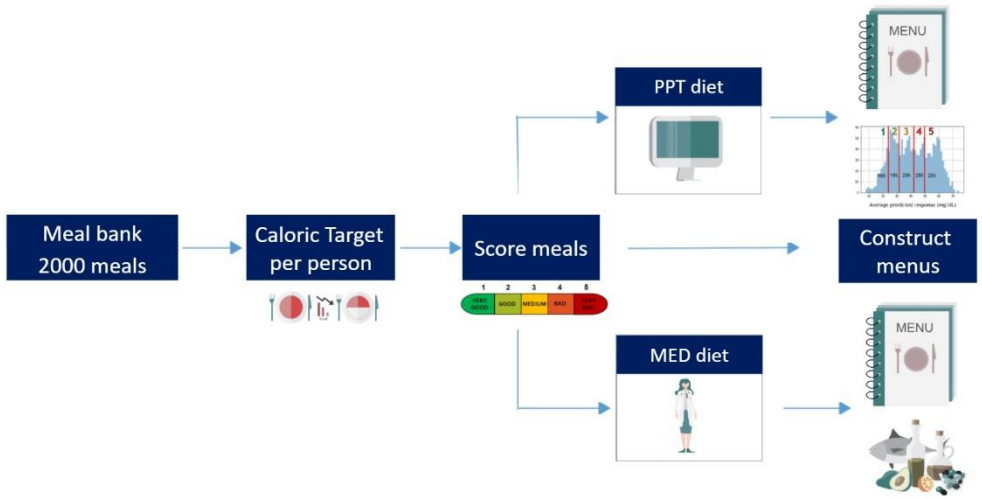

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The Breast Cancer Personalized Nutrition study (BREACPNT):

A phase 2 single-blinded randomized study of algorithm-based personalized nutrition intervention compared to standard diet intervention in patients treated with endocrine therapy for early stage, hormone receptor positive breast cancer

Study Protocol

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1. Purpose

The Breast Cancer Personalized Nutrition (BREACPNT) study will evaluate the effect of a microbiome based personalized diet intervention on control of weight gain, glycemic response, disease outcome and various biomarkers in hormone receptor early breast cancer patients receiving adjuvant endocrine treatment.

2. Background

Weight gain is a common incident in breast cancer survivors [1]. As many as 50–96% of women experience weight gain during treatment. Weight gain in breast cancer survivors is complex and influenced by many factors such as tumor type, socio-demographic characteristics, and menopausal status [2,3]. Most breast cancer patients (~75%) are diagnosed with hormone receptor-positive (HR+) tumors and receive endocrine treatment for a period of at least 5 years. Endocrine treatment was identified as a risk factor for weight gain in several studies [2]. Weight gain during endocrine therapy was highest in women who were premenopausal or had previous chemotherapy [4].

Weight gain may decrease adherence to long-term hormonal therapy and increase risk for metabolic syndrome and cardiac disease. Importantly, post-diagnosis weight gain has been implicated as a risk factor in breast cancer recurrence and survival. Hence, weight management for breast cancer survivors is important for increasing adherence to therapy and lowering recurrence risk [5].

The essential role of the gut microbiota in modulating immune and metabolic functions in health and disease is increasingly recognized. Dysbiosis, a disruption in the balance of gut bacterial communities, is associated with many conditions [6]. The entire bacteria population in the digestive tract (microbiome) consists of ~1,000 species with a genetic repertoire of ~3 million different genes. The homeostasis of intestinal microbiota can be influenced by internal factors, such as genetic, age-related and hormonal, as well as by external factors, such as nutrition, stress, lifestyle and antibiotics [7]. The microbiome is directly affected by our diet and directly affect the body's response to food [8,9]. Particularly in breast cancer (BC), diet plays an important role in creating a microbiome

environment involved in estrogens metabolism [10]. High systemic estrogen levels contribute to breast cancer risk in postmenopausal women. Estrogen levels in the blood are regulated in part via enterohepatic recirculation, involving bacterial enzymatic pathways and deconjugation[11]. Indeed, profiling gut microbiota in postmenopausal breast cancer patients revealed altered composition and estrogen-independent low diversity of their gut microbiota compared to healthy controls [12]. Thus, the gut microbial community may affect estrogen-related breast cancer [13].

A recent study linked the gut microbiome diversity to weight gain [14] and microbiome alterations were found to contribute to post-dieting weight regain [15]. In addition it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol [16]. The Personalized Nutrition Project, conducted at Eran Segal's group in the Weizmann Institute of Science, recently showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food [17]. Most importantly, it emphasized the involvement of functional microbial pathways and bacterial taxa in host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on many personal measurements, including blood tests, personal lifestyle and gut bacteria profiles.

Continuing studies demonstrate that short-term dietary interventions change the microbiome and are beneficial to the host in maintaining glucose levels. Moreover, Low glycemic index (GI) diets may be important in weight management [18]. In a small-scale pilot study using this algorithm, personally tailoring dietary interventions to healthy and pre-diabetic people, showed a significantly improved PPGRs accompanied by consistent alterations to the gut microbiota (personal communication) . These results suggest that individually tailored dietary interventions help maintain normal blood glucose levels and influence microbiome diversity, which, in turn, can control weight changes.

3. Hypothesis Objectives and Endpoints

Study Hypothesis

Algorithm based personalized diet will be superior to standard low fat diet for controlling weight gain and glycemic response in breast cancer patients treated with endocrine therapy.

Primary Objective

To evaluate the efficacy of a personalized diet compared to a standard low fat diet to control body mass as measured by changes in body mass.
Endpoint: Body weight changes will be defined as the net body weight gained/lost in the 6 months' intervention period.

Secondary Objective

1. To evaluate the efficacy of the personalized diet compared to a standard low fat diet to control glycemic response.
   Endpoint: glycemic response control measured by the area under the glucose curve (AUC) during continuous glucose monitoring (CGM) period.

Exploratory objective

1. Evaluate disease outcomes as measured by disease free survival, Breast cancer recurrence in study subjects. Endpoint: 5 years Disease free survival (DFS), 5 years Breast cancer specific recurrence.
2. To investigate microbiome composition and modulation during the diet intervention period and assess if there are differences in modulations between the personalized diets as compared to the standard diet.
3. Investigate the mutual effects of gut microbiome and blood metabolites during the diet intervention period and search for possible biomarkers for dietary treatment efficacy.
4. Investigate inflammation parameters and immune profiles of patients (lymphocytes, T-cell receptor repertoire, antibodies profiling using phage display libraries) of HR positive patients undergoing intervention and their correlations to microbiome modulations.
5. Test whether patients have better compliance and adherence to algorithm-based personalized diets, compared to standard diets advised for weight control. The compliance to the diets will be measured by: Compliance questionnaire, 3-day dietary log, Number of study meetings attended.
6. Test whether HR-positive breast cancer patients have better adherence to hormonal treatment following weight-control diets. This compliance will be tested for 5 years post treatment.

4. Study Design

This is a phase 2 randomized trial in hormone receptor positive breast cancer patients receiving adjuvant endocrine therapy. Figure 1 illustrates the study schedule.

200 HR+ breast cancer patients, eligible for adjuvant endocrine therapy will be recruited to the study. Upon recruitment, subjects will provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for 2 weeks. Thereafter, patients will be randomly assigned in a 1:1 ratio to receive a personalized diet recommendation or a standard low-fat diet for 6 months. The algorithm is based on patients' microbiome analyses and glucose monitoring results. Patients will be monitored by continuous glucose monitoring (CGM) at least 2 times during the 6 months' intervention period. At the end of the 6 months' period patients will undergo a second course of CGM for 2 weeks and provide a second stool sample for microbiome analysis. Patient clinical records will be followed 2-3 times yearly for 5 years for DFS and BC recurrence.

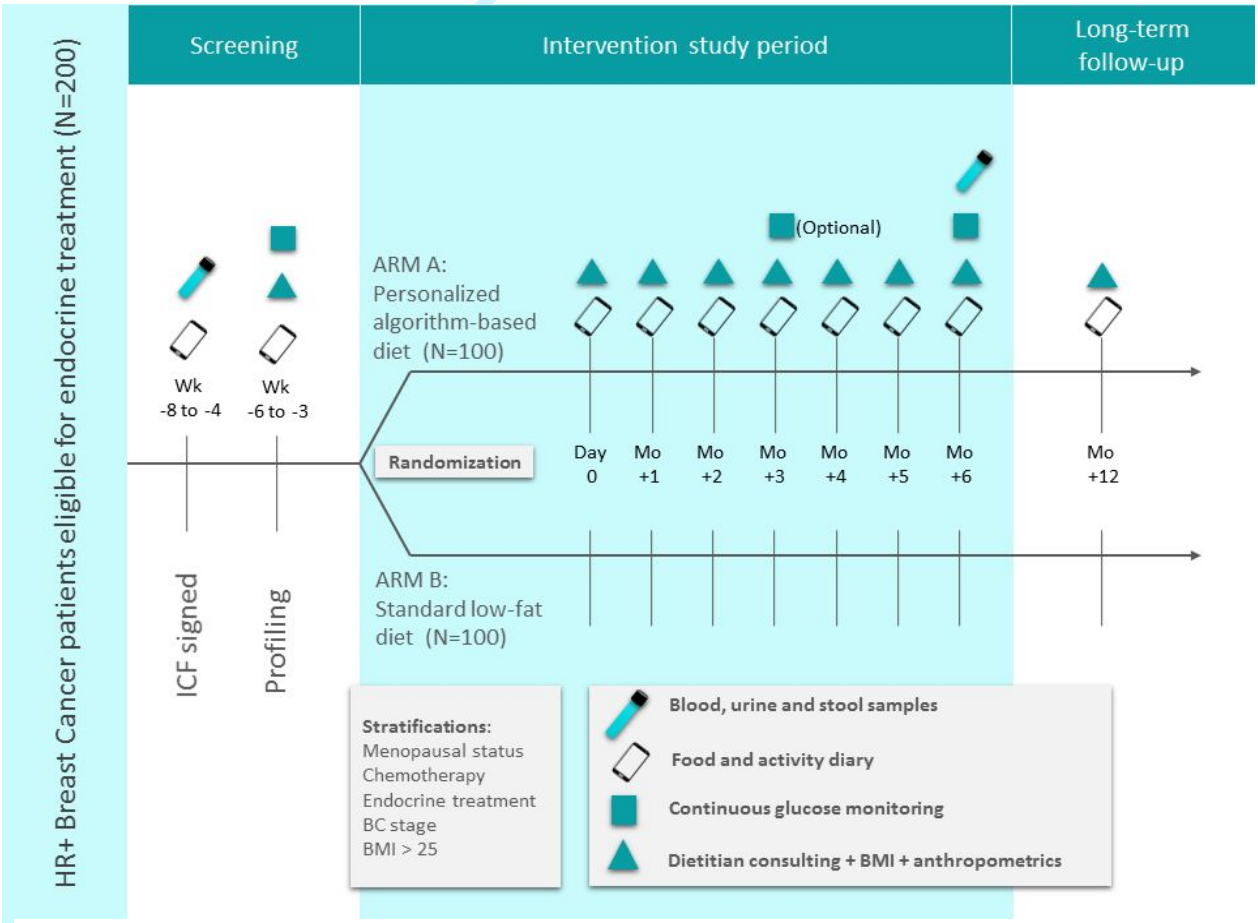

Figure 1: Study design and schedule

Inclusion Criteria:

- Female patients, Age 18-70
- Patients diagnosed with stage 1-3 breast cancer, who underwent surgery, have finished their neo/adjuvant chemotherapy and radiotherapy if these were indicated and are treated with adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists).
- Patients are at least 60 days after finishing their last non-endocrine oncology treatment (i.e. definitive surgery, radiation or chemotherapy – whichever is last), have received at least 30 days of endocrine therapy (tamoxifen or aromatase inhibitor) but no more than 24 months.
- Patients treated with neoadjuvant endocrine therapy are eligible provided they had undergone surgery, are at least 60 days post their last non endocrine therapy (definitive surgery or radiation and chemotherapy, if these were indicated), are continuing their endocrine therapy but did not receive more than 24 months post-surgery.
- Are willing to work with smartphone application

Exclusion Criteria:

- Oral Antibiotics/antifungal use in the previous 3 months to profiling stage (these patients will be able to join the study at a later point)
- Use of anti-diabetic and/or weight-loss medication
- BMI<18.5
- People under another diet regime and/or a dietitian consultation/another study?
- Pregnancy, breast feeding
- HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
- Psychiatric disorders (Schizophrenia, Bipolar Disorder)
- Known diagnosis of IBD (inflammatory bowel diseases)
- Patients that underwent Bariatric surgery
- Known Alcohol or substance abuse
- Known Diagnosis of diabetes

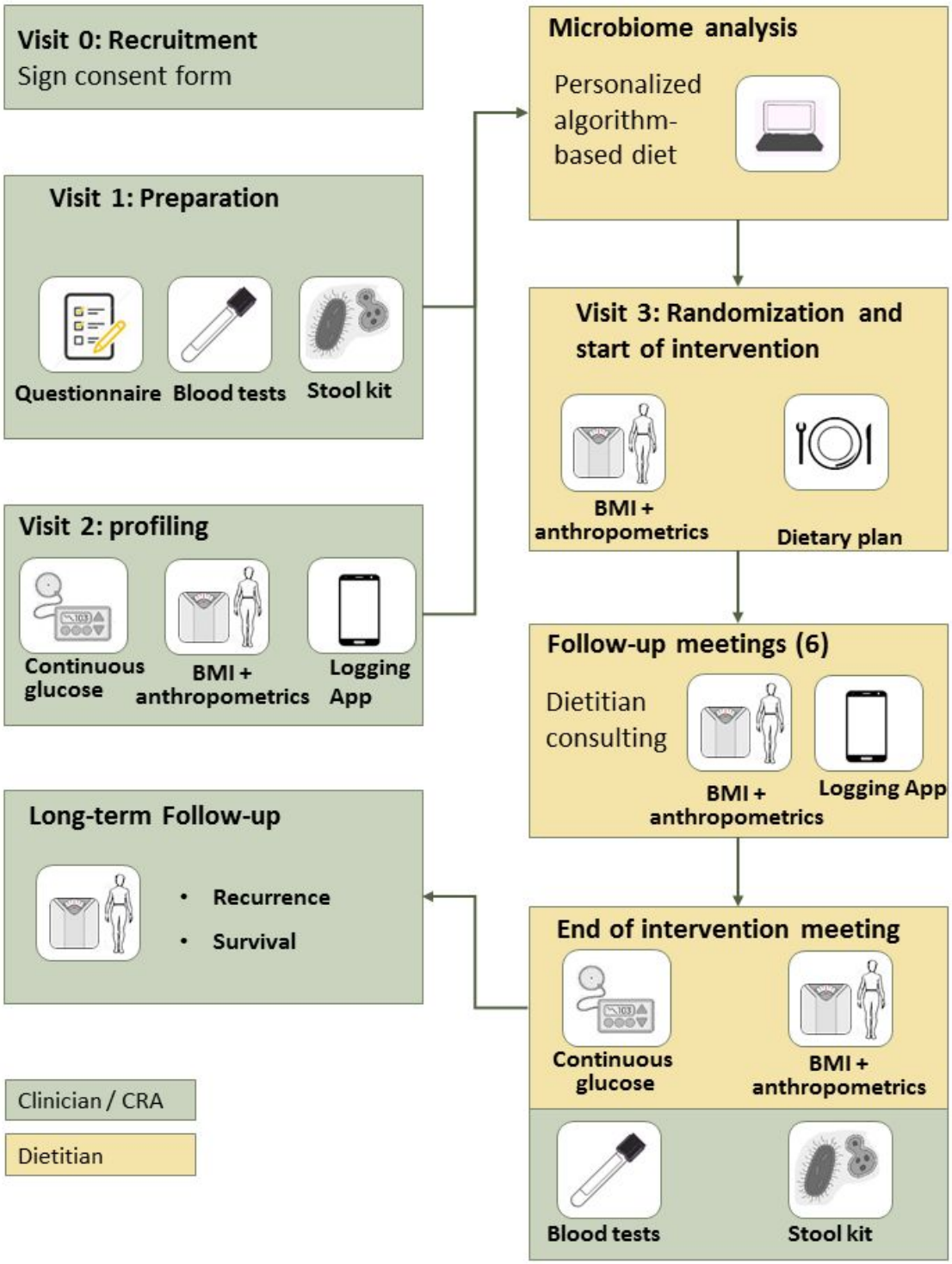

Figure 2: Study scheme

Patient Recruitment

Breast cancer patients will be recruited to the study through their regular clinic visit at the Breast Oncology Unit at Sheba Medical Center. Patients eligible for the study will sign an informed consent. This recruitment process will be ongoing until the designated number of study patients is reached.

Screening and profiling stage (-3 Months to Day -1_)

During this stage consenting patients will:

1. Meet a study coordinator and complete questionnaires regarding their medical background, nutritional habits and lifestyle activities (filled online using the REDcap software, using a dedicated tablet computer). Patients will provide blood samples for CBC, blood chemistry including liver function, lipid profiling, HBA1C, TSH, CRP, LH FSH and future exploratory analyses, and urine samples for estradiol derivatives. All patients will receive a code from the software and their data will be anonymized.

2. Patients will be asked to log a three-day food diary using a designated mobile phone application.

3. Patients will receive a designated stool kit to collect the stool sample at home. In the next meeting (profiling stage) patients will return the stool sample which will be used for microbiota profiling.

4. Meet with a certified dietitian to build a menu for the "profiling period" based on the three-day food diary of dietary habits provided by the patient. This meeting will include anthropometrics measurements (weight, height, waist and hip circumference) and connection to the glucose measurement device (Abbott Freestyle Libre). The CGM kit includes a sensor affixed to the back of the arm, which continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels. Patients will be connected to a CGM for two weeks and will be asked to follow their diet plan as given to them by the dietitian, according to their regular habits and lifestyle. During the two weeks of connection patients will be instructed to use a dedicated application, in which they will

log in real-time their food diary, exercise, sleep, wake up, special events. Patients will return the CGM kit via a courier service from the patients' home upon measurement completion. The stool sample will be processed for microbiome profiling. The resulting data and data provided by the CGM kit will be analyzed to provide a personal profile for each patient.

Randomization and Intervention

Following the profiling stage: patients will be randomly assigned to one of two arms of the study. Approximately 100 subjects will be randomized to each arm. Patients will be blinded to the arm to which they were assigned .Randomization will be done by a computer program, taking into account the following stratification factors:

1. Menopausal status at study entry
2. Previous chemotherapy
3. Endocrine treatment type (Tamoxifen/Aromatase inhibitor)
4. Breast Cancer stage
5. BMI above/below 25

The intervention arm will be an 'algorithm-based' arm in which patients will receive personally tailored dietary recommendations. The prediction algorithm is based on gradient boosting regression model and is capable of accurately predicting personalized postprandial glycemic responses to arbitrary meals based on microbiome, CGM data from the profiling period (two weeks of CGM connection data) and other clinical data such as blood tests and lifestyle features. This model predicts PPGRs using the sum of thousands of different decision trees. Trees are inferred sequentially, with each tree trained on the residual of all previous trees and making a small contribution to the overall prediction. The features within each tree are selected by an inference procedure from a pool of 187 features representing meal content (e.g., energy, macronutrients, micronutrients); daily activity (e.g., meals, exercises, sleep times); blood parameters (e.g., HbA1c%, HDL cholesterol); CGM-derived features; questionnaires; and microbiome features (metagenomic relative abundances and KEGG pathways)[17]. The algorithm was developed using a standard leave-one-out scheme to rank every meal of each participant in the profiling period (i.e.,

the PPGR to each predicted meal will be hidden from the predictor). The model was validated in an independently collected 100-person cohort . [17]

The control arm will receive nutritional recommendations according to the standard Israeli dietary approach Mediterranean-style low-fat diet. In order to provide patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as average between:

1. Estimated Energy Requirements (EER) calculated with the use of Mifflin equation for Resting Energy Expenditure (REE) [19]. The result from this equation will be divided by 0.7 (as REE represent ~70% of total energy expenditure).

2. Average daily caloric intake obtained from patient's log in the app during the profiling stage. For overweight patients (BMI>25) a total of 500Kcal will be reduced from their reported caloric intake to allow weight loss as accepted according to the American Association of Clinical Endocrinologists guidelines.

The diet recommendations for both arms will be provided and explained by a certified dietitian which will meet the patients from both arms at Day 0 and monthly thereafter for a total of 6 scheduled monthly visits. Weight and other anthropometric measurements (height, waist and hip circumference) will be taken at this and all following meetings with the dietitian. Participants will be asked to document their food intake and daily activities including exercise and sleep using a dedicated smartphone app throughout the intervention period.

Intervention Meetings:

Patients from both arms will be invited during the intervention period to monthly follow-up meetings with a certified dietitian (total of 6 meetings). Meetings will include evaluation of patients' compliance to the dietary recommendations they received and additional advice will be provided if needed. During the follow up meetings anthropometric measurements will be taken (height, weight, hip and waist circumference). We will also follow up on patients via phone, email, text massage, in order to increase compliance. During the monthly meeting at the beginning of Month 4 of the intervention period patients will be

offered to be reconnected to CGM for 2 weeks (optional). Data from CGM connections will be analyzed at the end of the intervention

End of intervention

At the end of the 6 months intervention period, patients will be invited to a meeting in which they will undergo anthropometrics measurement, urine, blood samples and stool sample. Additionally, patients will be connected to CGM for 2 weeks (mandatory).

Patients will be followed up at 12 months following the start of the intervention. They will attend a follow up meeting with the study coordinator in which BMI, anthropometrics, and a 3 day food diary will be recorded.

Long term follow up

Long-term clinical follow-up will be collected from the electronic medical records for recurrence and survival calculation purposes for a period of up to 5 years post recruitment.

5. Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer translational Research laboratory at Sheba Medical Center. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored encoded with no identifying information. Identifying details and codes will be kept in a file stored at Sheba medical center. Encoded stool samples will be transferred to the Segal laboratory at the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team

Future research:

Samples will be stored for up to 10 years. All future use of stool and blood samples will be subject to IRB approval.

6. Safety Endpoints

No safety endpoints planned for this study

7. Statistical Considerations

Sample size determination. To estimate the required sample size, we performed power analysis while estimating effect size using the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. [20], the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis (brief summary). All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variable, the spearman correlation coefficient will be used. To compare parameters for continuous variables in 2 time points the paired-samples t-test will be performed (or Wilcoxson test for non-normally distribute variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous or categorical variables in number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

8. Possible Benefits

Patients will receive counseling and close monitoring by a certified dietitian throughout the study, regardless of the research arm to which they were assigned

Patients will have the opportunity to evaluate their blood glucose levels in response to food that they tend to eat, exercise, etc., throughout the CGM connection.

Patients will receive an analysis of their glucose response to the foods that they ate.

Patients will have access to different nutritional tools that will be available to them on a secure website or on their mobile phone (App).

At the end of the study all patients will be given access to their personal tailored dietary recommendations, built for them by the study team based on their personal data, regardless of the arm they were assigned to during the study.

9. Possible Risks and Analysis of Risk/Benefit Ratio

When blood tests are taken, there is no risk except a slightly discomfort associated with the prick, hematoma or local infection it the prick area.

In order to monitor glucose levels patients will be connected to a continuous glucose monitor (CGM). The CGM includes a sensor that will be inserted using a small needle into the body. There is ultra-low risk of inserting the sensor including mild discomfort associated with inserting the sensor, a local infection in the prick area, a mild redness at the patch area. We consider this risk to be quite low. Continuous Glucose Monitoring may reveal a previously undiagnosed diabetes. These patients will be excluded from the trial and the patient and treating oncologist will be notified to provide appropriate therapy and inform the patient's general physician.

Caloric restriction will be provided only to patients who are overweight or obese (BMI>25) and not to patients with BMI at the normal range (18.5-25). Patients with BMI lower than the normal range will be excluded from the study.

10. Risk Management Procedures

Confidentiality

Patients will be identified by a numerical study ID. Only the designated research staff at Sheba Medical center will have access to the patient's fully identified medical information. The information that matches the code to the identifying information will be kept in a safeguarded database that is password protected.

11. Subject Payment/Costs

Subjects will not be directly remunerated for participation in the study. There is no cost to the subject for study participation.

12. End of Study definition

It is estimated that accrual will be completed in approximately 24 months
Time from initiation of intervention to last post intervention meeting – 12 months.
End of the study is the date of the last visit for the last patient which will be approximately 36 months from first patient intervention

Clinical endpoints will be collected up to 5 years after end of intervention.

13. Consent Procedures

Study purpose, methods, materials, risks, benefits, and alternatives will be provided in a detailed description in the consent form and will be discussed with the patient by the investigator or authorized designee. Patients will be told they are free to refuse to participate and may withdraw their consent at any time for any reason. The consent forms will be signed and dated by the patient before his or her participation in the study. The informed consent forms and process shall be documented in the patients' clinical records. A copy of the signed consent form will be provided to the patient.

14. Privacy

If patients wish to review or discuss their results this information will be discussed in private consultation with the study team medical personnel.

15. Data Security

The collection and processing of personal data from subjects enrolled in this study will be limited to the data needed to investigate this study's hypothesis. Access to identifiable data will be limited to Sheba Medical Center designated personnel; patient level de-identified data will be available only to investigators authorized by the Principal Investigator.

Data files are stored on a password-protected computer/database and will be accessible only to the designated investigators and research staff. Only the research staff will have the link that can match the code to traditional identifying information. The data sets used

for analysis will be coded and not contain any traditionally used identifying information that could be used to identify the patient.

16. Study/Intervention Discontinuation

Patients will be discontinued from study intervention in the following circumstances:

1. The patient is enrolled in any other clinical trial involving any investigational product or any other type of medical research judged not to be scientifically or medically compatible with this study.
2. Investigator decision: the investigator decides that the patient should be discontinued from the study or study intervention if the patient, for any reason, requires treatment with a therapeutic agent that effects study indication/intervention or for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP).
3. Patient decision: If the patient requests to be withdrawn from study intervention but agrees to stay in the study she will be evaluable for the endpoint if she attended at least one follow-up meeting post randomization. If the patient wishes to withdraw participation in the study she can do so at any time and in such a case data and samples will be destroyed
4. Disease recurrence.
5. Discontinuation of Inadvertently Enrolled Patients: If the investigator identifies a patient who did not meet enrollment criteria and was inadvertently enrolled, a decision on whether or not the patient may remain on intervention will be made and documented. Patients will be evaluable for the primary endpoint if they were randomized and attended at least one follow up meeting post start of intervention.

17. Monitoring

Sheba Medical Center will monitor the study. Source documents will be reviewed to ensure all subjects have properly signed and dated the informed consent forms. All information will be reviewed to ensure eligibility criteria as per the protocol, and supporting source data will be verified.

18. Record Retention

Research records with patient identification will be kept for 10 years after study completion. The collected data and related de-identified health information may be kept indefinitely. Record retention will comply with the specific requirements of the Sheba Medical Center IRB. No personal health information will be retained.

19. Publication

The results of this research will be presented at meetings or in publication. However, the subject's identity will not be disclosed in those presentations.

20. Facilities and Personnel

All study activities will occur within the patient's home and breast cancer institute clinic at Sheba Medical Center. All communications with patients will be through the Sheba Medical Center.

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Appendix 1: **Samples Collection, Storage and Analysis**

Blood: during the study we will collect blood samples 2 times : At the initial screening, and at the end of the intervention,at time points 0 and 6m. Two blood samples will be taken- one sample for immediate analysis and a second sample (blood and plasma) would be stored in deep freeze (-80°C) for future metabolomics testing (blood tests are detailed in schedule of activities table, see Appendix 2).

Stool: Patients will be asked to provide several stool sample at 2 time points throughout the study. Stool samples are required for the study, and will be collected at baseline and at the end of intervention. Stool samples will be stored at Sheba Medical center and transfered to Segal lab at the Weizmann Institute. The samples will be stored encoded with no identifying information. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored for 10 years. Identifying details and codes will be kept by the principal investigator and designated personnel. All future use of stool samples will be subject to Helsinki approval.

Urine: urine samples will be taken from every patient at 2 time points, including at the beginning and at the end of the intervention, in order to characterize estradiol derivatives.

Appendix 2: Schedule of activities

Trial period	Screening	Profiling	Intervention						End of treatment	Followup	
Scheduling window	week -8 to -4	week -4 to -3	Day 0 (M1)	M2	M3	M4	M5	M6	M7	M12	Every 6 months (5years)
Signed ICF	X										
Review of eligibility	X										
Medical History	X										
Nutrition/Lifestyle/Medical Questennaire	X								X		
Weight		X	X	X	X	X	X	X	X	X	
Height		X	X	X	X	X	X	X	X	X	
Other Anothropometrics		X	X	X	X	X	X	X	X	X	
Provision of stool kit	X										
CBC		X							X		
Blood Chemistry including total serum protein and albumin and fasting glucose		X							X		
Lipid profiling		X							X		
Liver profiling (GGT, Bilirubin, Alkaline Phosphatase, AST,ALT)		X							X		
TSH		X							X		
CRP		X							X		
HbA1C		X							X		
Hormonal Profiling (LH, FSH)		X									
Urinalysis (estradiol derivatives)		X							X		
Whole blood sample for expolratory analysis		X							X		
Dietitian Consult		X	X	X	X	X	X	X	X	X	
Profiling stage menu		X									
Food and Activity Diary log in	X	X	X	X	X	X	X	X	X	X	
Continuous Glucose Monitoring Connection (2 weeks)		X				X (optional)			X		
Survival And Breast Cancer recurrence follow up										X	X (from EMR)
Hormonal Treatment Adherence Follow up	X								X	X	X (from EMR)
Diet Adherence follow up (weekly phone, text or email followup)			X	X	X	X	X	X	X		

List of features - PPGR predictions
Blood tests
HbA1C%
Hemoglobin
FastingGlucose
Anthropometric (measured at profiling)
BMI
BodyFat
Waist
Weight
Hips
Dietary components of the meal
Alanine_g
Alcohol_g
Arginine_g
Caffeine_mg
Calcium_mg
Carbohydrate_g
Cholesterol_mg
Energy_kcal
Fructose_g
Galactose_g
Glucose_g
Isoleucine_g
Lactose_g
Leucine_g
Magnesium_mg
Maltose_g
Niacin_mg
Phenylalanine_g
Protein_g
Sodium_mg
Starch_g
Sucrose_g
SugarsTotal_g
Thiamin_mg
TotalDietaryFiber_g
TotalLipid_g
TotalMonounsaturatedFattyAcids_g
TotalPolyunsaturatedFattyAcids_g
TotalSaturatedFattyAcids_g
TotalTransFattyAcids_g
VitaminC_mg
VitaminD_IU
VitaminE_mg
Water_g
Zinc_mg
Health questionnaire
Age
Currently_smokes
Evening_Hunger
Ever_smoked
Gender

General_Hunger
Is_pregnant
Midday_Hunger
Morning_Hunger
Physical_activity_-_freq
Physical_activity_-_mins
Regular_defecation
Sleep_quality
Stress
Work_activity
Microbiome features
s_Acidaminococcus_unclassified
s_Adlercreutzia_equolifaciens
s_Akkermansia_muciniphila
s_Alistipes_finegoldii
s_Alistipes_indistinctus
s_Alistipes_onderdonkii
s_Alistipes_putredinis
s_Alistipes_senegalensis
s_Alistipes_shahii
s_Anaerostipes_hadrus
s_Anaerotruncus_unclassified
s_Bacteroidales_bacterium_ph8
s_Bacteroides_caccae
s_Bacteroides_cellulosilyticus
s_Bacteroides_clarus
s_Bacteroides_dorei
s_Bacteroides_eggerthii
s_Bacteroides_faecis
s_Bacteroides_finegoldii
s_Bacteroides_fragilis
s_Bacteroides_intestinalis
s_Bacteroides_massiliensis
s_Bacteroides_nordii
s_Bacteroides_ovatus
s_Bacteroides_plebeius
s_Bacteroides_salyersiae
s_Bacteroides_stercoris
s_Bacteroides_thetaiotaomicron
s_Bacteroides_uniformis
s_Bacteroides_vulgatus
s_Bacteroides_xylanisolvens
s_Barnesiella_intestinihominis
s_Bifidobacterium_adolescentis
s_Bifidobacterium_animalis
s_Bifidobacterium_bifidum
s_Bifidobacterium_catenulatum
s_Bifidobacterium_longum
s_Bifidobacterium_pseudocatenulatum
s_Bilophila_unclassified
s_Bilophila_wadsworthia
s_Burkholderiales_bacterium_1_1_47
s_Catenibacterium_mitsuokai

s_Clostridium_bartlettii
s_Clostridium_bolteae
s_Clostridium_leptum
s_Collinsella_aerofaciens
s_Coprobacter_fastidiosus
s_Coprococcus_catus
s_Coprococcus_comes
s_Coprococcus_sp_ART55_1
s_Desulfovibrio_desulfuricans
s_Desulfovibrio_piger
s_Dorea_formicigenerans
s_Dorea_longicatena
s_Eggerthella_unclassified
s_Erysipelotrichaceae_bacterium_6_1_45
s_Escherichia_coli
s_Escherichia_unclassified
s_Eubacterium_biforme
s_Eubacterium_eligens
s_Eubacterium_hallii
s_Eubacterium_ramulus
s_Eubacterium_rectale
s_Eubacterium_siraeum
s_Eubacterium_ventriosum
s_Faecalibacterium_prausnitzii
s_Flavonifractor_plautii
s_Gordonibacter_pamelaeae
s_Haemophilus_parainfluenzae
s_Holdemania_unclassified
s_Lachnospiraceae_bacterium_1_1_57FAA
s_Lachnospiraceae_bacterium_2_1_58FAA
s_Lachnospiraceae_bacterium_3_1_46FAA
s_Lachnospiraceae_bacterium_5_1_63FAA
s_Lachnospiraceae_bacterium_7_1_58FAA
s_Lachnospiraceae_bacterium_8_1_57FAA
s_Lactobacillus_ruminis
s_Lactococcus_lactis
s_Megamonas_unclassified
s_Methanobrevibacter_smithii
s_Odoribacter_splanchnicus
s_Oscillibacter_unclassified
s_Oxalobacter_formigenes
s_Parabacteroides_distasonis
s_Parabacteroides_goldsteinii
s_Parabacteroides_johnsonii
s_Parabacteroides_merdae
s_Parabacteroides_unclassified
s_Paraprevotella_clara
s_Paraprevotella_unclassified
s_Paraprevotella_xylaniphila
s_Parasutterella_excrementihominis
s_Peptostreptococcaceae_noname_unclassified
s_Phascolarctobacterium_succinatutens
s_Prevotella_copri

s_Roseburia_hominis
s_Roseburia_intestinalis
s_Roseburia_inulinivorans
s_Roseburia_unclassified
s_Ruminococcus_albus
s_Ruminococcus_bromii
s_Ruminococcus_callidus
s_Ruminococcus_gnavus
s_Ruminococcus_lactaris
s_Ruminococcus_obeum
s_Ruminococcus_sp_5_1_39BFAA
s_Ruminococcus_torques
s_Streptococcus_parasanguinis
s_Streptococcus_salivarius
s_Streptococcus_thermophilus
s_Subdoligranulum_unclassified
s_Sutterella_wadsworthensis
s_Veillonella_parvula

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a, We added the MOH identifier
Protocol version	#3	Date and version identifier	Protocol attached
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16-17
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	16
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4, figure 1
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1, page 7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6-7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	12-13; Figure 2c
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1, page 7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline,	14, Box 1

		final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1 ; 8-12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate	11

		document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires,	9-11

		laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Figure 2A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8,15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14-15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14-15
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15

Notes:

- 2b: n/a, We added the MOH identifier
- 8: 4, figure 1
- 11c: 11; figure 2c

- 12: 12-13, Box 1

- 13: Figure 1 ; 6-9 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 24. February 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

BREAst Cancer Personalized NuTrition (BREACPNT) - dietary intervention in breast cancer survivors treated with endocrine therapy: A protocol for a randomized clinical trial

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	NUTRITION & DIETETICS, Breast tumours < ONCOLOGY, Microbiology < NATURAL SCIENCE DISCIPLINES

BREAst Cancer Personalized NuTrition (BREACPNT) - dietary intervention in breast cancer survivors treated with endocrine therapy: A protocol for a randomized clinical trial

Michal Rein[3,4], Maya Dadiani[1], Anastasia Godneva[3,4], Michal Bakalenik-Gavry[1], Dana Morzaev-Sulzbach[1], Yaeli Vachnish[1], Dmitry Kolobkov[3,4], Maya Lotan-Pompan[3,4], Adina Weinberger[3,4], Eran Segal[3,4] and Einav Nili Gal-Yam[2]

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Word count: 3986

ABSTRACT

Introduction: Breast cancer survivors treated with adjuvant endocrine therapy commonly experience weight gain, which has been associated with low adherence to therapy and worse breast cancer prognosis. We aim to assess whether a personalized postprandial glucose targeting diet will be beneficial for weight management as compared to the recommended Mediterranean diet in this patient population

Methods and Analysis: The BREAst Cancer Personalized NuTrition (BREACPNT) study is a phase-2 randomized trial in Hormone Receptor positive (HR+) breast cancer patients, treated with adjuvant endocrine therapy. The study objective is to assess whether dietary intervention intended to improve postprandial glycemic response to meals results in better weight and glycemic control in this population as compared to the standard recommended Mediterranean diet. Consenting participants will be assigned in a single blinded fashion to either of two dietary arms (Mediterranean diet or an algorithm-based personalized diet). They will be asked to provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for two weeks, at the initiation and termination of the intervention period. Microbiome composition data will be used to tailor personal dietary recommendations. After randomization and provision of dietary recommendations, participants will be asked to continuously log their diet and lifestyle activities on a designated smartphone application during the 6-month intervention period, during which they will be monthly monitored by a certified dietitian. Participants' clinical records will be followed twice yearly for five years for treatment adherence, disease free survival and recurrence.

Ethics and dissemination: The study has been approved by the ethics committee in the Sheba medical center (file 5725-18-SMC, Ramat Gan, Israel) and the Weizmann Institutional Review Board (file 693-2, Rehovot, Israel). The findings of this study will be published in a peer reviewed publication.

ClinicalTrials.gov Identifier: NCT04079270

MOH Identifier: 2019-03-28_006056

Strengths and limitations of this study

- A single blinded study where patients are being assigned to one out of two dietary arms (Mediterranean diet or an algorithm-based personalized diet).
- The personalized diet involves advanced machine-learning analysis of multi-omics dataset, including microbiome, continuous glucose monitoring, metabolomics features and full dietary records.
- A homogenous study population including HR+, early stage breast cancer survivors treated with adjuvant endocrine therapy, albeit representing mostly women from the center of Israel.
- Patients are being randomized into the two study arms and stratified by stage, treatment type, menopausal status and BMI.
- The study design includes daily use of smartphone application for logging dietary intake and lifestyle events. This may lead to exclusion of patients inaccessible to smartphone app on a daily basis.

INTRODUCTION

The majority (~75%) of breast cancer patients are diagnosed with hormone receptor-positive (HR+) tumors and are assigned adjuvant endocrine treatment (ET) for a period of at least 5 years, which was shown to improve survival. However, adjuvant ET is associated with distressing side-effects which may be long lasting and substantially impair patients' quality of life and adherence to treatment. These side effects include weight gain and body composition changes, which are common in breast cancer survivors and are experienced by many women during treatment and for years after diagnosis(1–3). Weight gain in this population is complex and is associated with various factors such as tumor type, menopausal status(4), pre-diagnosis body mass index (BMI)(5) and neo-adjuvant/adjuvant treatment type including chemotherapy and ET(2,6). Importantly, weight gain after breast cancer

diagnosis is associated with increased risk for metabolic syndrome and cardiac disease (7,8), and was reported as a risk factor for breast cancer recurrence and shorter survival(4,9,10). Therefore, weight management strategies including diet, regular physical activity and cognitive behavioral therapy are recommended for controlling weight gain in breast cancer patients. Previous studies showed that weight loss interventions, incorporating diet, exercise and psychosocial support, in overweight or obese breast cancer survivors appear to result in decreased body weight, BMI and waist circumference and improvement in overall quality of life(11). We chose the Mediterranean (MED) diet as a control diet because it is commonly recommended in different countries including Israel(12) and was suggested to improve metabolic health in the general population as well as within breast cancer survivors(13–15). Still, the optimal weight loss intervention method and the impact of weight loss on survival outcomes is unclear. Furthermore, the interaction between the microbiome of breast cancer patients and dietary intervention has not been assessed.

The comprehensive role of the gut microbiome in modulating immune and metabolic health is increasingly recognized. Dysbiosis, referring to the disruption in the balance of gut bacterial communities, is associated with many conditions(16). The gut microbiome homeostasis can be influenced by internal factors, such as genetic, age-related and hormonal-related, as well as by external factors, such as stress, lifestyle and antibiotics(17). In addition, the microbiome is directly affected by the individual diet which in turn affects the body's response to food (18,19). Particularly relevant to breast cancer, diet plays an important role in creating a microbiome environment involved in estrogen metabolism(20). High estrogen levels contribute to breast cancer risk in postmenopausal women(21). In a recent study, gut microbiome diversity was linked to weight gain(22) and microbiome alterations were found to contribute to post-dieting weight regain(23). In addition, it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol (24). In a previous study we showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food(25). Importantly, this study emphasized the involvement of functional microbial pathways and bacterial taxa in

host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on personal measurements, including blood tests, personal lifestyle and gut bacteria profiles. In a following study implementing a 6-month dietary intervention plan in individuals with prediabetes, the Personalized Postprandial-glucose-response Targeting (PPT) approach significantly improved glycemic control and reduced PPGRs as compared to the commonly recommended Mediterranean diet (26).

In this study we seek to evaluate the clinical efficacy of the PPT diet combined with caloric restriction, compared to the Mediterranean diet, in promoting weight maintenance or weight loss and glycemic control in HR+ early stage breast cancer survivors treated with adjuvant ET.

METHODS

Study design

This study is a two-arm, parallel group, single-blinded, randomized controlled trial in early stage HR+ breast cancer patients treated with adjuvant endocrine therapy. Eligible participants will undergo a 6-month nutrition intervention program which will include dietary recommendations, daily logging and monthly follow up meetings provided by a certified dietician. Upon trial entry and after profiling (described below) participants will be randomly and equally assigned to the Personalized Postprandial-glucose-response Targeting (PPT) dietary (arm A) or to the Mediterranean-style (MED) dietary (arm B). All meetings will take place in the Breast Oncology Institute at the Sheba Medical Center. The primary objective of the study is to evaluate the efficacy of the PPT arm vs the MED arm in controlling body mass changes in the patient population during the intervention period (see summarized study endpoints in **BOX 1.** For complete SPIRIT checklist and the full protocol see Supplemental material no.1 and 2 respectively)

BOX 1 – STUDY ENDPOINTS

Primary endpoint

Body weight changes defined as the net body weight gained/lost in the 6–month intervention period

Secondary endpoint

Glycemic response as measured by the area under the glucose curve (AUC) during continuous glucose monitoring (CGM) period pre-intervention and during the intervention.

Exploratory endpoint

- Five years Disease free survival (DFS)
- Microbiome and blood metabolites modulation during the diet interventions- tested using the samples taken at profiling and 6month time points.
- Adherence to algorithm-based personalized diets, compared to standard diets advised for weight control – assessed by monthly compliance questionnaire.
- HR+ breast cancer patients' adherence to hormonal treatment
- Translational studies

Patient and public involvement

Research questions and outcome measures were partially based on numerous encounters of ENG in the clinic with breast cancer patients voicing concerns regarding weight gain and optimal diet while on endocrine treatment for breast cancer. Furthermore, Patients are being involved in the recruitment effort by actively publishing the study recruitment information and sharing their own experience during the study, via social networks and breast cancer survivors' groups. The patients are being followed up in the clinic for a long period of time (~10 years). Accumulating study results will be summarized periodically, transferred to the study team including treating and recruiting physicians and through them transferred to patients during clinic visits. Patients will also be informed regarding publications and specific results through the cancer institute social network and digital

resources (such as the Sheba oncology web application and Sheba oncology Facebook page).

Study population

This trial will enroll breast cancer survivors treated with ET and followed at the Breast Oncology Institute at the Sheba Medical Center. Eligibility criteria (inclusion and exclusion criteria) are detailed in **Table 1**. Potentially eligible participants will be identified and recruited to the study by the medical team during regular clinic visits or via database search and phone calls by the clinical study coordinator (SC). Information leaflets and a poster describing the study design and contact information will be available at the institute's reception and waiting area. Additionally, a video explaining the study and its aims will be shown on screens at the institute's reception and waiting area and will be sent to potential participants (https://youtu.be/kxrqONj3KGM). All participants will assign informed consent.

Table 1: Eligibility criteria

Inclusion Criteria	Exclusion Criteria
Female patients	Oral Antibiotics/antifungal use in the previous one month to profiling stage *
Age ≥ 18 and ≤ 80	Known Diagnosis of diabetes or the use of anti-diabetic and/or weight-loss medication
Diagnosis of stage 1-3 HR+ breast cancer, who underwent surgery	BMI<18.5
At least 60 days after last non-endocrine oncology treatments (i.e. definitive surgery, radiation or chemotherapy – whichever is last) if these were indicated.	Patients under another diet regime and/or a dietitian consultation/ clinical study
Adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists) taken for at least 30 days but no more than 24 months.	Pregnancy, breast feeding
Willing to operate a smartphone application	HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
	Known diagnosis of psychiatric disorders (Schizophrenia, Bipolar Disorder)
	Known diagnosis of IBD (inflammatory bowel diseases)
	Patients that underwent Bariatric surgery
	Known Alcohol or substance abuse

*Patients will be offered to join the study at a later point

Study Procedures and Intervention

ICF signed (wk -8 to -4)

Eligible participants will be invited to sign an inform consent at the oncologic clinic in Sheba medical center (as shown in **Figure 1**).

Profiling stage (wk -6 to -3)

Consenting patients will proceed to the profiling stage. During this stage they will undergo the following procedures:

1. Meeting with the SC and completion of questionnaires detailing relevant medical background, nutritional habits and lifestyle activities. Questionnaires will be filled online using the REDCap (27) software (a secure web application for managing online surveys and clinical trials).
2. Participants will provide blood samples after a night fast (12 hours) for complete blood count and blood chemistry, including liver function, lipid profiling, Fasting Plasma Glucose (FPG) and HbA1c. LH, FSH and Estradiol will be measured only in pre-menopausal patients. Participants will provide urine sample for estradiol derivatives for future exploratory analyses.
3. Anthropometrics measurements, including weight, height, waist and hip circumference will be taken at this meeting.
4. Stool sample: Patients will receive a designated stool kit (Genotek OMR200) to collect stool at home. The SC will instruct them how to provide the stool samples and will ask to return this kit during the following week for further processing of the microbiome data. Microbiome sequenced data are essential for the algorithm predictions, thus stool sample is obligatory for participation in the study.
5. Continuous glucose monitoring (CGM) connection: Patients will be connected to a CGM (Abbott Freestyle LibrePro) for 2 weeks. The CGM kit includes a sensor affixed to the back of the arm that continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels.

6. Food diary: Patients will be instructed to download the study dedicated smartphone application ('personalized nutrition project') for food logging. They will log in real-time their food intake, physical activities, sleep duration and quality and special events. During the profiling period, patients will be asked to follow their regular dietary and lifestyle habits (see examples of logging activities in **Figure 2A**). All participants will receive a registration code and their data will be anonymized.
7. Data collected during the profiling period, including microbiome, anthropometrics, blood parameters and questionnaires will be analyzed and used by the PPT algorithm to provide personal dietary recommendations for each participant.

Randomization

After completion of the profiling stage patients will be randomly assigned to one of two arms of the study by one programmer from the trial personnel who had no contact with participants. Approximately 100 subjects will be assigned to each arm using a blinded randomization algorithm and the following stratification factors: (1) menopausal status at study entry (post/pre) (2) received/not received chemotherapy prior to study entry (3) ET type (Tamoxifen/Aromatase inhibitor) (4) Breast Cancer stage at diagnosis and (5) BMI above/below 27. Notably, we only used the stratification factors to minimize differences between groups in the allocation process and did not analyze the data according to the stratification factors. Patients and part of the study team (oncologists and study coordinators), excluding the dietitian, will be blinded to the study arm assigned. At the end of intervention, dietary assignment was revealed, and participants were asked to continue following their respective diets for 6 additional months.

Recommendation meeting (Day 0)

Upon menus construction, patients from both arms will be invited to a recommendations meeting (hereafter 'day-0') with the dietitians. Patients will receive general information regarding their menu and will be instructed to consume and log

their meals according to it. In order to ensure accurate logging the dietitians will schedule an online follow up two weeks after 'day-0'. Anthropometrics measurements, including weight, hip and waist circumference, taken at this meeting will be used as baseline measurements.

Follow up Meetings (Mo +1 up to Mo +5)

All patients will participate in monthly follow-up meetings with a dietitian (total of 6 meetings) in order to evaluate their compliance to the dietary recommendations they received and provide additional advice if needed. Anthropometric measurements (weight, hip and waist circumference) will be taken at each time point. Furthermore, patients will be asked to fill a follow-up questionnaire and report any changes within their lifestyle and treatment. At the beginning of Month 4 of the intervention period, patients will be offered to be reconnected to CGM for two weeks (optional). At the monthly meeting before the end of intervention patients will receive a stool kit, to be returned at the end of intervention meeting.

End of intervention (Mo +6)

At the end of the 6-month intervention period, patients will be invited to a meeting in which anthropometrics measurement will be taken, as well as urine, blood and stool samples. Additionally, participants will be connected to CGM for 2 weeks (mandatory) for the third time (Figure 1). When patients return CGM they will be unblinded to their assigned intervention arm by the study dietitian.

Long term follow up (Mo +12)

At 12-month time point, patients will be invited to a follow up meeting and will be asked to fill follow-up questionnaires, including food frequency questionnaire. Anthropometric measurements and a 3-day food diary on the study app will be recorded. Patients will receive the menu of the other study arm and will be offered to follow either one of the diets. Long-term clinical follow-up information will be collected from the electronic medical records twice yearly for treatment adherence, recurrence and survival calculation purposes, for a period of up to five years post randomization.

Menus construction

Before randomization, menus will be constructed for each patient and will be adjusted for, patients' caloric target and clinical data. The menus construction flow is presented in **Figure 2B**.

Meal bank (list)

The menus provided to patients in this study are constructed from a meal bank that we previously generated(26), with over 2,000 meals representative of the Israeli typical diet and with a variety of different food combinations. We divided the meals in the meal bank into four meal types (breakfast, lunch, dinner and snacks) and labeled them according to meal categories (dairy; meat; fish etc.) in order to generate menus according to patients' personal preferences.

Caloric target calculation

In order to provide the patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as an average between:

1. Estimated Energy Requirements (EER) calculated with the use of the Mifflin equation for Resting Energy Expenditure (REE), using their weight, height, age and gender, and multiplied by Physical Activity (PA) factor, based on the level of PA that the person performs on a regular basis (28).

2. Energy expenditure assessed by Basal Metabolic Rate (BMR) value measured by body composition analyzer (Tanita). The result from this equation will be divided by 0.7 (as REE represents ~70% of total energy expenditure).
3. Average daily caloric intake obtained from the patients' dietary records during the profiling stage, to account for the subject's dietary habits prior to the intervention.

Furthermore, for individuals with BMI>25 a total of 500Kcal will be reduced from their calculated caloric target, but not less than 1200 calories/day, to allow weight loss according to common recommendations for weight loss (29,30).

Diets

Mediterranean-style (MED) diet

In this arm we included meals that were scored by four external dietitians according to the Mediterranean-style dietary recommendations. Meals were binary scored as recommended (=1) or not recommended (=0) and we applied scores 1 to 5 to all meals, depending on how many dietitians marked the meal as recommended or not. The diet is based on recommended foods such as vegetables, fruits, legumes, whole grain products, unsaturated fats such as olive oil and nuts, fish, poultry and low-fat dairy products. Consumption of red meat, high fat dairy products, processed foods and sweet pastries, was discouraged as part of the diet. Additionally, menus in this arm were designed with the following target for daily macronutrient composition: 45-65% of calories from carbohydrates; 15-20% from protein; and 20-35% from fat, with up to 10% from saturated fat. Menus include only meals that received scores 1 and 2. Participants will be encouraged to consult with the dietitian regarding meals that may not appear in the constructed menu.

Personalized Postprandial-glucose-response Targeting (PPT) diet

In this arm, dietary recommendations will be based on the algorithm predictions of the postprandial glucose responses(25), shown to improve glycemic control and metabolic health in healthy individuals or in individuals with prediabetes and diabetes(26,31). Notably, these interventions were not caloric restricted as in the current study. Among the features used to predict PPGR to meals were anthropometrics, blood tests (FPG, HbA1c% and Hemoglobin), lifestyle features derived from questionnaires, microbiome (abundances of species estimated by MetaPhlAn2 and meal features (macro- and micronutrient composition) were used (see Supplementary table 1 for the full list). Since no events around the meal were

used for prediction, trained predictor could predict response for any profiled participant to any given meal.

All logged meals will be scored from 1-5 based on a unique scoring method that we developed and tested in previous studies, and study participants will be asked to consume only meals with score 1 or 2. Importantly, the PPT diet, by definition, was not aimed to have a predetermined macronutrient distribution, In contrast to the Med-diet

Adherence to the study recommendations

The adherence to the prescribed diets during the intervention will be evaluated by the dietitian by a close monitoring of the patients' self-recorded dietary intake in the logging application, as well as by monthly electronic follow-up questionnaires that participants will be asked to fill out. In order to encourage dietary adherence and self-monitoring, we will generate a bi-weekly semi-automatic feedback reports that will include composite grades on a scale of 0-100 (from worse to best) for diet composition, calorie intake and dietary fiber intake separately, for the entire two-week period (**Figure 2C**).

- *MED-diet composition grade:* indicates how well the participant sticks to the dietary recommendations based on the MED approach including Carbohydrate (as % of daily caloric intake), fats in general (as % of daily caloric intake) and specifically saturated fat intake below and above 10% of caloric intake. Dietary fiber intake per each of 1000 kCal per day will be also calculated as part of the score.
- *PPT-diet composition grade:* indicates how well the participant sticks to predictor-based meal scores. Each meal score was assigned with a grade as follows: meal score 1=grade 100; meal score 2=80; meal score 3=50; meal score 4=25; meal score 5=0. The grades are averaged calorie-wise (with food energy trimmed to be within (100,500) kcal interval) - Σ kcal(i) $\cdot$ grade(i). For example, if a participant eats three meals: 600 kcal of meal score 2, 1000 kcal

of meal score 5 and 80 kcal of meal score 1, feedback grade would be: (500*80+500*0+100*100) / (500+500+100) = 45. If too few (100 by default) calories are logged (overall), we did not compute a score.

- *Calories grade:* indicates how well the participant sticks to the prescribed caloric target. When caloric intake deviates within 15% of caloric target (CT) the applied grade is 100; when caloric intake deviation exceeds 60% of CT the applied grade is 0; when caloric intake deviation is between 15% and 60%, a linear penalty is applied to the grade depending on the deviation.
- *Dietary fiber grade:* indicates if participants consumed the recommended amount of dietary fibers (set to 14 gram for every 1000 kcal/day for both arms) from the diet at the referred time When fiber intake in grams per day reaches the recommended amount, or higher the applied grade is 100 and when it is below the recommended amount a linear penalty is applied to the grade.

In addition to grades, feedback reports also included a list of recommended meals and non-recommended meals (by meal score) to highlight the best and worst meals consumed on that time period (as logged by the participant). The best and worst meals lists will be generated systematically and be reviewed by a dietitian from the study team.

STATISTICAL ONSIDERATION

Sample size determination

To estimate the required sample size we performed power analysis, using an unpaired t-test assuming normal distribution of the primary outcome (weight change), estimating the effect size based on the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. (32), the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis

All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variables, the Spearman correlation coefficient will be used. To compare parameters for continuous variables in two time points, the paired-samples t-test will be performed (or Wilcoxson test for non-normally distributed variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous/categorical variables in the number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer Translational Research laboratory at Sheba Medical Center. The blood and urine samples will be stored at -80°C and bacterial DNA samples will be stored at -20°C. The samples will be encoded with no identifying information. Identifying details and codes will be kept in an encrypted file stored at Sheba medical center. Encoded stool samples will be transferred to the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team. Samples will be stored for up to 10 years. All future use of stool and blood samples will be subjected to IRB approval.

Ethics and dissemination

The study has been approved by the Sheba medical center Institutional Review Board (IRB 5725-18) and the Weizmann institute of science Institutional Review Board. The findings of this study will be published in a peer reviewed publication. De-identified individual participant data and applicable supporting clinical trial documents will be available upon request for 12 months after publication.

CURENT STATUS

Enrollment and recruitment initiated on July 2019. To date (February 2022), 120 participants have been recruited, out of them 60 completed the 6-month intervention period including 38 participants who completed the 12-month time point.

DISCUSSION

Dietary interventions are the first-line treatment for weight management within breast cancer survivors and have beneficial results. Yet, the ability to maintain these outcomes is questionable and require further research(11,33,34). In this trial, we aim to assess the effect of a PPT diet on weight maintenance as compared to Mediterranean-style diet in early stage HR+ breast cancer patients, taking ET.

This study has several strengths and limitations. Advantages of this study design include a comprehensive profiling of each participant, which allow us to better understand participants' metabolic baseline and to assess the effect of the dietary changes. Additionally, the continuous food logging by the study patients using a designated smartphone app can provide us with insights on the patients' compliance to the dietary recommendations in both arms. However, this may limit the study population to individuals who are able to work with smartphone application on a daily basis. Furthermore, the study participants are being closely followed by a dietitian from the study team who monitor their food intake and meet them on a monthly basis in order to increase compliance during the first 6-months. However, on the long term, without intensive monitoring, the feasibility of the PPT diet and the ability to follow the diet recommendations should be investigated. Notably, Ben-Yacov et al. (26), reported that prediabetes individuals following PPT diet were able to maintain the

results during 12-month follow up as compared to those who followed the MED diet. Additionally, as a novel tool, the algorithm is not available for general use which makes it difficult to replicate the intervention. Nevertheless, we do publish the full list of features we use to generate the menus, based on personal and microbiome data (Supplementary table 1).

Lastly, microbiome composition and pathways were recently associated with weight changes and metabolic health parameters, as well as with risk for breast cancer diagnosis and recurrence (35). This may allow us to further explore whether gut microbiome composition and pathways have a predictive role in weight management, metabolic health parameters, glycemic control and even disease recurrence on the next 5 years after the intervention within breast cancer patients, although for disease recurrence differences the sample size may not be large enough.

Taken together, our rich dataset including deep phenotyping of each patient may allow us to deeply investigate associations between clinical and Omic data to DFS in early stage HR+ breast cancer patients and may pave the way to larger studies.

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AUTHOR CONTRIBUTIONS

MR, MD and AW conceived the study and designed the intervention. MR and MD wrote the manuscript. AG is responsible for directing the computational aspects of the study. DK is responsible for the feedback reports and summary reports being sent to participants. MBG, DMS and YV coordinate participants' recruitment and management throughout the intervention and follow-up. AW developed the protocols and directed and performed the microbiome sample sequencing with the help of MLP. ES and ENG conceived the study, designed the intervention and wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST STATEMENT

ENG reports Honoraria and Consulting fees from Pfizer, Novartis, Roche Eli-lilly and AstraZeneca. ES reports scientific consultant fees from Day Two Inc. No pharmaceutical manufacturers or other companies from the industry contributed to the planning, design, or conduct of the trial. No other potential competing interest are relevant to this article were reported.

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treated by adjuvant chemotherapy for breast and gynecological malignancies. BMC Med. 2020 Oct 21;18(1):281.

FIGURES LEGEND

Figure 1. An illustration of the study design

Figure 2. Study application and menus construction. **(A)** The food logging application, examples of logging activities and information available on the application. **(B)** Menus construction flow. **(C)** An example of the bi-weekly feedback report that will be sent to participants.

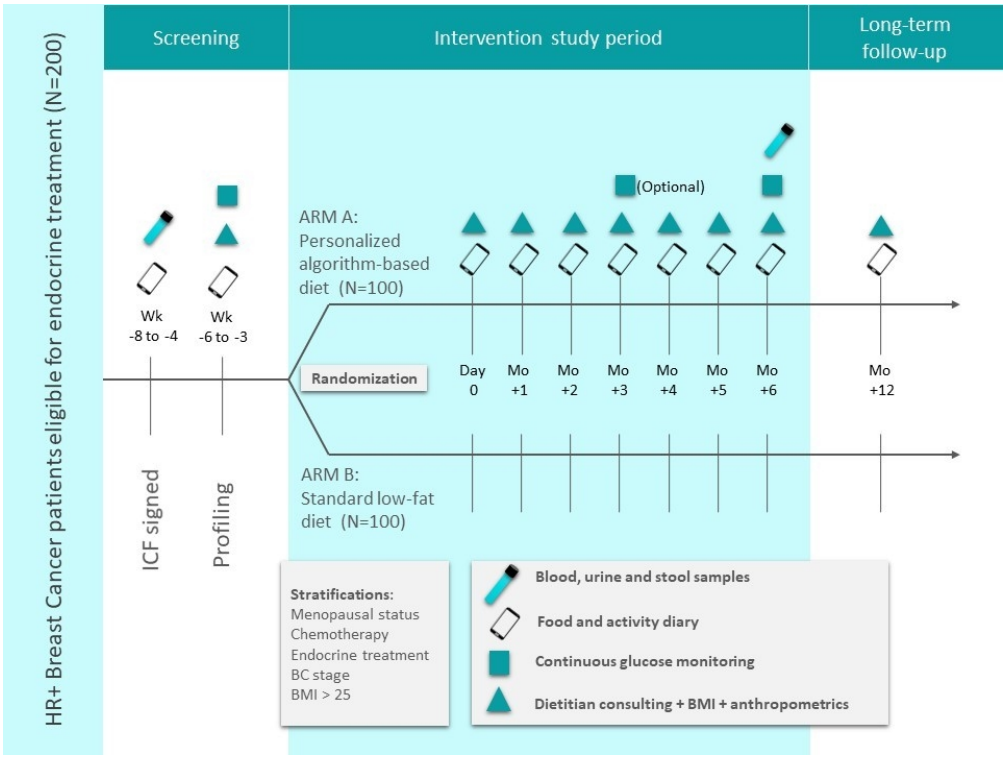
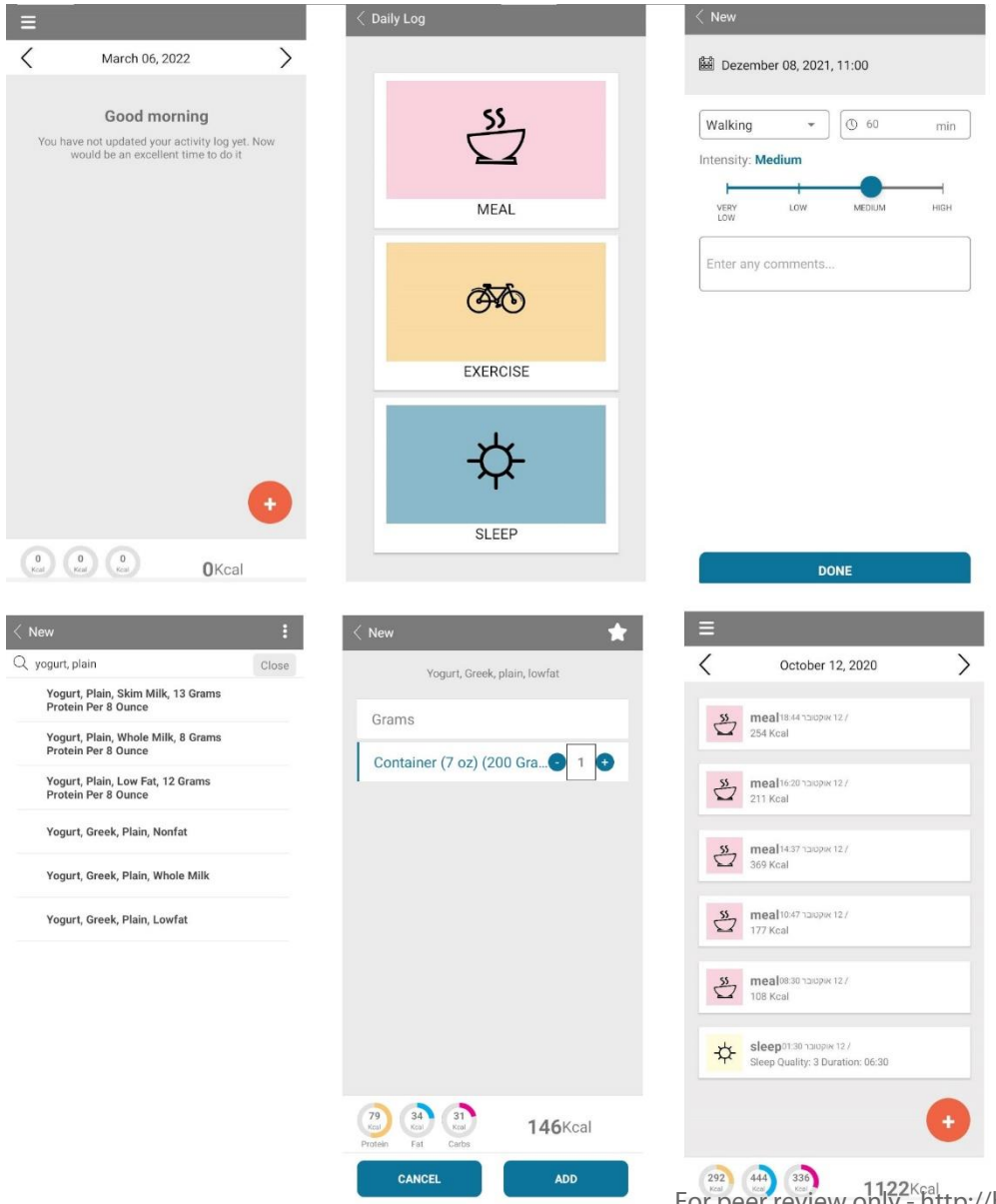


Figure 1. An illustration of the study design

254x190mm (96 x 96 DPI)

A



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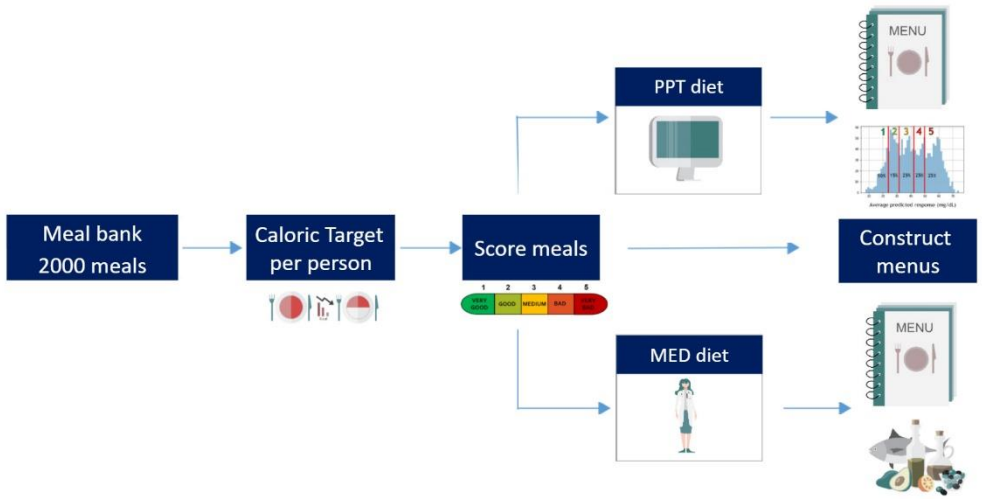

C

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a, We added the MOH identifier
Protocol version	#3	Date and version identifier	Protocol attached
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16-17
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	16
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including	16

		whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4, figure 1
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1, page 7

Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6-7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	12-13; Figure 2c
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1, page 7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14, Box 1
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1 ; 8-12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			

Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	Figure 2A

		collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	8,15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14-15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14-15
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other	15

		data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15

Notes:

- 2b: n/a, We added the MOH identifier
- 8: 4, figure 1
- 11c: 11; figure 2c
- 12: 12-13, Box 1
- 13: Figure 1 ; 6-9 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 24. February 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

The Breast Cancer Personalized Nutrition study (BREACPNT):

A phase 2 single-blinded randomized study of algorithm-based personalized nutrition intervention compared to standard diet intervention in patients treated with endocrine therapy for early stage, hormone receptor positive breast cancer

Study Protocol

SMC -5725-18

February 7, 2019

Version 1.3

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Table of Contents

1. Purpose

The Breast Cancer Personalized Nutrition (BREACPNT) study will evaluate the effect of a microbiome based personalized diet intervention on control of weight gain, glycemic response, disease outcome and various biomarkers in hormone receptor early breast cancer patients receiving adjuvant endocrine treatment.

2. Background

Weight gain is a common incident in breast cancer survivors [1]. As many as 50–96% of women experience weight gain during treatment. Weight gain in breast cancer survivors is complex and influenced by many factors such as tumor type, socio-demographic characteristics, and menopausal status [2,3]. Most breast cancer patients (~75%) are diagnosed with hormone receptor-positive (HR+) tumors and receive endocrine treatment for a period of at least 5 years. Endocrine treatment was identified as a risk factor for weight gain in several studies [2]. Weight gain during endocrine therapy was highest in women who were premenopausal or had previous chemotherapy [4].

Weight gain may decrease adherence to long-term hormonal therapy and increase risk for metabolic syndrome and cardiac disease. Importantly, post-diagnosis weight gain has been implicated as a risk factor in breast cancer recurrence and survival. Hence, weight management for breast cancer survivors is important for increasing adherence to therapy and lowering recurrence risk [5].

The essential role of the gut microbiota in modulating immune and metabolic functions in health and disease is increasingly recognized. Dysbiosis, a disruption in the balance of gut bacterial communities, is associated with many conditions [6]. The entire bacteria population in the digestive tract (microbiome) consists of ~1,000 species with a genetic repertoire of ~3 million different genes. The homeostasis of intestinal microbiota can be influenced by internal factors, such as genetic, age-related and hormonal, as well as by external factors, such as nutrition, stress, lifestyle and antibiotics [7]. The microbiome is directly affected by our diet and directly affect the body's response to food [8,9]. Particularly in breast cancer (BC), diet plays an important role in creating a microbiome

environment involved in estrogens metabolism [10]. High systemic estrogen levels contribute to breast cancer risk in postmenopausal women. Estrogen levels in the blood are regulated in part via enterohepatic recirculation, involving bacterial enzymatic pathways and deconjugation[11]. Indeed, profiling gut microbiota in postmenopausal breast cancer patients revealed altered composition and estrogen-independent low diversity of their gut microbiota compared to healthy controls [12]. Thus, the gut microbial community may affect estrogen-related breast cancer [13].

A recent study linked the gut microbiome diversity to weight gain [14] and microbiome alterations were found to contribute to post-dieting weight regain [15]. In addition it was found that the increase in breast cancer risk with increasing BMI among postmenopausal women is associated with an increase in estrogens, particularly bioavailable estradiol [16]. The Personalized Nutrition Project, conducted at Eran Segal's group in the Weizmann Institute of Science, recently showed in an unprecedented scale of 800 people that individuals vary greatly in their glycemic response to the same food [17]. Most importantly, it emphasized the involvement of functional microbial pathways and bacterial taxa in host glucose metabolism. This unique dataset yielded an algorithm capable of accurately predicting personalized postprandial glycemic response (PPGR) to arbitrary meals. The algorithm's predictions are based on many personal measurements, including blood tests, personal lifestyle and gut bacteria profiles.

Continuing studies demonstrate that short-term dietary interventions change the microbiome and are beneficial to the host in maintaining glucose levels. Moreover, Low glycemic index (GI) diets may be important in weight management [18]. In a small-scale pilot study using this algorithm, personally tailoring dietary interventions to healthy and pre-diabetic people, showed a significantly improved PPGRs accompanied by consistent alterations to the gut microbiota (personal communication) . These results suggest that individually tailored dietary interventions help maintain normal blood glucose levels and influence microbiome diversity, which, in turn, can control weight changes.

3. Hypothesis Objectives and Endpoints

Study Hypothesis

Algorithm based personalized diet will be superior to standard low fat diet for controlling weight gain and glycemic response in breast cancer patients treated with endocrine therapy.

Primary Objective

To evaluate the efficacy of a personalized diet compared to a standard low fat diet to control body mass as measured by changes in body mass.
Endpoint: Body weight changes will be defined as the net body weight gained/lost in the 6 months' intervention period.

Secondary Objective

1. To evaluate the efficacy of the personalized diet compared to a standard low fat diet to control glycemic response.
   Endpoint: glycemic response control measured by the area under the glucose curve (AUC) during continuous glucose monitoring (CGM) period.

Exploratory objective

1. Evaluate disease outcomes as measured by disease free survival, Breast cancer recurrence in study subjects. Endpoint: 5 years Disease free survival (DFS), 5 years Breast cancer specific recurrence.
2. To investigate microbiome composition and modulation during the diet intervention period and assess if there are differences in modulations between the personalized diets as compared to the standard diet.
3. Investigate the mutual effects of gut microbiome and blood metabolites during the diet intervention period and search for possible biomarkers for dietary treatment efficacy.
4. Investigate inflammation parameters and immune profiles of patients (lymphocytes, T-cell receptor repertoire, antibodies profiling using phage display libraries) of HR positive patients undergoing intervention and their correlations to microbiome modulations.
5. Test whether patients have better compliance and adherence to algorithm-based personalized diets, compared to standard diets advised for weight control. The compliance to the diets will be measured by: Compliance questionnaire, 3-day dietary log, Number of study meetings attended.
6. Test whether HR-positive breast cancer patients have better adherence to hormonal treatment following weight-control diets. This compliance will be tested for 5 years post treatment.

4. Study Design

This is a phase 2 randomized trial in hormone receptor positive breast cancer patients receiving adjuvant endocrine therapy. Figure 1 illustrates the study schedule.

200 HR+ breast cancer patients, eligible for adjuvant endocrine therapy will be recruited to the study. Upon recruitment, subjects will provide a stool sample for microbiome analysis and will undergo continuous glucose monitoring for 2 weeks. Thereafter, patients will be randomly assigned in a 1:1 ratio to receive a personalized diet recommendation or a standard low-fat diet for 6 months. The algorithm is based on patients' microbiome analyses and glucose monitoring results. Patients will be monitored by continuous glucose monitoring (CGM) at least 2 times during the 6 months' intervention period. At the end of the 6 months' period patients will undergo a second course of CGM for 2 weeks and provide a second stool sample for microbiome analysis. Patient clinical records will be followed 2-3 times yearly for 5 years for DFS and BC recurrence.

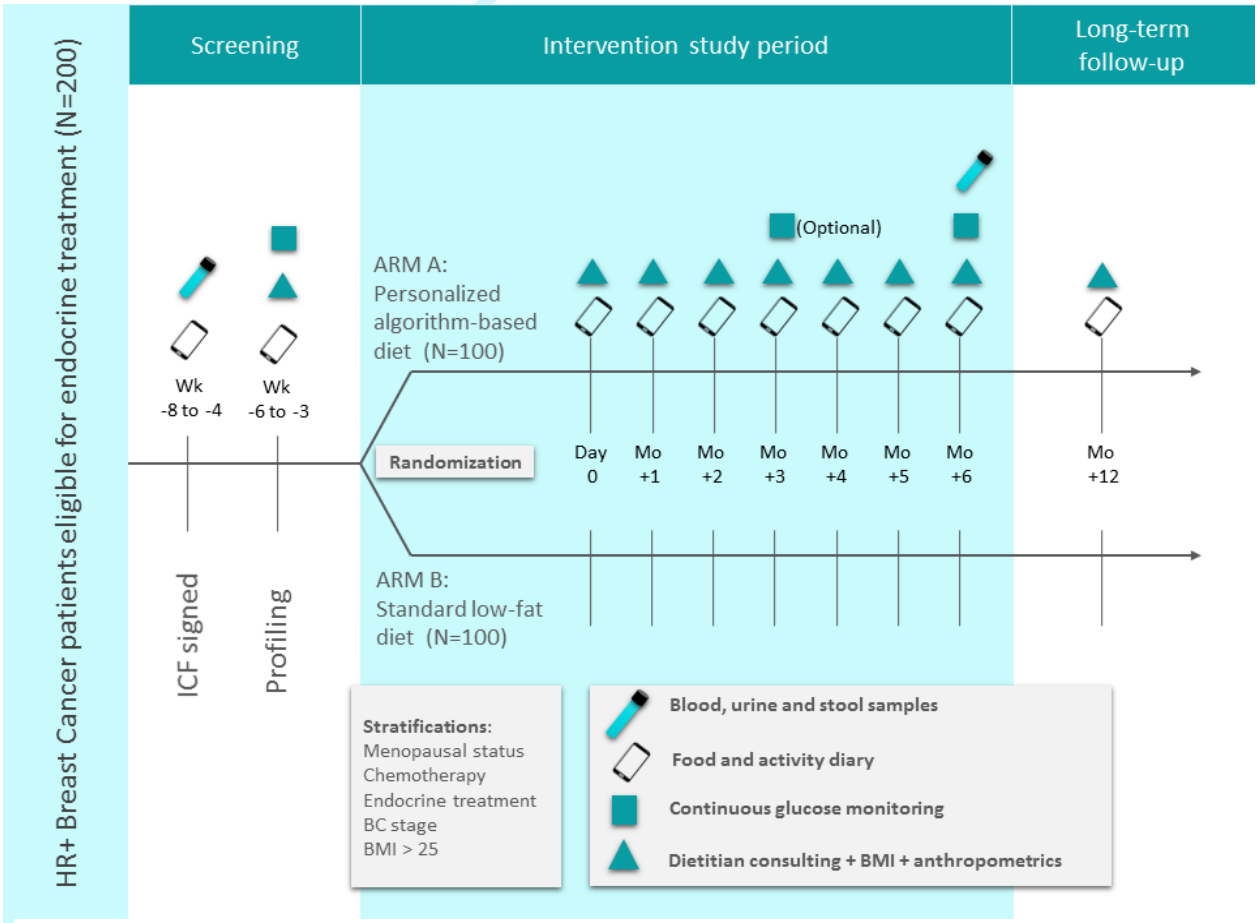

Figure 1: Study design and schedule

Inclusion Criteria:

- Female patients, Age 18-70
- Patients diagnosed with stage 1-3 breast cancer, who underwent surgery, have finished their neo/adjuvant chemotherapy and radiotherapy if these were indicated and are treated with adjuvant endocrine therapy (either Tamoxifen or Aromatase inhibitor +/- GNRH agonists).
- Patients are at least 60 days after finishing their last non-endocrine oncology treatment (i.e. definitive surgery, radiation or chemotherapy – whichever is last), have received at least 30 days of endocrine therapy (tamoxifen or aromatase inhibitor) but no more than 24 months.
- Patients treated with neoadjuvant endocrine therapy are eligible provided they had undergone surgery, are at least 60 days post their last non endocrine therapy (definitive surgery or radiation and chemotherapy, if these were indicated), are continuing their endocrine therapy but did not receive more than 24 months post-surgery.
- Are willing to work with smartphone application

Exclusion Criteria:

- Oral Antibiotics/antifungal use in the previous 3 months to profiling stage (these patients will be able to join the study at a later point)
- Use of anti-diabetic and/or weight-loss medication
- BMI<18.5
- People under another diet regime and/or a dietitian consultation/another study?
- Pregnancy, breast feeding
- HIV carriers, Cushing syndrome, Chronic Kidney Disease, acromegaly, hyperthyroidism, liver cirrhosis
- Psychiatric disorders (Schizophrenia, Bipolar Disorder)
- Known diagnosis of IBD (inflammatory bowel diseases)
- Patients that underwent Bariatric surgery
- Known Alcohol or substance abuse
- Known Diagnosis of diabetes

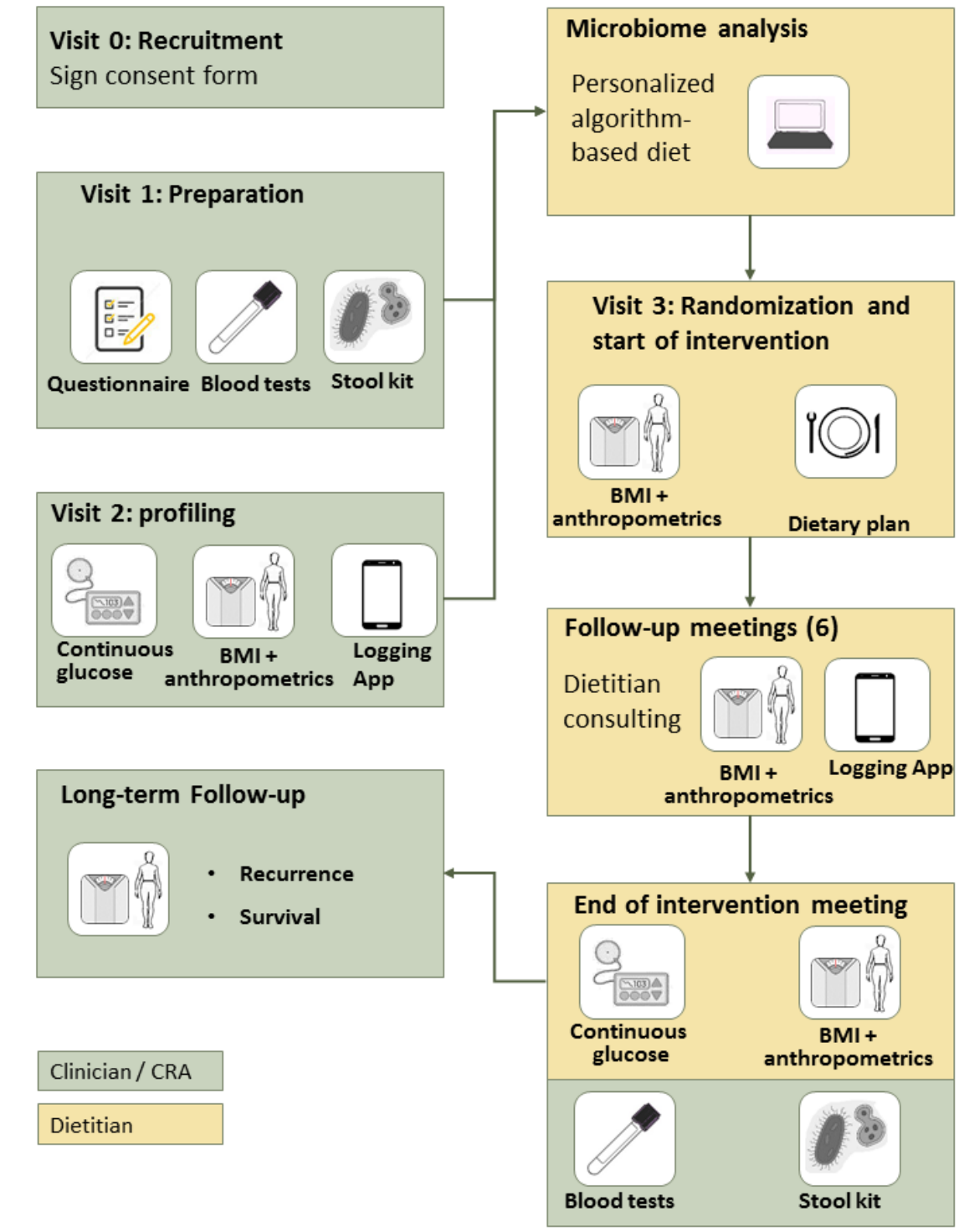

Figure 2: Study scheme

Patient Recruitment

Breast cancer patients will be recruited to the study through their regular clinic visit at the Breast Oncology Unit at Sheba Medical Center. Patients eligible for the study will sign an informed consent. This recruitment process will be ongoing until the designated number of study patients is reached.

Screening and profiling stage (-3 Months to Day -1_)

During this stage consenting patients will:

1. Meet a study coordinator and complete questionnaires regarding their medical background, nutritional habits and lifestyle activities (filled online using the REDcap software, using a dedicated tablet computer). Patients will provide blood samples for CBC, blood chemistry including liver function, lipid profiling, HBA1C, TSH, CRP, LH FSH and future exploratory analyses, and urine samples for estradiol derivatives. All patients will receive a code from the software and their data will be anonymized.

2. Patients will be asked to log a three-day food diary using a designated mobile phone application.

3. Patients will receive a designated stool kit to collect the stool sample at home. In the next meeting (profiling stage) patients will return the stool sample which will be used for microbiota profiling.

4. Meet with a certified dietitian to build a menu for the "profiling period" based on the three-day food diary of dietary habits provided by the patient. This meeting will include anthropometrics measurements (weight, height, waist and hip circumference) and connection to the glucose measurement device (Abbott Freestyle Libre). The CGM kit includes a sensor affixed to the back of the arm, which continuously monitors glucose levels in the interstitial fluid, translates and records blood glucose levels. Patients will be connected to a CGM for two weeks and will be asked to follow their diet plan as given to them by the dietitian, according to their regular habits and lifestyle. During the two weeks of connection patients will be instructed to use a dedicated application, in which they will

log in real-time their food diary, exercise, sleep, wake up, special events. Patients will return the CGM kit via a courier service from the patients' home upon measurement completion. The stool sample will be processed for microbiome profiling. The resulting data and data provided by the CGM kit will be analyzed to provide a personal profile for each patient.

Randomization and Intervention

Following the profiling stage: patients will be randomly assigned to one of two arms of the study. Approximately 100 subjects will be randomized to each arm. Patients will be blinded to the arm to which they were assigned .Randomization will be done by a computer program, taking into account the following stratification factors:

1. Menopausal status at study entry
2. Previous chemotherapy
3. Endocrine treatment type (Tamoxifen/Aromatase inhibitor)
4. Breast Cancer stage
5. BMI above/below 25

The intervention arm will be an 'algorithm-based' arm in which patients will receive personally tailored dietary recommendations. The prediction algorithm is based on gradient boosting regression model and is capable of accurately predicting personalized postprandial glycemic responses to arbitrary meals based on microbiome, CGM data from the profiling period (two weeks of CGM connection data) and other clinical data such as blood tests and lifestyle features. This model predicts PPGRs using the sum of thousands of different decision trees. Trees are inferred sequentially, with each tree trained on the residual of all previous trees and making a small contribution to the overall prediction. The features within each tree are selected by an inference procedure from a pool of 187 features representing meal content (e.g., energy, macronutrients, micronutrients); daily activity (e.g., meals, exercises, sleep times); blood parameters (e.g., HbA1c%, HDL cholesterol); CGM-derived features; questionnaires; and microbiome features (metagenomic relative abundances and KEGG pathways)[17]. The algorithm was developed using a standard leave-one-out scheme to rank every meal of each participant in the profiling period (i.e.,

the PPGR to each predicted meal will be hidden from the predictor). The model was validated in an independently collected 100-person cohort . [17]

The control arm will receive nutritional recommendations according to the standard Israeli dietary approach Mediterranean-style low-fat diet. In order to provide patients with diets that support their energetic needs and meet the recommendations for weight loss in people with overweight or obesity, the daily caloric target for each patient (in both arms) will be calculated as average between:

1. Estimated Energy Requirements (EER) calculated with the use of Mifflin equation for Resting Energy Expenditure (REE) [19]. The result from this equation will be divided by 0.7 (as REE represent ~70% of total energy expenditure).
2. Average daily caloric intake obtained from patient's log in the app during the profiling stage. For overweight patients (BMI>25) a total of 500Kcal will be reduced from their reported caloric intake to allow weight loss as accepted according to the American Association of Clinical Endocrinologists guidelines.

The diet recommendations for both arms will be provided and explained by a certified dietitian which will meet the patients from both arms at Day 0 and monthly thereafter for a total of 6 scheduled monthly visits. Weight and other anthropometric measurements (height, waist and hip circumference) will be taken at this and all following meetings with the dietitian. Participants will be asked to document their food intake and daily activities including exercise and sleep using a dedicated smartphone app throughout the intervention period.

Intervention Meetings:

Patients from both arms will be invited during the intervention period to monthly follow-up meetings with a certified dietitian (total of 6 meetings). Meetings will include evaluation of patients' compliance to the dietary recommendations they received and additional advice will be provided if needed. During the follow up meetings anthropometric measurements will be taken (height, weight, hip and waist circumference). We will also follow up on patients via phone, email, text massage, in order to increase compliance. During the monthly meeting at the beginning of Month 4 of the intervention period patients will be

offered to be reconnected to CGM for 2 weeks (optional). Data from CGM connections will be analyzed at the end of the intervention

End of intervention

At the end of the 6 months intervention period, patients will be invited to a meeting in which they will undergo anthropometrics measurement, urine, blood samples and stool sample. Additionally, patients will be connected to CGM for 2 weeks (mandatory).

Patients will be followed up at 12 months following the start of the intervention. They will attend a follow up meeting with the study coordinator in which BMI, anthropometrics, and a 3 day food diary will be recorded.

Long term follow up

Long-term clinical follow-up will be collected from the electronic medical records for recurrence and survival calculation purposes for a period of up to 5 years post recruitment.

5. Data Acquisition, Storage and Analysis

All samples will be stored at the Breast Cancer translational Research laboratory at Sheba Medical Center. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored encoded with no identifying information. Identifying details and codes will be kept in a file stored at Sheba medical center. Encoded stool samples will be transferred to the Segal laboratory at the Weizmann Institute of science. There, samples will be processed for bacterial DNA processing. All clinical data will be coded. Data will be transferred using the REDcap server and stored on Weizmann servers behind a protected firewall and be accessible only to the study team

Future research:

Samples will be stored for up to 10 years. All future use of stool and blood samples will be subject to IRB approval.

6. Safety Endpoints

No safety endpoints planned for this study

7. Statistical Considerations

Sample size determination. To estimate the required sample size, we performed power analysis while estimating effect size using the results of different studies describing controlled diet intervention aimed at weight control. Our goal is to detect a difference of at least 2 kg in net weight loss/gain (kg) between control group and experimental group. Based on the study of Shai et al. [20], the standard deviation of weight loss is 4.2 and the projected sample size with alpha of 0.05 and power of 0.8, with an estimated dropout rate of 10%, is 107 people for each arm totaling 214 individuals.

Primary, secondary and exploratory endpoint analysis (brief summary). All statistical analyses will be performed using Python 2.7. Continuous variables will be presented as mean±SD and dichotomous/categorical variables as proportions. The normality of the distribution of continuous variables will be tested by the Kolmogorov-Smirnov test. If normality will be rejected, non-parametric tests will be used. To test the association between continuous variables with normal distribution, the Pearson correlation coefficient will be performed and to test associations between continuous variables which do not distribute normally or for ordinal variable, the spearman correlation coefficient will be used. To compare parameters for continuous variables in 2 time points the paired-samples t-test will be performed (or Wilcoxson test for non-normally distribute variables), in dichotomous/categorical variables the McNemar test will be performed. To compare continuous variables in a number of time points ANOVA with repeated measures will be used. For comparison of dichotomous or categorical variables in number of time points the Cochran's Q test will be performed. P values < 0.05 will be considered significant.

8. Possible Benefits

Patients will receive counseling and close monitoring by a certified dietitian throughout the study, regardless of the research arm to which they were assigned

Patients will have the opportunity to evaluate their blood glucose levels in response to food that they tend to eat, exercise, etc., throughout the CGM connection.

Patients will receive an analysis of their glucose response to the foods that they ate.

Patients will have access to different nutritional tools that will be available to them on a secure website or on their mobile phone (App).

At the end of the study all patients will be given access to their personal tailored dietary recommendations, built for them by the study team based on their personal data, regardless of the arm they were assigned to during the study.

9. Possible Risks and Analysis of Risk/Benefit Ratio

When blood tests are taken, there is no risk except a slightly discomfort associated with the prick, hematoma or local infection it the prick area.

In order to monitor glucose levels patients will be connected to a continuous glucose monitor (CGM). The CGM includes a sensor that will be inserted using a small needle into the body. There is ultra-low risk of inserting the sensor including mild discomfort associated with inserting the sensor, a local infection in the prick area, a mild redness at the patch area. We consider this risk to be quite low. Continuous Glucose Monitoring may reveal a previously undiagnosed diabetes. These patients will be excluded from the trial and the patient and treating oncologist will be notified to provide appropriate therapy and inform the patient's general physician.

Caloric restriction will be provided only to patients who are overweight or obese (BMI>25) and not to patients with BMI at the normal range (18.5-25). Patients with BMI lower than the normal range will be excluded from the study.

10. Risk Management Procedures

Confidentiality

Patients will be identified by a numerical study ID. Only the designated research staff at Sheba Medical center will have access to the patient's fully identified medical information. The information that matches the code to the identifying information will be kept in a safeguarded database that is password protected.

11. Subject Payment/Costs

Subjects will not be directly remunerated for participation in the study. There is no cost to the subject for study participation.

12. End of Study definition

It is estimated that accrual will be completed in approximately 24 months
Time from initiation of intervention to last post intervention meeting – 12 months.
End of the study is the date of the last visit for the last patient which will be approximately 36 months from first patient intervention

Clinical endpoints will be collected up to 5 years after end of intervention.

13. Consent Procedures

Study purpose, methods, materials, risks, benefits, and alternatives will be provided in a detailed description in the consent form and will be discussed with the patient by the investigator or authorized designee. Patients will be told they are free to refuse to participate and may withdraw their consent at any time for any reason. The consent forms will be signed and dated by the patient before his or her participation in the study. The informed consent forms and process shall be documented in the patients' clinical records. A copy of the signed consent form will be provided to the patient.

14. Privacy

If patients wish to review or discuss their results this information will be discussed in private consultation with the study team medical personnel.

15. Data Security

The collection and processing of personal data from subjects enrolled in this study will be limited to the data needed to investigate this study's hypothesis. Access to identifiable data will be limited to Sheba Medical Center designated personnel; patient level de-identified data will be available only to investigators authorized by the Principal Investigator.

Data files are stored on a password-protected computer/database and will be accessible only to the designated investigators and research staff. Only the research staff will have the link that can match the code to traditional identifying information. The data sets used

for analysis will be coded and not contain any traditionally used identifying information that could be used to identify the patient.

16. Study/Intervention Discontinuation

Patients will be discontinued from study intervention in the following circumstances:

1. The patient is enrolled in any other clinical trial involving any investigational product or any other type of medical research judged not to be scientifically or medically compatible with this study.
2. Investigator decision: the investigator decides that the patient should be discontinued from the study or study intervention if the patient, for any reason, requires treatment with a therapeutic agent that effects study indication/intervention or for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP).
3. Patient decision: If the patient requests to be withdrawn from study intervention but agrees to stay in the study she will be evaluable for the endpoint if she attended at least one follow-up meeting post randomization. If the patient wishes to withdraw participation in the study she can do so at any time and in such a case data and samples will be destroyed
4. Disease recurrence.
5. Discontinuation of Inadvertently Enrolled Patients: If the investigator identifies a patient who did not meet enrollment criteria and was inadvertently enrolled, a decision on whether or not the patient may remain on intervention will be made and documented. Patients will be evaluable for the primary endpoint if they were randomized and attended at least one follow up meeting post start of intervention.

17. Monitoring

Sheba Medical Center will monitor the study. Source documents will be reviewed to ensure all subjects have properly signed and dated the informed consent forms. All information will be reviewed to ensure eligibility criteria as per the protocol, and supporting source data will be verified.

18. Record Retention

Research records with patient identification will be kept for 10 years after study completion. The collected data and related de-identified health information may be kept indefinitely. Record retention will comply with the specific requirements of the Sheba Medical Center IRB. No personal health information will be retained.

19. Publication

The results of this research will be presented at meetings or in publication. However, the subject's identity will not be disclosed in those presentations.

20. Facilities and Personnel

All study activities will occur within the patient's home and breast cancer institute clinic at Sheba Medical Center. All communications with patients will be through the Sheba Medical Center.

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Appendix 1: **Samples Collection, Storage and Analysis**

Blood: during the study we will collect blood samples 2 times : At the initial screening, and at the end of the intervention,at time points 0 and 6m. Two blood samples will be taken- one sample for immediate analysis and a second sample (blood and plasma) would be stored in deep freeze (-80°C) for future metabolomics testing (blood tests are detailed in schedule of activities table, see Appendix 2).

Stool: Patients will be asked to provide several stool sample at 2 time points throughout the study. Stool samples are required for the study, and will be collected at baseline and at the end of intervention. Stool samples will be stored at Sheba Medical center and transfered to Segal lab at the Weizmann Institute. The samples will be stored encoded with no identifying information. The samples will be stored at -80C, bacterial DNA samples will be stored at -20C. The samples will be stored for 10 years. Identifying details and codes will be kept by the principal investigator and designated personnel. All future use of stool samples will be subject to Helsinki approval.

Urine: urine samples will be taken from every patient at 2 time points, including at the beginning and at the end of the intervention, in order to characterize estradiol derivatives.

Appendix 2: Schedule of activities

Trial period	Screening	Profiling	Intervention						End of treatment	Followup	
Scheduling window	week -8 to -4	week -4 to -3	Day0 (M1)	M2	M3	M4	M5	M6	M7	M12	Every 6 months (5years)
Signed ICF	X										
Review of eligibility	X										
Medical History	X										
Nutrition/Lifestyle/Medical Questennaire	X								X		
Weight		X	X	X	X	X	X	X	X	X	
Height		X	X	X	X	X	X	X	X	X	
Other Anothropometrics		X	X	X	X	X	X	X	X	X	
Provision of stool kit	X										
CBC		X							X		
Blood Chemistry including total serum protein and albumin and fasting glucose		X							X		
Lipid profiling		X							X		
Liver profiling (GGT, Bilirubin, Alkaline Phosphatase, AST,ALT)		X							X		
TSH		X							X		
CRP		X							X		
HbA1C		X							X		
Hormonal Profiling (LH, FSH)		X							X		
Urinalysis (estradiol derivatives)		X							X		
Whole blood sample for expolratory analysis		X							X		
Dietitian Consult		X	X	X	X	X	X	X	X	X	
Profiling stage menu		X									
Food and Activity Diary log in	X	X	X	X	X	X	X	X	X	X	
Continuous Glucose Monitoring Connection (2 weeks)		X				X (optional)			X		
Survival And Breast Cancer recurrence follow up										X	X (from EMR)
Hormonal Treatment Adherence Follow up	X								X	X	X (from EMR)
Diet Adherence follow up (weekly phone, text or email followup)			X	X	X	X	X	X	X		

Supplementry Table1: Features for Prediction
List of features - PPGR predictions
Blood tests
HbA1C%
Hemoglobin
FastingGlucose
Anthropometric (measured at profiling)
BMI
BodyFat
Waist
Weight
Hips
Dietary components of the meal
Alanine_g
Alcohol_g
Arginine_g
Caffeine_mg
Calcium_mg
Carbohydrate_g
Cholesterol_mg
Energy_kcal
Fructose_g
Galactose_g
Glucose_g
Isoleucine_g
Lactose_g
Leucine_g
Magnesium_mg
Maltose_g
Niacin_mg
Phenylalanine_g
Protein_g
Sodium_mg
Starch_g
Sucrose_g
SugarsTotal_g
Thiamin_mg
TotalDietaryFiber_g
TotalLipid_g
TotalMonounsaturatedFattyAcids_g
TotalPolyunsaturatedFattyAcids_g
TotalSaturatedFattyAcids_g
TotalTransFattyAcids_g
VitaminC_mg
VitaminD_IU
VitaminE_mg
Water_g
Zinc_mg
Health questionnaire
Age

Currently_smokes
Evening_Hunger
Ever_smoked
Gender
General_Hunger
Is_pregnant
Midday_Hunger
Morning_Hunger
Physical_activity_-_freq
Physical_activity_-_mins
Regular_defecation
Sleep_quality
Stress
Work_activity
Microbiome features
s_Acidaminococcus_unclassified
s_Adlercreutzia_equolifaciens
s_Akkermansia_muciniphila
s_Alistipes_finegoldii
s_Alistipes_indistinctus
s_Alistipes_onderdonkii
s_Alistipes_putredinis
s_Alistipes_senegalensis
s_Alistipes_shahii
s_Anaerostipes_hadrus
s_Anaerotruncus_unclassified
s_Bacteroidales_bacterium_ph8
s_Bacteroides_caccae
s_Bacteroides_cellulosilyticus
s_Bacteroides_clarus
s_Bacteroides_dorei
s_Bacteroides_eggerthii
s_Bacteroides_faecis
s_Bacteroides_finegoldii
s_Bacteroides_fragilis
s_Bacteroides_intestinalis
s_Bacteroides_massiliensis
s_Bacteroides_nordii
s_Bacteroides_ovatus
s_Bacteroides_plebeius
s_Bacteroides_salyersiae
s_Bacteroides_stercoris
s_Bacteroides_thetaiotaomicron
s_Bacteroides_uniformis
s_Bacteroides_vulgatus
s_Bacteroides_xylanisolvens
s_Barnesiella_intestinihominis
s_Bifidobacterium_adolescentis
s_Bifidobacterium_animalis
s_Bifidobacterium_bifidum

s_Bifidobacterium_catenulatum
s_Bifidobacterium_longum
s_Bifidobacterium_pseudocatenulatum
s_Bilophila_unclassified
s_Bilophila_wadsworthia
s_Burkholderiales_bacterium_1_1_47
s_Catenibacterium_mitsuokai
s_Clostridium_bartlettii
s_Clostridium_bolteae
s_Clostridium_leptum
s_Collinsella_aerofaciens
s_Coprobacter_fastidiosus
s_Coprococcus_catus
s_Coprococcus_comes
s_Coprococcus_sp_ART55_1
s_Desulfovibrio_desulfuricans
s_Desulfovibrio_piger
s_Dorea_formicigenerans
s_Dorea_longicatena
s_Eggerthella_unclassified
s_Erysipelotrichaceae_bacterium_6_1_45
s_Escherichia_coli
s_Escherichia_unclassified
s_Eubacterium_biforme
s_Eubacterium_eligens
s_Eubacterium_hallii
s_Eubacterium_ramulus
s_Eubacterium_rectale
s_Eubacterium_siraeum
s_Eubacterium_ventriosum
s_Faecalibacterium_prausnitzii
s_Flavonifractor_plautii
s_Gordonibacter_pamelaeae
s_Haemophilus_parainfluenzae
s_Holdemania_unclassified
s_Lachnospiraceae_bacterium_1_1_57FAA
s_Lachnospiraceae_bacterium_2_1_58FAA
s_Lachnospiraceae_bacterium_3_1_46FAA
s_Lachnospiraceae_bacterium_5_1_63FAA
s_Lachnospiraceae_bacterium_7_1_58FAA
s_Lachnospiraceae_bacterium_8_1_57FAA
s_Lactobacillus_ruminis
s_Lactococcus_lactis
s_Megamonas_unclassified
s_Methanobrevibacter_smithii
s_Odoribacter_splanchnicus
s_Oscillibacter_unclassified
s_Oxalobacter_formigenes
s_Parabacteroides_distasonis
s_Parabacteroides_goldsteinii

s_Parabacteroides_johnsonii
s_Parabacteroides_merdae
s_Parabacteroides_unclassified
s_Paraprevotella_clara
s_Paraprevotella_unclassified
s_Paraprevotella_xylaniphila
s_Parasutterella_excrementihominis
s_Peptostreptococcaceae_noname_unclassified
s_Phascolarctobacterium_succinatutens
s_Prevotella_copri
s_Roseburia_hominis
s_Roseburia_intestinalis
s_Roseburia_inulinivorans
s_Roseburia_unclassified
s_Ruminococcus_albus
s_Ruminococcus_bromii
s_Ruminococcus_callidus
s_Ruminococcus_gnavus
s_Ruminococcus_lactaris
s_Ruminococcus_obeum
s_Ruminococcus_sp_5_1_39BFAA
s_Ruminococcus_torques
s_Streptococcus_parasanguinis
s_Streptococcus_salivarius
s_Streptococcus_thermophilus
s_Subdoligranulum_unclassified
s_Sutterella_wadsworthensis
s_Veillonella_parvula