# Clinical Study Protocol

## Metabolic Parameters and Quality of Life in Women on Neo-/Adjuvant Therapy for Breast Cancer: a Randomized Controlled Trial on the Effectiveness of an Intensive Dietary Intervention

## Promoter

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# 1. STUDY BACKGROUND AND RATIONALE

The correlation between breast cancer (BC) and obesity is an intriguing research topic since 1980s. As widely described in the literature, (3-10, 12, 13, 15, 17, 18) women on adjuvant therapy for BC, are prone to weight gain. On the other hand, weight gain and obesity seem to negatively influence BC outcome (1, 2, 9, 11, 12, 14, 16); increased body weight during/after chemo-therapyfor BC seems to be related to a higher risk of disease relapse and a lower disease free survival (DFS) and overall survival (OS).

BC adjuvant therapy is also associated with modifications of the patient's metabolic profile, and in particular with dyslipidaemia (reduction in the HDL cholesterol serum concentration and increase in total/LDL cholesterol, and triglycerides) (21). High plasma cholesterol levels have been suggested to enhance the risk of BC because of its role as a precursor of steroid hormones. Moreover, in post-menopause, the oestrogens produced from androgens through the activity of aromatase in the fat tissue, are a potent stimulus for mammary carcinogenesis.

# 2. OBJECTIVES AND ENDPOINTS

The present trial aims to compare the effectiveness of an "intensive" vs a "standard" nutritional intervention on body weight regulation and some metabolic parameters in women on adjuvant therapy for BC.

## 2.1 PRIMARY ENDPOINT

- To investigate the effects of an intensive nutritional intervention on body weight control (defined as weight changes ≤+5% of the initial body weight) at 12 months after the beginning of systemic treatment

## 2.2 SECONDARY ENDPOINTS

- To evaluate the effects of an intensive nutritional intervention at 6, 12 and 24 months after the beginning of systemic treatment on:

    - Body weight and Body Mass Index (BMI)
    - Body composition and Resting Energy Expenditure (REE)

- Serum metabolic variables
- Quality of life (QoL)

- To evaluate the effects of an intensive nutritional intervention on patient outcome and tolerance of systemic treatment:

  - Chemo-therapy side effects
  - Disease free survival (DFS)
  - Event free survival (EFS)
  - Overall survival (OS)

## 3. STUDY DESIGN

The effectiveness of an "intensive" versus "non-intensive" dietary intervention will be assessed in 120 patients on adjuvant therapy for BC in a prospective, randomized controlled study for a total of 3 years. The eligibility of participants will be verified according to the inclusion/exclusion criteria at the screening visit; therefore, after a detailed explanation of the study protocol, Informed Consent will be signed. After the recruitment, subjects will be randomized into an "intensive" (**ARM A**) and "non-intensive" (**ARM B**) dietary intervention group. After the 6 month treatment period, patients will be followed up for the following 18 months.

**ARM A**: Visits will be set up monthly in order to assess body weight, waist circumference, diet compliance (etc...) for all period of the BC adjuvant treatment (6 months).

**ARM B:** Visits will be set up only at the baseline, after 6 months namely at the end .

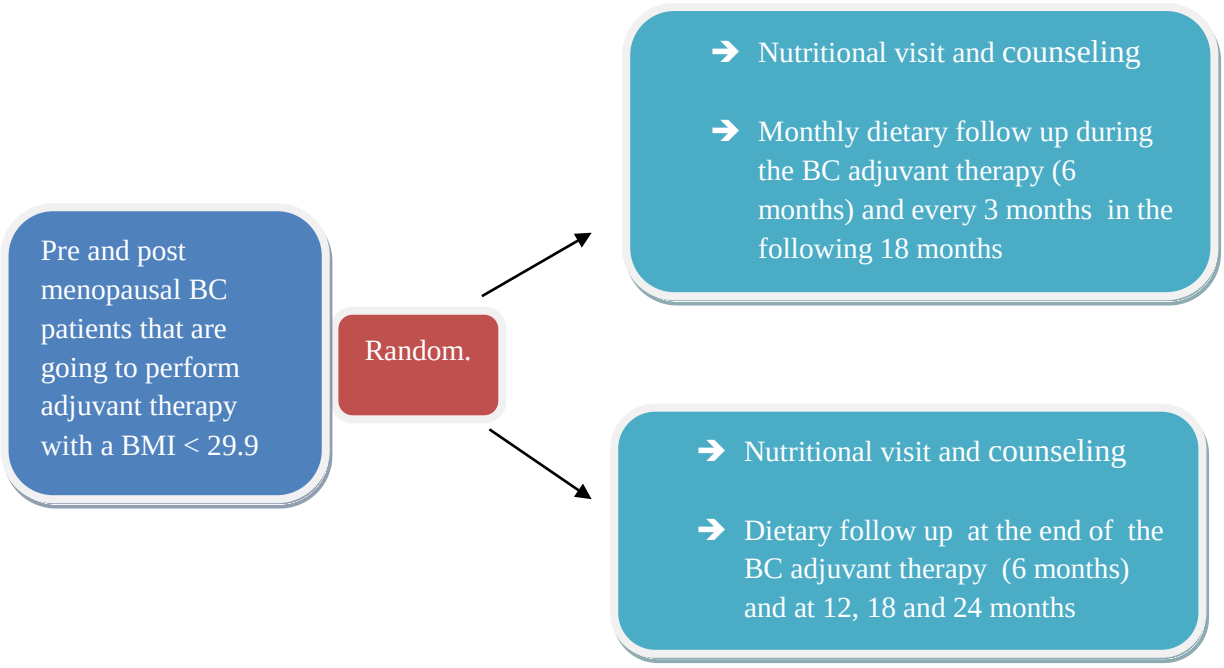


**Figure 1. Study Design**

This study will be carried out at the Department of Clinical Medicine and Surgery of Federico II University in Naples

On the day first, patients in a fasting condition will reach the Department at 8.30 a.m. by calm mode of transportation. On the day before, they will be asked to follow a standardized fasting procedure, i.e. abstention from alcohol and hard physical activity. After their arrival, blood samples will be taken and then anthropometrics variables, REE, body composition, handgrip and breast size will be measured. At the end of all measurements, they will receive a dietary prescription and a specific visit schedule, according to their randomization.

## 5. SELECTION OF PATIENTS

This study will be carried out in pre- and post-menopausal women with operable stage I-III breast cancer, candidate to receive adjuvant/neoadjuvant therapy for BC at the Department of Clinical Medicine and Surgery of Federico II University in Naples. Patients will be randomized to the treatment with a ratio 1:1, according to an assignment list previously generated by a statistician.

### 5.1 INCLUSION CRITERIA

- Women ≥18 years old
- BMI < 30 kg/m$^2$
- Caucasians

- Histological diagnosis of invasive breast cancer
- Starting an adjuvant systemic treatment for breast cancer
- Available past medical history
- Informed consent to participate to the study

## 5.2 EXCLUSION CRITERIA

- Metastatic disease
- BMI >30 $kg/m^2$
- Presence of a serious medical condition that could alter food absorption or prognosis
- Refusal to provide informed consent to participate in the study

## 6. MEASUREMENT

### 6.1 Tumor characteristics

Histological type, tumour dimension (T), nodal status (N), grading (G), Ki67, estrogen receptor status (ER), progesterone receptor status (PgR), c-erbB2 (HER2) evaluation will be collected at study entry at basal visit.

### 6.2 Clinical history

Past history including weight history, co-morbidities and current medications. Information about breast size will be also collected. All these informations will be collected at basal visit.

### 6.3 Blood sample

Venous blood sampling for routine biochemistry (fasting glucose, serum insulin concentration, C-peptide, glycosylated haemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, urea, creatinine, urate, AST, ALT, GGT, ALP, VES, PCR, TSH, FT3, FT4, vitamin D) will be collected at patients enrolment and thereafter every 6 months.

### 6.4 Body weight, height and BMI

Body weight will be measured at each visit. The participants will be weighed dressed in light clothing or underwear after emptying the bladder to the nearest 0.05 kg and height using a wall-mounted stadiometer to the nearest 0.5 cm, then noted in the source data (Seca 709; Seca, Hamburg, Germany). The participants' height should be measured without shoes and only at baseline. BMI was calculated as weight (kg) divided by squared of height (m).

### 6.5 Waist and hip circumference, waist-to-hip ratio

Waist and hip circumferences will be measured according to the established methods. Waist circumference will be taken at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest; while hip measurement will be taken around the widest portion of the buttocks, using a plastic metric tape.

The circumference will be measured on subjects without heavy outer garments with all tight clothing, including the belt, loosened and with pockets emptied. Subjects should stand with their feet close together (no more than 15 cm apart) with their weight equally distributed on each leg. Subjects should be asked to breathe normally and at the time of the reading of the measurements asked to breath out gently. This will prevent subjects from contracting their muscles or from holding their breath.

### 6.6 Handgrip strength

Functional assessment of muscle status will be performed by handgrip strength using a dynamometer. The participant will be in a standing position, arms at their side, not touching their body, keeping the elbow bent slightly. Then, it will be administer the test on the non-dominant hand and will be asked the participant to squeeze the dynamometer with as much strength as possible, being careful to squeeze only once for each measurement. Three measurements will be made with a pause of about 10-20 seconds between each repetition to avoid the effects of muscle fatigue.

### 6.7 Body composition

Body composition will be assessed with Bio-impedance analysis (BIA) at 50 kHz (Human Im Plus II, DS Medica) at room temperature of 22-25 °C. Measurements will be carried out on the non-

dominant side of the body in the post-absorptive state, after being in the supine position for 20 minutes, subjects will void prior to measurements. The measured BIA variables are resistance (R) and reactance (Xc); Fat Free Mass (FFM) and Fat Mass (FM) will be estimated using the prediction equations developed by Kushner and phase angle (PA).

### 6.8 **Resting Energy Expenditure**

Resting Energy Expenditure will be measured (MREE) by indirect calorimetry using a canopy system (V max29, Sensor Medics, Anaheim, U.S.A.) at an ambient temperature of 23-25 °C. The instrument is checked by burning ethanol while oxygen and carbon dioxide analyzers are calibrated using nitrogen and standardized gases (mixtures of nitrogen, carbon dioxide and oxygen). Subjects will be in the post-absorptive condition (12-14 h fasting), lying down on the bed, in a quiet environment. Females of child bearing age are evaluated within 5-10 days from the onset of menstrual cycle. After a 15 minutes adaptation period, oxygen consumption and carbon dioxide production will be determined for 45 minutes. The inter-day coefficient of variation (as determined in six individuals on subsequent days) was less than 3%. Energy expenditure is then calculated with the abbreviated Weir's formula, neglecting protein oxidation.

### 6.9 Food records

At baseline, a Food Frequency Questionnaire (FFQ) will assess the habitual food intake of participants enrolled in the study; while, the compliance to the prescribed diet, according to the study design, will be assessed by 24h recall that will record all meals consumed during 24 h prior to the visit.

In addition, a face-to-face interview with the participants will be performed at baseline and at the end of the study by dietitians to assess the adherence to the Mediterranean diet using the validated 14-item Questionnaire of Mediterranean diet adherence, developed by Martínez-González MA et al. 2002 (PREDIMED study) (**File 1**).

### 6.10 Physical activity

Physical activity will be assessed using the self-administered short form of the International Physical Activity Questionnaires (IPAQ) that takes into account three specific types of activity such as walking, moderate-intensity activities and vigorous intensity activities through simple questions. IPAQ is self-administered (**File 2**).

# 7. TREATMENT

## 7.1 Investigational Treatment

No drug treatment will be administered in this study

## 7.2 Dietary Treatment

### 7.2.1 Nutritional counseling

Dietary recommendations will be given by two registered dietitians and will be based on a Mediterranean diet, which includes whole cereals, legumes, seeds, blue fish, extra virgin olive oil, vegetables, and other Mediterranean seasonal food; taking into account all recommendations provided by the World Cancer Research Fund International as well **(File 3)**.

According to randomization, outpatient visits will be scheduled monthly or half-yearly.

Dietitians will explain the main goal of the dietary advices in order to balance energy intake and promote healthy lifestyle. They will provide information about the dietary changes, the correct choice of food pairings, portion control and energy density of food as well as how to manage the feeling of hunger; they will illustrate how to understand nutritional labels, select seasonal and traditional foods, increase use and variety of typical Mediterranean foods.

Dietitians will advise patients about the benefit of physical activity, the timing and type of physical exercise, the consequences of a sedentary lifestyle.

## 7.3 Concomitant Medications

Patients' current medications will be collected before study randomization at first visit. The patient will inform the investigational site about any new medications she takes after the start of the study treatment.

# 8. STUDY SCHEDULE

| | **Treatment Phase** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit name** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** | **V10** | **V11** | **V12** | **V14** |
| **Month** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **9** | **12** | **15** | **18** | **21** | **24** |
| Inclusion/Exclusion Criteria | X | | | | | | | | | | | | |
| Obtain Informed Consent | X | | | | | | | | | | | | |
| Randomization | X | | | | | | | | | | | | |
| Patient cancer history, co-morbidities and current medications | X | | | | | | | | | | | | |
| Tumor histology, disease staging | X | | | | | | | | | | | | |
| Age, menopausal status | X | | | | | | | | | | | | |
| Weight, height, BMI, WC, HC, WHR, handgrip strength | X | X* | X* | X* | X* | X* | X | | X | | | | X |
| Laboratory assessment with venous blood collection | X | | | | | | X | | X | | | | X |
| BIA and Indirect calorimetry | X | | | | | | X | | X | | | | X |
| Dietary questionnaire | X | | | X | | | X | | X | | | | X |
| Quality of life assessment | X | | | X | | | X | | X | | | | X |
| | **Nutritional visit:** | | | | | | | | | | | | |
| Interventional group | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Control group | X | | | | | | X | | X | | X | | X |

X *: only body weight will be assessed monthly during neo-/adjuvant treatment

### 8.1 Study Flow and Visit Schedule

All of the assessments and indicates with an “X”, the visits when they are performed. All data obtained from these assessments must be supported in the patient’s source documentation. Tests, procedures and visits should occur on schedule whenever possible, the +/- 14 days window is allowed.

## 9. DATA COLLECTION AND MANAGEMENT

### 9.1 Data Confidentiality

Information about study subjects will be kept confidential and managed under the applicable laws and regulations. The data collection system for this study uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorized access to confidential participant information.

## 10. STATISTICAL METHODS AND DATA ANALYSIS

### 10.1 STUDY SIZE DEFINITION

Assuming a percentage of women who experienced a weight changes >+5% of the initial body weight after twelve months of adjuvant therapy for BC, equal to 30% and considering as clinically relevant that such percentage become equal to 10% or less, a sample size of 60 women for each treatment arm will allow to detect such difference, if truly exists, with a power of 80% and a two sided significance level equal to 5%.

### 10.2 STATISTICAL ANALYSIS

In all analyses, a 2-sided significance level of 5% (p-value < 0.05) will be used to determine if the difference between the two treatment groups is statistically significant. No formal test of normality will be carried on, but all the statistical assumption will be checked by visual inspection of the distribution of the outcome variables. All statistical analyses will be performed using the Statistical Platform R (vers. 3.2.3 or next).

Demographic and clinical data measured at baseline visit will be analysed using standard descriptive statistics and compared between group (without reporting statistical significance) in order to assess whether good balance of baseline characteristics was achieved by randomization. Frequencies and Percentages of missing data will be also reported.
Treatment success, defined as weight changes ≤+5% of the initial body weight at 12 months after the beginning of systemic treatment, will be evaluated by computing the crude odds ratio, with the corresponding 95% Confidence Interval, with respect the treatment group. A logistic regression model will be also developed in order to adjust the odds ratio for the baseline weight of patients. Longitudinal trajectories of body weight will be analysed using a linear mixed model (LMM) approach with random term for both intercept and slope. The fixed effects of the model will include the group factor, the time coded continuously (defined as months from baseline) and the interaction term group x time. Time of the baseline observation of the outcome variable will be coded as 0. All the other comparisons will be based on standard statistical method for comparing numerical and categorical factors between two groups.

### 10.3 RANDOMIZATION

Allocation of women to the intervention or control arm will be based on a block randomization schema with constant block size of 6. Each block will thus be composed of three codes for the treatment arm and three codes for control arm.

## 11.ADMINISTRATIVE FEATURES

This is a randomized clinical trial promoted by the Dipartimento di Medicina Clinica, UOC di Oncologia Clinica, Università Federico II di Napoli, Italy, which is the not-profit Sponsor.
This clinical study was designed, shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC), and with the ethical principles laid down in the Declaration of Helsinki.

Data deriving from this clinical trial are intended only for scientific and educational purposes, which include presentation and scientific meetings, congresses and symposia and/or publication in scientific journals. These data are the property of the Dipartimento di Medicina Clinica, UOC di

Oncologia Clinica, Università Federico II di Napoli, Italy, which share it with all participating researchers.

## 12. REFERENCES


1. Tao MH, Shu XO, Ruan ZX, Gao YT, Zheng W. Association of overweight with breast cancer survival. Am J Epidemiol. 2006; 163(2):101–7.

2. Whiteman MK, et al. Body mass and mortality after breast cancer diagnosis. Cancer Epidemiol Biomarkers Prev. 2005;14(8): 2009–14

3. Demark-Wahnefried W, Rimer BK, Winer EP 1997 Weight gain in women diagnosed with breast cancer. J Am Diet Assoc 97:519 –526, quiz 527–528, 529

4. Huntington MO 1985 Weight gain in patients receiving adjuvant chemotherapy for carcinoma of the breast. Cancer 56:472–474

5. Freedman R.J. Weight and Body Composition Changes during and after Adjuvant Chemotherapy in Women with Breast Cancer, The Journal of Clinical Endocrinology & Metabolism 89(5):2248–2253

6. Berrino F. Reducing Bioavailable Sex Hormones through a Comprehensive Change in Diet: the Diet and Androgens (DIANA) Randomized Trial, Cancer Epidemiol Biomarkers Prev2001;10:25-33.

7. Goodwin P. Adjuvant Treatment and Onset of Menopause Predict WeightGain After Breast Cancer Diagnosis, J ClinOncol 17:120-129. r 1999

8. Demark-Wahnefried W. Changes in Weight, Body Composition, and Factors Influencing Energy Balance Among Premenopausal BreastCancer Patients Receiving Adjuvant Chemotherapy, J ClinOncol 19:2381-2389. 2001

9. Gadea E. Importance of metabolic changes induced bychemotherapy on prognosis of early-stage breast cancer patients: a review of potential mechanisms, obesity reviews (2012) 13, 368–380

10. Heideman W.H. The frequency, magnitude and timing of post-diagnosis bodyweight gain in Dutch breast cancer survivors, European Journal of Cancer 45 (2009) 119- 126

11. Ballard-Barbash R. Physical Activity, Weight Control, and Breast Cancer Risk and Survival: Clinical Trial Rationale and Design Considerations, J Natl Cancer Inst 2009;101: 630 – 643

12. Kroenke H. Weight, Weight Gain, and Survival After Breast Cancer Diagnosis, J ClinOncol 23:1370-1378. 2005

13. Makari-Judson G. Longitudinal Patterns of Weight Gain after Breast Cancer Diagnosis: Observations beyond the First Year, The Breast Journal, Volume 13 Number 3, 2007 258–265

14. Nichols B. Body Mass Index Before and After Breast Cancer Diagnosis:Associations with All-Cause, Breast Cancer, and Cardiovascular Disease Mortality, Cancer Epidemiol Biomarkers Prev2009;18:1403-1409.

15. Saquib N. Weight gain and recovery of pre-cancer weight after breastcancer treatments: evidence from the women's healthy eatingand living (WHEL) study, Breast Cancer Res Treat (2007) 105:177–186

16. Thivat E. Weight change during chemotherapy changesthe prognosis in non metastatic breast cancer for the worse, BMC Cancer 2010, 10:648

17. Tredan O. Body weight change in women receiving adjuvant chemotherapyfor breast cancer: A French prospective study, Clinical Nutrition 29 (2010) 187–191

18. Vance V. Weight gain in breast cancer survivors: prevalence,pattern and health consequences, obesity reviews (2011) 12, 282–294

19. Villarini A. Preventing weight gain during adjuvant chemotherapyfor breast cancer: a dietary intervention study, Breast Cancer Res Treat (2012) 135:581–589
20. Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One. 2012;7:e43134

21. Grazia Arpino, Carmine De Angelis, Giuseppe Buono et al. Metabolic and anthropometric changes in early breast cancer patients receiving adjuvant therapy. Breast Cancer Res Treat (2015) 154: 127-132

22. Minder CM, Shaya GE, Michos ED, Keenan TE, Blumenthal RS, Nasir K, Carvalho, JA, Conceição RD, Santos RD, Blaha MJ. Relation between self-reported physical activity level, fitness, and cardiometabolic risk. Am J Cardiol. 2014 Feb 15;113(4):637-43.