Randomized trial of diet, physical activity and breast cancer recurrences: the DIANA-5 study.
DIANA (DIet and ANdrogens)-5: randomized controlled trial to test the efficacy of dietary change and physical activity to prevent or delay the development of recurrences in breast cancer (BC) patients estimated to be at high risk based on their hormonal or metabolic milieu

NCT

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Background

Prospective epidemiological studies consistently showed that high serum levels of testosterone predict the subsequent occurrence of BC, both before and after menopause. After menopause also high oestrogen and low sex hormone-binding globulin (SHBG) levels are associated with increased BC risk, but the association with testosterone persists upon adjustment for estrogens. Also high serum levels of insulin and insulin-like growth factor-I (IGF-I) are associated with increased BC risk. Insulin has gonadotropic properties and stimulates the synthesis of androgens in the ovary; moreover, it inhibits the liver synthesis of SHBG and of two IGF-binding proteins (IGFBP1 and 2), thus increasing the bio-availability of both sex hormones and IGF-I.

In BC patients, overweight and high serum levels of insulin and testosterone are associated with a significantly increased risk of recurrences. We also showed an increased recurrence rate in patients with metabolic syndrome, defined as the presence of three or more of the following: abdominal obesity, hypertension, hyperglycemia, high triglycerides or low HDL cholesterol values.

Our previous DIANA randomized trials showed that an insulin lowering diet, based on traditional Mediterranean ad macrobiotic recipes, significantly decreases body weight, serum testosterone, insulin, and the bioavailability of estrogens and IGF-I, in both healthy women and BC patients. A significant decrease in insulin resistance and serum testosterone also occurred in a randomized trial of moderate physical activity. Consistently, several observational studies suggested that physical activity and weight reduction help preventing both BC and BC recurrences. The WINS randomized trial of low versus high total fat consumption in BC patients showed a significant 24% reduction of recurrences over 5 years.

We now propose a randomized dietary and physical activity intervention trial to reduce BC recurrences (local, distant, or second BC) in BC patients at high risk because of high serum testosterone (>0.4 ng/ml), or insulin (>50 pmol/L), or the presence of metabolic syndrome. In these patients the estimated 5-year cumulative recurrence rate is 30%. Participants will be recruited through hospitals, patients' associations, and the media. We plan to recruit 1,200 patients and to randomize them in two groups: 600 shall receive standard recommendation for healthy lifestyle without, however, any active support; 600 shall receive a combination of individual and group contacts over the course of one year, including kitchen courses, gym and dance classes, common meals and reinforcing meetings, with emphasis on a comprehensive dietary change including low saturated fat and low refined carbohydrates, and high whole grain cereals and pulses consumption. Compliance will be monitored through weight change and plasma glucose, triglycerides, cholesterol and testosterone.

The main analysis will be by intention to treat. Under the hypothesis of reducing recurrence rate by 25% or 33% the statistical power of the study is 80% or, respectively, 90%, ($P<0.05$, 5-year follow-up).

Background

There is increasing scientific evidence that endogenous hormones and metabolic factors effect breast cancer incidence and prognosis (Figure 1).

Figure 1. Hormonal, metabolic and dietary correlates of breast cancer risk and recurrences

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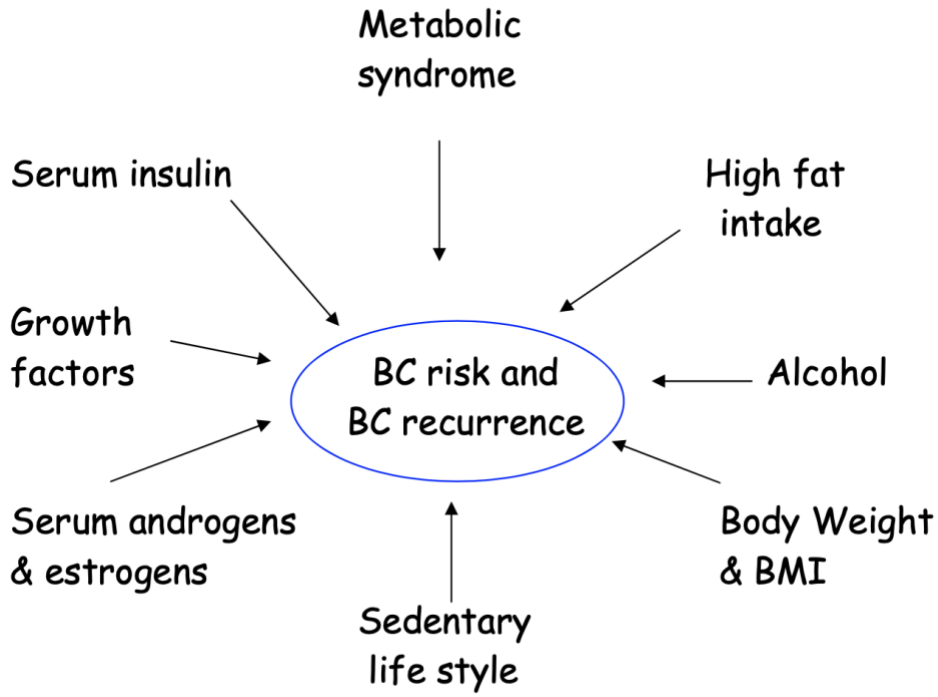


Serum testosterone levels effect BC risk

Prospective epidemiological studies consistently showed that high serum levels of testosterone predict the subsequent occurrence of BC, both before (1-3) and after menopause (4-6). The relative risk (RR) increases linearly with increasing testosterone levels, and the women in the top quartile or quintile of testosterone distribution are 2 to 4 times as likely to develop BC compared with women in the bottom quantile. After menopause also, high estrogen and low SHBG levels are associated with increased BC risk. When testosterone and estradiol are included in the same model, however, the effect of testosterone is greater than that of estradiol (4-6). Consistently with the elevation in risk with increasing endogenous testosterone level, menopausal women using estrogens and testosterone as hormonal replacement therapy (HRT) have a significantly increased BC risk compared with estrogen only therapy and greater risk also compared with estrogen and progestogens therapy (7). Moreover, HRT with synthetic testosterone-derived progestogens are associated with a significantly greater BC risk compared with those with natural (micronized) progesterone (8). The mechanism underlying the association between androgens and BC risk is not well understood. Androgens may act directly, stimulating the growth and division of BC cells via binding to the androgen receptor or the estrogen receptor (9), or indirectly, via conversion to estrogens in target tissues (10), or by increasing the bioavailability of estradiol because of the greater affinity of testosterone and dihydrotestosterone with SHBG.

Insulin and related metabolic factors effect BC risk

Overweight and sedentary lifestyle are associated with insulin resistance. Women who gain weight in adulthood and overweight postmenopausal women have a greater risk for BC than lean women (11, 12). Postmenopausal women who engage in regular exercise (> 3 hours per week) have a reduced risk for BC compared with inactive women (13, 14). Several epidemiological studies found that high serum insulin and IGF-I levels are associated with increase BC risk (15-20), with, however, some inconsistencies as for the effect modification of age and menopausal status.

Insulin and testosterone effect prognosis in BC patients

In BC patients, high serum levels of insulin (21) and testosterone (22 and Micheli et al, submitted) are significantly associated with increased risk of recurrences; after adjustment for traditional prognostic factors (cancer stage at diagnosis, ER and PR) the RRs for BMI and for insulin in the upper quartile were about 2, while the RRs for testosterone in the upper tercile ranged from 2 to 7. We also found a borderline significant association of ER+ cancer with serum levels of estradiol, which, however, disappeared upon adjustment for testosterone levels (22). Testosterone levels are higher than estradiol levels, are easier to measure reproducibly and have lower intra- individual variability (23-25). For this reason, and because it proved to be predictive at any age, we chose testosterone as the main hormonal indicator of BC risk.

We also showed an high risk of recurrence in patients with metabolic syndrome, defined as the presence of three or more of the following factors: abdominal obesity (waist circumference >88 cm), hypertension (SBP>130 and /or DBP >85), hyperglycemia (>110mg/100ml), high triglycerides (>150 mg/100ml) or low HDL cholesterol (<50 mg/100ml) (26) (Table 1). Consistently, several observational studies suggested that sedentary habits (28) and overweight (27) are associated with increased BC incidence and BC recurrences.

Table 1. Hazard ratio of developing recurrences during 5.5-year follow-up of 110 BC patients according to the presence or absence of metabolic syndrome and serum testosterone levels above or under the median value (26).

Metabolic syndrome	Testosterone >0.40 ng/ml	Recurrence Yes	Recurrence No	Hazard Ratio	95% C.I
No	No	7	46	1	
Yes	No	1	3	2.2	0.2-19.4
No	Yes	17	24	3.8	1.5-9.5
Yes	Yes	7	5	6.7	2.3-19.8

Healthy diet and physical activity decrease insulin and testosterone levels

The DIANA (DIet and ANdrogens) randomized controlled trials showed that a highly satiating and insulin lowering diet, based on traditional Mediterranean ad macrobiotic recipes, significantly decreases body weight, serum testosterone and the bioavailability of both estrogens and IGF-I, in healthy postmenopausal women (29, 30) and in BC patients (22). In these trials' testosterone decreased by 18% and, respectively, 10% (tables 2 and 3).

A randomized trial of 12-month physical activity versus stretching in healthy postmenopausal women proved that moderate intensity sports/recreational activity can reduce serum insulin (31), testosterone (32), and, to a lesser extent, estrogen levels (33). Among exercisers who lost >2% body fat, testosterone declined by 10% and free testosterone by 12% ($P < 0.01$ compared with controls).

In a randomized trial, Mediterranean-style diet with increased consumption of whole grains, vegetables, fruits, nuts, and olive oil, succeeded in significantly reducing the prevalence of metabolic syndrome and insulin resistance (34).

Table 2. DIANA-1: Hormonal changes after 5 months of insulin lowering diet in healthy postmenopausal women: % change in the intervention group and P value with respect to the change in control group (52 women each) (29,30)

	% change	P		% change	P
Testosterone	-18	**	Glucose	-6	*
Estradiol	-18	ns	Insulin	-10	ns
Free testosterone	-29	**	Insulin AUC([a])	-8	*
Free estradiol	-23	*	C-peptide	-19	*
SHBG	+25	**	IGF-I	-6	ns
Triglycerides	-1	ns	IGFBP-1	+12	**
Total cholesterol	-14	**	IGFBP-2	+30	**
BMI	-6	**	Waist circumfer.	-5	**

* P < 0.05 ** P < 0.01 ([a]) area under the curve during a glucose tolerance test

Table. 3 - DIANA-2: Hormonal changes after 12 months of insulin lowering diet in breast cancer patients (N =110) (22 and unpublished)

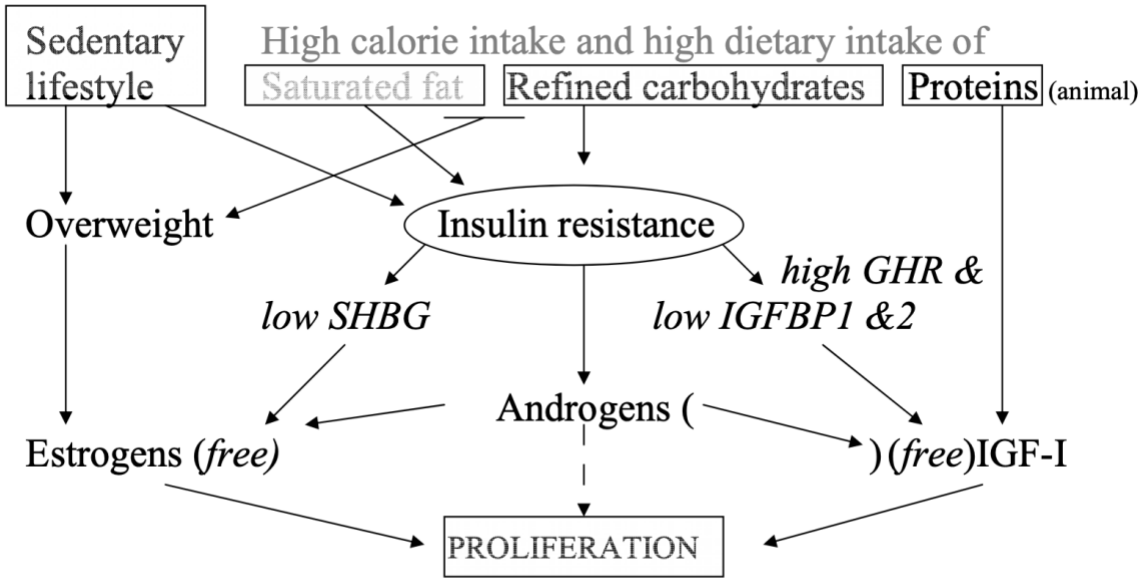
	% change	P		% change	P
Testosterone	-10	*	Glucose	-5	*
Estradiol	-6	*	Insulin	-17	*
SHBG	+5	*	IGF-I	-4	ns
Triglycerides	-14%	*	PDGF	-38	**
Total cholesterol	-11%	*	Systolic BP	-1	*
BMI	-5	**	Waist	-4	**

* P < 0.05 ** P < 0.01 with respect to baseline values

Mechanistic considerations

Various mechanism by which diet and life-style may promote increased risk for and progression of breast cancer, summarized in figure 2, have been recently reviewed (16, 27, 35). In short, sedentary lifestyle, overweight, hyper-caloric diet, high saturated fat and high glycemic index food are major determinants of metabolic syndrome, which in turn is associated with insulin resistance and increased androgenic activity. Insulin stimulates the synthesis of androgens in the ovary and the expression of GH receptors, and inhibits the liver production of SHBG and IGFBP1 and 2, thus increasing the bio-availability of both sex hormones and IGF-I. IGF-I is increased by a diet rich in protein, in particular milk protein (36). Alcohol intake increases the synthesis of androgens and estrogens (37). Postmenopausal overweight is associated with increased concentrations of total and free androgens, increased peripheral conversion of androgens into estrogens, decreased SHBG, and increased insulin levels. Sedentary lifestyle is associated with higher levels of estrogens, androgens (38) insulin (39) and C-peptide (40).

Figure 2. Mechanistic relationship between diet, physical activity and hormones



Previous randomized dietary intervention trials to decrease BC recurrences

Two randomized trials carried out in the eighties on a small number of BC cases (about 100 each) suggested that calorie restriction may reduce BC recurrences (41) and total mortality (42). The Women's Intervention Nutrition Study (WINS), a large randomized controlled trial investigating the role of dietary fat reduction on relapse-free survival in postmenopausal women with early-stage resect able BC (n = 2,700), found that patients on the reduced-fat diet had a lower risk of recurrence (hazard ratio = 0.76, 95% CI = 0.60-0.98) than women on the standard diet (39). A further large-scale trial aimed at reducing fat and increasing fruit and vegetable intake in women diagnosed with BC is currently ongoing (43).

Study aims and design

We propose a randomized intervention trial of diet and physical activity to reduce BC relapse (local, distant), and second ipsilateral or contralateral BC, in BC patients at high risk of recurrence because of biochemical markers of increased risk, namely high serum testosterone and/or high fasting insulin and/or metabolic syndrome. Several other markers of increased recurrence risk are available, such as cancer stage at diagnosis, histological grade, hormonal receptors and other gene expression profile. All these will be registered, but we are specifically interested in markers of the host that can be modified through life-style.

Secondary aims include assessing:

- the effect of the combined dietary and physical activity change on the prevalence of metabolic syndrome and on biomarkers that are hypothesized to be intermediate factors in the association of diet and physical activity with BC
- the effect of the intervention on the development of other life-style related health conditions, such as other cancers, diabetes, hypertension and dyslipidemia, and on total mortality.

Phases of the study and milestones:

Identification of eligible patients, Recruitment, Randomization, Intervention, Baseline and yearly measurements, Compliance evaluation, Follow up, Statistical analysis

Identification of eligible patients

Inclusion criteria:

1) Mastectomy or conservative surgery for invasive breast carcinoma, any type, in the last five years (we expect to recruit mostly patients at the time of diagnosis or at the end of the first follow-up year after surgery, i.e when chemotherapy has been concluded and hormonal therapy, if necessary, started)

2) Absence of signs or symptoms suggestive of recurrences

Presence of one or more of the following endocrine/metabolic indicators:

- serum testosterone level ≥ 0.4 ng/ml (1 nmol/ml), corresponding to the median value in BC patients, or
- serum insulin ≥ 7 uU/ml (50 pmol/L), corresponding to the upper quartile of insulin distribution in BC patients, or
- metabolic syndrome, present in about 15% of BC patients

Exclusion criteria:

1) Metastatic disease, or previous relapse

2) Age >70

3) Physical or mental handicaps that would impede to engage in moderate physical activity or participate in kitchen classes

Setting: The study will be carried out in collaboration by two institutes in Milan (the National Cancer Institute and the European Institute of Oncology), and one in Palermo (the Department of Oncology of the ARNAS-Civico Hospital).

Recruitment

Potential participants will be recruited at the time of diagnosis or through follow-up clinics, patients' associations, or the media. Patients will be requested to sign an informed consent, including authorization for getting blood samples, for storing samples for future studies on the effect of diet on blood parameters, and on DNA polymorphisms that may modify the response to dietary changes, and for follow-up. We shall collect demographic information, measure body weight, height, waist circumference, and blood pressure, and take an early morning (0800-1000h) fasting blood sample to measure blood glucose, LDL and HDL cholesterol, triglycerides, testosterone and insulin. Eligible patients shall fill in a baseline questionnaire on medical history, medication use, reproductive and body weight history, usual physical activity (frequency, duration and intensity), and a validated semi-quantitative food frequency questionnaire.

Biological bank: Every about 12 month we shall collect and store at -80°C one aliquot of whole blood and three 2-ml serum aliquot. In a sample of cases we shall also preserve blood with RNAlater solution to allow the expression assay of several relevant genes.

Randomization

We plan to recruit 1,200 high risk patients (400 per year) and to randomize them in two groups: 600 (control group) shall receive general standard written recommendation for healthy lifestyle without, however, any active support; 600 (intervention group) shall receive a combination of individual and group contacts over the course of one year, including kitchen courses, gym and dance classes, common meals and reinforcing meetings, with emphasis on a comprehensive dietary and life-style change.

Randomization will be carried out within strata of age, treatment group (no adjuvant therapy, chemotherapy only, hormonal therapy only, both hormonal and chemotherapy) and axillary nodal status at diagnosis. Allocation will be done centrally and blindly.
Patients not fulfilling the high-risk criteria will be given the same general recommendations as the control group, and will be followed up as an external low risk group.

Intervention

The intervention shall aim at increasing physical activity, controlling weight, and promoting healthy, low calorie diet.

a) Physical activity. The goals of the physical activity intervention are:
- Achieve and maintain regular participation in a moderate intensity physical activity (approximately 3 to 5 METs) program of 210 minutes/week (30 min on average per day) over at least 3 days /week
-Decrease sedentary behaviors by 30 minutes/day on at least 5 days/week. During the first 12 months one group physical activity session per month will be offered to enhance program adoption. Women who wish to take up vigorous sports will be encouraged to do so. For those who do not progress to more vigorous activity, the focus will be on maintaining moderate intensity activities, such as walking. For self-monitoring and compliance enhancement, study participants will use logs and fill-in questionnaires

b) Weight control. Reducing energy intake relative to expenditure is the primary dietary focus for promoting weight loss in overweight or obese participants, and maintaining a healthy energy balance is the primary focus for normal weight participants. Energy density of the diet has emerged as a dietary characteristic that can be easily manipulated to maintain volume and satiation despite reduced energy intake. Participants will be encouraged to include whole grains and high-fiber vegetables, which add bulk and volume to the meal, as major components of their diet, to choose cooked cereals rather than bread or dry crackers, eat a vegetable salad, vegetables or soup before eating higher energy foods, choose fresh fruit rather than juice, and plan the meals in order to limit the opportunities to wrestle with food choices, and to reduce overeating prior to the eating situation itself.

c) Healthy diet .In order to reduce glycemic and insulinemic response, recommendations will include:
- reducing calorie intake, through the preferred consumptions of highly satiating foods, such as unrefined cereals, legumes and vegetables.
- reducing high glycemic index food, such as refined flours, potatoes, white rice, corn flakes, and high insulinemic foods, such as sugar and milk, preferring instead whole grain rice, barley, millet, oat, spelt, quinoa and buckwheat, legumes (any type including traditional soy products), vegetables (any type, except potatoes)
- reducing sources of saturated fat (red and processed meat, milk and dairy products) preferring instead unrefined vegetable fats, such as olive oil, nuts and oleaginous seeds.
- reducing protein intake, mainly animal protein (except fish)

To reach these goals a multiple intervention strategy will be developed, based on the following: a) actions for both the intervention and the control group

1. invitation leaflet, explaining the rationale of the study and including basic life-style recommendations, based on the 1997 WCRF recommendations (to be updated in 2007) and the Italian National Institute of Nutrition food pyramid.
2. dissemination of the information on the study by media
3. yearly follow-up questionnaire on breast events and dietary and physical activity change

b) actions for the intervention group

4. Individual advice on diet
5. Individual advice on physical activity
6. Kitchen courses to teach basic Mediterranean and macrobiotic recipes (two 1-day course plus, ten 3-h courses associated with common dinner) (15-20 participants at a time)
7. Fortnightly common lunch or dinner (50-60 participant at a time)
8. Basic gymnastic course (twelve – monthly- 2-hour courses)
9. Organization of "Solidal Buying Groups" in order to buy recommended food and other commonly used goods directly from the producers or from big retailers at a discounted rate.
10. Agreement with healthy food shops and organic farmers to obtain special price
11. Agreement with fitness centers, yoga or martial arts centers, and dance schools
12. Study newsletter, with scientific information, kitchen recipes, study facilities
13. DIANA-5 website and chat line to exchange recipes and experience
14. Periodic conferences on diet and health
15. Periodic reinforcement meetings with common meals, gymnastic sessions, and dancing, after the first year
16. Periodic body weight assessment (weekly self measurement and monthly measurement at the study center)
17. Discounted rate for advanced kitchen courses
18. Psychological support groups
19. Individual advise on the basis of the changes in body weight and blood parameters at follow-up examinations

Baseline and yearly measurements

Height and weight: electronic scale with women in light clothes and without shoes. In a sample of cases body fat and lean mass will be measured with bioelectric impedance.
Blood pressure: electronic device.
Serum glucose, triglycerides and cholesterol: standard quality controlled laboratory techniques
Serum Testosterone and Insulin: Radioimmunoassay (RIA). For testosterone we shall use a direct RIA kit which has been validated by comparison with indirect assay after organic extraction of serum samples and celite purification (44).
For a sample of patients, we plan to apply for further financial support to measure several other metabolic, endocrine and inflammation markers (estrogens, SHBG, IGF-I, IGFBP 1, 2 and 3, C-reactive protein, leptin and adiponectin) as well as the DNA polymorphism and RNA expression of several relevant gene, in collaboration with the Dept of Oncology of the Ospedale Civico ARNAS in Palermo and at the Institute of Endocrinology at the Milan University.

Compliance

Compliance will be monitored through lifestyle compliance questionnaires, dietary questionnaires, weight change and plasma glucose, triglycerides, cholesterol, insulin, testosterone changes after one year of intervention. As adjuvant treatment may alter these biomarkers' level and confound the effect of the intervention, we shall compare blood samples collected in the same treatment phase: at least 3 months after the end of chemotherapy, if any, and 3 months after the start of hormonal therapy, if any, and one year later.
Altering the proportion of energy from dietary carbohydrate and fat has been shown to affect plasma lipid concentrations in controlled feeding studies. As we recommend to obtain carbohydrates from low glycemic index food, however, we do not expect any increase in

triglycerides. We expect a substantial reduction of total and LDL cholesterol and fasting glycaemia, minor changes in HDL cholesterol and triglycerides, and a substantial reduction of body weight and waist circumference in the intervention group compared with the control group. Total cholesterol change is an estimate of saturated fat intake change. Testosterone and insulin are also expected to decrease in subsequent samples, which will be assayed in the same batch. A compliance score will be computed, based on the direction of change in all these biomarkers.

Follow-up and outcome events

The main outcome will be new BC events including: - new primary breast cancer
- local/regional recurrence
- distant/metastatic recurrence

The follow up will be based on the routine clinical follow-up at the collaborating hospitals, the periodic questionnaires to study participants, the regional cancer registry and hospital discharge diagnosis system, and death certificates

Statistical power

The survival of BC patients in Italy is dramatically increasing: 5-year relative survival increased from 80.6% for patients diagnosed in the early 1990s (Berrino et al, EUROCARE-3) (45) to 85.6% for patients diagnosed in the early 2000s (EUROCARE-4, unpublished). For these patients the estimated relapse free survival is 81.9 (EUROCARE high resolution study, unpublished). Such a survival improvement is accompanied by a postponement of the incidence of relapse (table 4).

Table 4. incidence rate of local + distant recurrences (EUROCARE, unpublished)

Follow-up year	relapse rate %	95% confidence interval
1st	3.4	2.0 -5.8
2nd	4.7	3.0-7.5
3rd	5.1	3.2-8.1
4th	4.7	2.9-7.8
5th	2.1	0.9-4.6
6th to 8th	2.2	1.4-3.0

Based on EUROCARE data we estimate that the 5-year distant recurrences plus second ipsilateral or contralateral BC for patients recruited in the first few years after diagnosis will be about 20%. In the subgroup of patients selected on the basis of high insulin and/or testosterone levels (50% of cases with RR of the order of 3), we estimate that about 30% of patients will develop a recurrence or a second primary BC within 5-year follow-up: 360 cases out of 1200. Considering that the WINS randomized study obtained a 24% reduction of recurrence rate changing a single dietary factor (total fat) (39), and that observational studies suggested that a moderate physical activity may reduce recurrence rate by over 40% (28), we hypothesize that a comprehensive dietary intervention plus physical activity will reduce BC recurrence rate by 33% or more, but we consider also a more prudent estimate of 25% reduction.

The following table gives the number of patients (per arm) that we should recruit in order to have 90% or 80% power to detect a significant difference with $\alpha < 0.05$, two sided, or one sided ($\alpha < 0.10$).

Power (1-β)	0.9	0.9	0.8	0.8
α	*0.05*	*0.10*	*0.05*	*0.10*
33% reduction	412	339	313	251
25% reduction	748	614	566	451

Our aim is to recruit 1,200 high risk patients in order to have 600 patients per arm, which shall guarantee 80% chance of getting a significant difference also with 25% reduction, and allowing for 90% compliance in the intervention group and 10% contamination of the control group.

Statistical analysis

We shall first assess the baseline association between androgens, insulin, and several measures of adiposity including body mass index, waist circumference and (in a sample of cases) percentage body fat, with Spearman correlation coefficients. We shall then compare the change in food consumption, body weight, and geometric means of hormone end points from baseline to 12 months in the intervention and control group.

The main analysis of the intervention effect on the incidence of recurrences will be by intention to treat, i.e. based on assigned treatment at the time of randomization, regardless of adherence. As a secondary analysis we shall assess the effect by change in body weight and biomarkers. We shall compute total survival, disease free survival and relapse free survival.

Hazard ratios and confidence intervals will be computed by the Cox proportional hazard model, with standard clinical-pathological prognostic covariates as potential confounders. Separate analyses will be carried out by ER, PR and erbB2 status. Interim analyses will be carried out as soon as the total follow-up person-years reach 2,500 (corresponding to α =0.10 and β= 0.20 for a 33% reduction of recurrence rate).

Feasibility

The principal investigator has long term experience in carrying on and coordinating epidemiological studies, including small-scale dietary intervention studies (22, 29, 30). The European Institute of Oncology has long term experience in chemoprevention studies. The Milan National Cancer Institute is fully equipped with facilities for kitchen courses, dedicated cafeteria, gym facilities. The Palermo oncology center has access to kitchen facilities in a nearby technical institute and has already carried out a dietary intervention study with very high compliance. The above institutions treat, respectively, about 3,000, 1,000 and 200 BC patients per year.

Control of potential biases

Selection bias: the randomization will be carried out blindly, without any possibility of changing the allocation, within strata of age, date of diagnosis, treatment allocation (depending on stage and hormone receptor status) and number of positive axillary nodes at diagnosis (none, 1-3, 3 or more).

Performance bias: patients will be recruited after the main treatments have been planned or delivered. In no case the allocation to the intervention or control group or the compliance will affect treatment.

Measurement bias: patients and researchers cannot be blinded, but the ascertainment of outcome will be carried out by clinicians that are not involved in the study.

Attrition bias: Based on previous studies we expect a fairly high compliance in the intervention

group (>90%) but also some modification in the control group. After the main analysis by intention to treat, therefore, secondary analyses will be carried out by compliance score.

Milestones and timing:

1st)	3rd	Month:
2nd)	12th	Month:
3rd)	16th	Month:

testosterone by 10% or more and serum glucose and insulin by 5% or more in at least 50% of participants.

Operative study protocol and information system

Recruitment of at least 300 patients in the first year.

Compliance evaluation. The aim is to decrease serum cholesterol and

4th) 24th Month: Test of follow-up procedures and comparison of observed and expected number of recurrences

5th) 35th Month: First outcome analysis

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