Eur J Nutr
DOI 10.1007/s00394-017-1489-9

ORIGINAL CONTRIBUTION

Serum antioxidant capacity, biochemical profile and body composition of breast cancer survivors in a randomized Mediterranean dietary intervention study

Maria Skouroliakou[1] · D. Grosomanidis[2] · P. Massara[1] · C. Kostara[3] · P. Papandreou[3] · D. Ntountaniotis[4] · G. Xepapadakis[2]

Received: 14 August 2016 / Accepted: 13 June 2017

Abstract
Purpose Increasing evidence suggests that Mediterranean Diet (MD) is correlated with reduced risk of breast cancer (BC) and cancer mortality, since it modifies patients' serum antioxidant capacity, body composition and biochemical parameters. The aim of the study was to investigate whether a dietary intervention based on MD has a beneficial effect on these factors.
Methods In this intervention study, seventy female BC survivors were randomly assigned to (1) the intervention group (personalized dietary intervention based on MD) and (2) the control group (received the updated American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention and ad libitum diet). Both groups were assessed twice [beginning, end of study (after 6 months)] regarding their anthropometric and biochemical parameters, serum vitamin C, vitamin A, a-tocopherol and CoQ10 levels, dietary intake and adherence to MD. An additional intermediate analysis was conducted on participants' body composition and biochemical profile.
Results Concerning the intervention group, body weight, body fat mass, waist circumference, body mass index as well as HDL-cholesterol were significantly decreased ($P < 0.2\%$). An increase was observed in the vitamin C levels in blood ($P < 0.2\%$). In the control group, body weight, body fat mass and serum total cholesterol rose ($P < 0.2\%$). At the end of the study the two groups were significantly different considering blood glucose, vitamin C, polyunsaturated fatty acids, vitamin A and a-tocopherol levels.
Conclusions This randomized dietary intervention based on MD managed to ameliorate serum antioxidant capacity, body composition, adherence to MD and glycemic profile of postmenopausal BC survivors.

Keywords Breast cancer survivors · Mediterranean diet · Dietary antioxidants · Body composition · Biochemical profile

Introduction

The steadily increasing primary incidence of breast cancer (BC) coupled with advancements in medical therapy have rendered BC survivors the largest group of cancer survivors worldwide [1, 2]. In Greece, BC had the greatest incidence among all other cancers in women during 2012 (27.95%) [3] and a 10-year survival rate over 75% [4]. Based on prospective studies, BC survivors have about two to five times higher risk of second primary BC incidence [5–7], which remains significantly increased up to 20 years after the initial diagnosis [6]. In addition to a second BC diagnosis, BC survivors have an increased risk to gain weight when following a BC treatment [8] and develop other comorbidities, such as cardiovascular disease (CVD), which directly affect the long-term prognosis of these patients [9].

Electronic supplementary material The online version of this article (doi:10.1007/s00394-017-1489-9) contains supplementary material, which is available to authorized users.

✉ Maria Skouroliakou
mskour@hua.gr

[1] Department of Nutrition and Dietetics, Harokopio University of Athens, Kallithea, 17671 Athens, Greece

[2] "REA" Hospital, 17564 Palaio Faliro, Greece

[3] "IASO" Maternity Hospital, Marousi, 15123 Athens, Greece

[4] Department of Chemistry, National and Kapodistrian University of Athens, Zografou, 15771 Athens, Greece

Published online: 20 June 2017

Environmental and lifestyle factors have a significant role in both BC's prevention and progression [10]. Existing evidence strongly supports that post-diagnosis physical activity levels (e.g., walks, exercise program at low intensity, biking, canoeing, general gardening, stretching, volleyball, tennis, swimming, ballroom and line dancing) are associated with a declined risk of BC recurrence [11], which, according to a recent meta-analysis, has been approximately estimated to 24% [12]. On the other hand, overweight and excessive body fat mass are connected with secondary malignancies, cancer recurrence, metabolic disorders and mortality [13–16]. Moreover, systemic oxidative stress has been implicated in the pathogenesis and progression of the disease via various mechanisms, including the genomic instability coupled with significant resistance to anticancer treatment, the activation of signaling cells involved in the proliferation of cancer cells and the increased tumor cell migration [17–19]. It is noteworthy that many anticancer treatments, including chemotherapy and radiotherapy, are based on the generation of free radicals to induce cancer cells destruction [20].

Various studies examined the association of BC with serum antioxidants, including vitamin A (retinol), coenzyme Q_{10} (CoQ10), vitamin E (a-tocopherol) and vitamin C, however, they resulted in conflicting conclusions [21]. For example the Finnish Mobile Clinic Health Examination Survey showed a positive association between beta-carotene and breast cancer risk [22]. On the contrary, a cross-sectional study indicated that serum antioxidants were inversely associated with tumor size [23]. Furthermore, an observational study concluded that individuals with BC were more likely to have lower plasma CoQ10 concentration than healthy controls [24]. Moreover, a prospective study reported an inverse relationship between circulating levels of CoQ10 and BC risk [25]. A nested case–control study evaluated the association between plasma carotenoid, retinol, a-tocopherol and vitamin C concentrations and risk of BC with an oversampling of estrogen receptor-negative (ER−) cases [26]. The conclusion was that high concentrations of several carotenoids and vitamin C may be protective against the development of hormone receptor specific BC, without examining this effect on secondary BC cases [26].

Antioxidants derived from diet, including vitamin A, CoQ10, vitamin E and vitamin C, have shown a significant role against oxidative stress [27] and various studies have observed an association between the consumption of single foods or food groups and plasma/serum antioxidant capacity [26, 28, 29]. However, food and nutrients are not consumed alone, and they constitute a complex network of interrelated and interacting dietary, biochemical and behavioral factors [29], which could have antagonistic or synergistic effect on health outcome. For that reason, it has been suggested to conduct an evaluation of complete dietary patterns, which also include dietary habits that preempt nutritional confounding [30].

Mediterranean diet (MD), a dietary pattern prevalent in the olive growing areas of the Mediterranean region, including Greece, [31] is characterized by a high intake of (1) vegetables and fruits, (2) legumes, (3) nuts, (4) whole grain cereals, (5) moderately high intake of fish, (6) high intake of monounsaturated lipids coupled with reduced intake of saturated fat, (7) moderate intake of dairies, (8) low consumption of meat products, and (9) regular, but moderate alcohol intake [32]. Current evidence indicates that greater adherence to a MD pattern (a) could reduce BC risk [33–35], (b) as well as overall cancer mortality [36] and (c) has a beneficial influence on health and longevity [37].

Various mechanisms have been proposed to explain the beneficial effect of MD on BC and cardio metabolic diseases' risk, including its richness in fruits and vegetables, significant sources of antioxidants (e.g., vitamins C, A and E) [38, 39] and its role in patients biochemical profile amelioration [40, 41]. However, to the best of our knowledge, the impact of the Mediterranean dietary pattern on lifestyle factors of BC survivors linked with cancer recurrence and comorbidities, such as antioxidant capacity, biochemical profile and body composition has rarely been investigated. The aim of the study was to investigate whether these factors are beneficially affected by a dietary intervention based on MD.

Materials and methods

Study design and participants

The study was a randomized two-arm dietary intervention conducted in Athens, Greece, in "Rea" maternity clinic between March 2014 and June 2015. The study protocol was submitted and received approval from the Scientific and Ethics Committee of the same hospital in accordance to the Declaration of Helsinki of the World Medical Association. Prior to the collection of any information, participants were informed about the aims and procedures of the study and provided their signed consent.

The volunteers were females suffering from BC with a histological confirmed diagnosis of invasive BC stage I–IIIA (diagnosed up to 3 months before recruitment) [42]. The inclusion criteria of the study are given in Fig. 1.

The exclusion criteria were: (a) multivitamin or simple vitamin supplementation; (b) a previous or current history for a second cancer; (c) active infection; (d) other severe coexisting medical conditions (e.g., obstructive ileus) that would hamper the ability of the patient to follow a

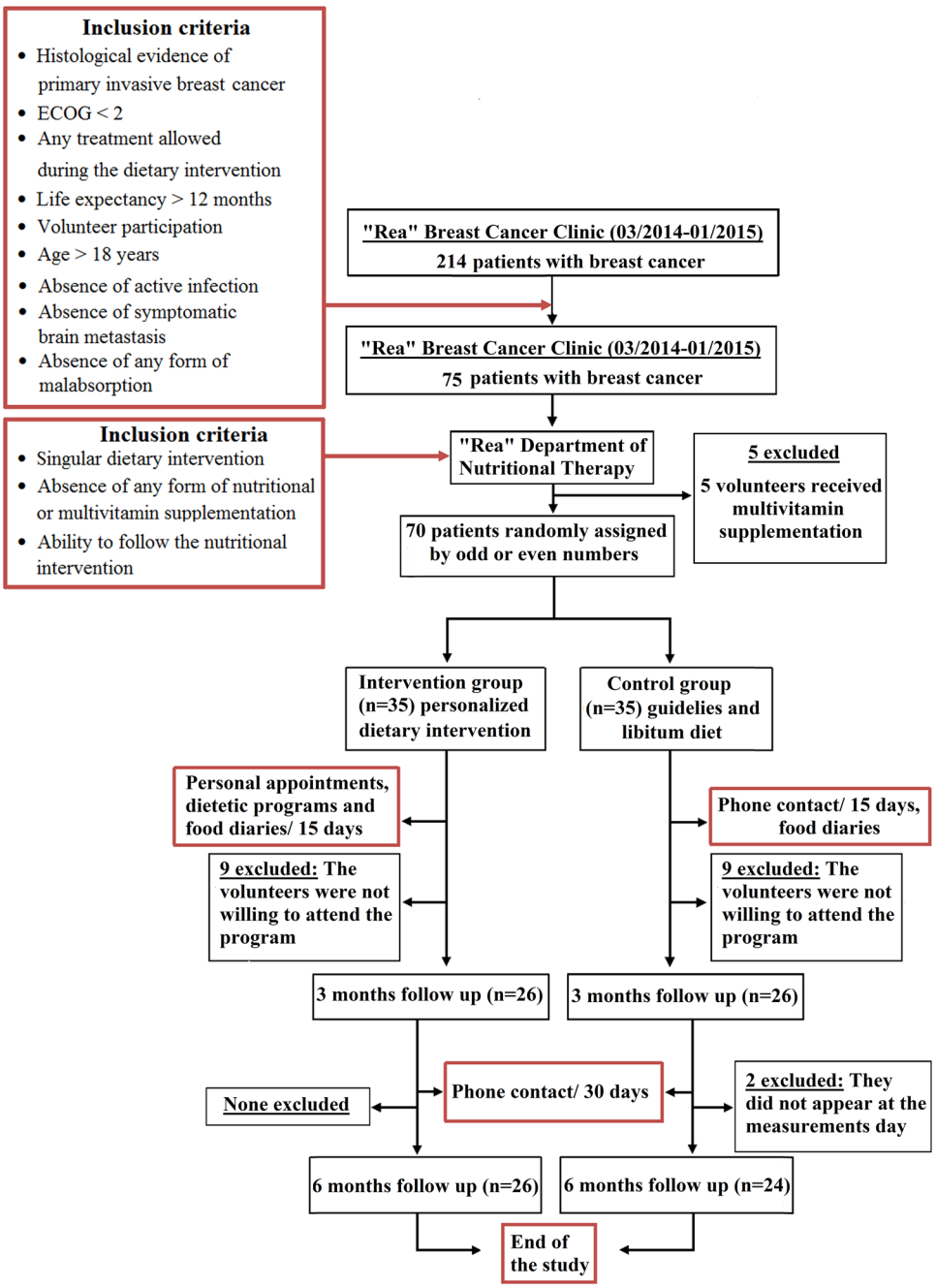

Fig. 1 Schematic overview of the design of the study

treatment program; (e) symptomatic brain metastases; (f) malabsorption; (g) refusal to comply with the nutritional program and physical activity recommendations. The eligible participants were randomly allocated between two groups: (1) the intervention group treated with personalized dietary intervention based on MD and (2) the control group received the updated American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention [43] and ad libitum diet. Recommendations from the American Cancer Society regarding physical activity were also provided to both groups.

The dietary intervention lasted 6 months. During the first 3 months of the study, regular 15-day personal appointments were scheduled for the intervention group and regular 15-day phone interviews for the control group. The idea was that an impersonal communication via phone calls could reduce the bias of contacting the same researchers. [e.g., if both, (the intervention group and the control group) had personal appointments with the same researchers, then due to human error, there is greater possibility to impart to the control group more detailed information and recommendations to improve the lifestyle and dietary habits. This

human error could happen for example, if a participant asks a question and the dietitian answers imparting information. Phone calls lead to reduced personal interaction and emotions.] After that period, the dietitians contacted with patients of both groups via phone calls at the end of month 4 and 5 [but a final personal appointment was scheduled at the end of month 6 (end of the study)]. Additionally the intervention group continued to receive a personalized dietary program via e-mail. The dietitians contacted with the control group via phone call generally, except from three appointments [at the beginning of the study, after 3 months and at the end of the study (after 6 months)].

Anthropometric measurements

Body weight (BW), body fat mass (BFM), percentage body fat mass (PBFM) were measured for both groups at the beginning, after 3 months and at the end of the study with the method of Air Displacement Plethysmography for research and clinical applications [BOD POD® Body Composition Tracking Systems, Life Measurement, Inc. (LMI)]. Two hours before measurements the examinees had not performed exercise, not consumed any foods or drinks, and they were dressed only in their underwear. Jewelry, glasses and mobile phones were not permitted in the test room. Participants' height was also measured at the beginning of the study with a calibrated stadiometer. The body mass index (BMI-kg/m^2) was calculated for every patient based on the aforementioned Bod Pod's and height's measurement. Waist circumference (WC) measurements were made with a tape made of fiberglass, around a patient's bare midriff, after the patient exhaled while stranded without shoes and with both feet touched and arms hanged freely. Threefold WC measurements were typically carried out and their average calculated to the nearest 0.1 cm. All the anthropometric measurements were conducted by two trained registered dietitians.

Dietary intervention and dietary intake assessment

The intervention group received tailor-made personalized dietary intervention, specific for BC survivors, conducted by two trained registered dietitians. It was based on MD and was enriched with olive oil and foods with specific health benefits to BC survivors [44, 45]. The patients were counseled to consume: (1) one tablespoon of flaxseed oil or four tablespoons grounded flaxseed per day, (2) three cups of green tea or Greek Mountain Tea (also known as "Shepherd's Tea") per day, (3) seasonal fruits and vegetables with high antioxidant capacity [i.e., broccoli, grapes, pomegranate, strawberries, cauliflower, blueberries, blackberries (more information about the antioxidant capacity of vegetables and fruits is available in Supplementary material)] [44]. Individual counseling was given every 15 days with daily dietary program with specific meals, products, recipes and food portions in grams and it was coupled with educational booklets, food diaries and individual nutritional advices.

The volunteers in the intervention group were also trained on meal preparation techniques such as cooking without added salt, sugar or butter, cooking in low temperature with tomato sauce [46], and promoted the use of traditional Mediterranean herbs, such oregano, rosemary, etc. Some health problems related to the participants' medical history, e.g., constipation, colitis and esophageal reflux, were taken into consideration to the dietary intervention to promote patients' health and compliance to the study. On the other hand, the control group received the updated American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention and ad libitum diet. The same guidelines were also incorporated into the educational booklet of the intervention group.

The targets of the intervention were (a) PBFM. 21–32.99% for ages 20–40 years and PBFM 23–33.9% for ages 40–60 years [47], (b) 10% loss of body weight in 6 months for those women who exceeded this range [48] and (c) adherence to MD's dietary pattern [49]. On the other hand, weight maintenance as well as the improvement of dietary habits were set as goals for the participants with normal PBFM.

Dietary intake of both groups was assessed with (1) a valid and reliable for the Greek population semiquantitative Food Frequency Questionnaire (FFQ) designed to assess the role of dietary habits and eating behaviors in the development of BC [50], (2) a shorter valid semiquantitative FFQ as supplementary to the previous one [51] and (3) weekly weighted food diaries (FD). The FFQs were used at the beginning and at the end of the study, while the food diary records and the nutritional programs were additionally assessed during the study. Diet Analysis Plus (version 6.1, Wadsworth 2003) was used to analyze and evaluate the dietary programs, the FFQs and the FDs. Diet Analysis Plus is based on the USDA database for the US. The users of this software have also the opportunity to add new food items to the program's food list. For this study the database from [52] was used. The book includes a detailed analysis of macronutrient and micronutrient of various foods consumed in Greece. These tables include an extended list of chemically analyzed Greek foods and traditional dishes and they are also available online from the Hellenic Health Foundation [2007, http://www.hhf-greece.gr/tables/ (more information about Diet Analysis Plus is available in Supplementary material)]. Participants' dietary intake in vitamin C, retinol, a-tocopherol, saturated-monosaturated and polysaturated fat was estimated based on the analysis of the average food intake as recorded in FFQs and the FDs.

Patients' conformity to the traditional MD was assessed via a validated 0–9 points score [53]. The score relied on nine dietary components frequently consumed in the traditional MD: fruits, vegetables, legumes, nuts, cereals, dairy, red and processed meat, fish and seafood. The diet score was calculated for each participant according to the daily median consumption of the aforementioned food groups. A value was assigned based on the estimated food portions from the FFQs and the literature [53]. Higher values of the MD score indicate a greater adherence to the MD (more information about Mediterranean Diet Score is available in Supplementary material).

Laboratory examinations

All serum samples were stored at −80 °C immediately after blood collection and were never previously thawed before extraction to minimize the outcome variations due to storage conditions (such as temperature and storage duration from collection to processing).

Serum Vitamin C, vitamin A (retinol), a-tocopherol and CoQ10 were analyzed for both groups at the beginning and at the end of the study using High Pressure Liquid Chromatography (HPLC). Subsequently the levels of fat soluble vitamins A and E in serum were determined with ClinRep® reagents for HPLC (Recipe, Munich 2015) based on the manufacture's references (VITAE_E_KUR.DOC, Version 6).

In brief, all procedures prior to storage of extracted analytes in amber vials were carried out under subdued light to avoid degradation of analytes. All solvents and serum samples were kept on ice (−5 °C) during work-up. An aliquot of 150 µL of serum was mixed with 150 µL precipitant P (contained 3 µg Internal Standard). After centrifuging for 5 min at 10,000*g*, 100 µL of supernatant and 100 µL of Stabilising Reagent S (cooled at 4 °C) were mixed shortly with Avanti™ 30, Beckman vortex mixer. HPLC analysis of vitamin A and vitamin E was performed after the injection of 50 µL of the supernatant on an Agilent, 1100 Series system. Before the analysis both a test and calibration run were performed based on the HPLC-system and regiments references. The analytical method for the simultaneous determination of vitamins A and E, as dictated by the particular kit, is described by the following parameters (1) solution flow: 1.5 mL/min, (2) column temperature: 30 °C, (3) injection interval: 8 min, (4) injection volume add: 50 mL, (5) UV-detector: 325, 295 nm, (initial eluting retinol with UV detector at 325 nm and then changing the detector wavelength of 295 nm, so that it is possible to determine the vitamin's concentration).

The biochemical evaluation was carried out in the hospital's laboratory and followed the criteria of the World Health Organization Reference Laboratories. Blood lipid examinations, serum total cholesterol (TC), high density cholesterol (HDL-C), low density cholesterol (LDL-C), triacyloglycerol (TG), and fasting blood glucose (BG) concentrations were measured for both groups with the use of a chromatographic enzyme method in an automatic analyzer (RA-1000; Mecon Ltd, Athens, Greece) at the beginning, after 3 months and the end of the study.

Questionnaires

Information on physical activity and patience performance status were assessed through two validated and reliable questionnaires, the International Physical Activity Questionnaire (IPAQ) [54] and the Eastern Cooperative Oncology Group (ECOG) Performance Status [55], respectively. IPAQ takes into account both the daily activities, e.g., household tasks as well as organized physical exercise performed during leisure time (e.g., participation in a sport activity). The classification of physical activity was based on the concept of metabolic equivalent (MET). One MET is equal with resting metabolic rate. Consequently, the intensity of physical activity was expressed as a multiple of MET. MET-mins are the product of the physical activity's METs for the time period it lasts. The aforementioned measurements were used for both groups at the beginning and at the end of the present study. Additionally a detailed nutritional and medical history of every participant was kept during the 6-months study for both groups.

Statistical analysis

All analyses were performed with the SPSS statistical software (version 21.0, SPSS, Inc, IBM, Chicago, IL, USA). Statistical significance was set at $P < 0.05$. Continuous data were expressed as medians and ranges (non-Gaussian distribution, Wilk test) and dichotomous variables as counts and proportions. Comparisons between the two study groups were performed using the Student's *t* test and the Mann–Whitney test for continuous variables, and Fisher's exact test for dichotomous variables. Analyses of covariance were applied to estimate mean levels of vitamin C of the two groups at the end of the study adjusted for BMI, smoking and vitamins C beginning levels. The analysis was also performed between both groups for blood glucose levels at the end of the study, adjusted for BMI and estimated weekly MET-mins. Pearson's and Spearman's ρ was used as a measure of correlation. A one way Anova with repeated measures, a paired *t* test and a Wilcoxon test were performed to conduct intragroup comparisons for the different time periods of the study. Bonferroni corrections have been applied for all variables (Tables 1, 2, 3) and the new threshold of statistical significance was set on 0.2% (more information about Bonferroni correction is available in Supplementary material).

Results

First 139 out of the 214 women (who entered the study) were excluded, because they did not meet the inclusion criteria and only 75 referred to the dietitians. Additionally, five women were excluded because they received supplements (Centrum, Eviol vitamin A or E). During the first 3 months of the intervention, nine women withdrew from the intervention group stating mostly failure to comply with the program. In addition two women did not appear at the date of the second measurement after 3 months of the intervention without, however, withdrawing the study. In the control group, 11 women were not able to finish the study for personal reasons. Overall, the withdrawal rate from the study was 35.7% (25 women). Moreover, a statistically significant correlation was observed between the study's total dropout rate (35.7%)

Table 1 Characteristics of the intervention and the control group at the end of the study

	Intervention group ($n = 26$)	Standard deviation	Control group ($n = 24$)	Standard deviation	P value
BMI (kg/m^2)	27.55	(±4.69)	27.73	(±5.70)	0.97
>18.5	0		0		
18.5–24.9	9 (34.6%)		9 (25.7%)		
25–29.9	11 (42.3%)		8 (22.9%)		
≥30	6 (23.0%)		7 (20.0%)		
Weight (kg)	72.69	(±13.83)	72.53	(±15.61)	0.89
Body fat mass (kg)	27.91	(±9.65)	28.02	(±11.19)	0.93
% Body fat mass	37.56	(±6.95)	36.84	(±6.95)	0.62
Waist circumference (cm)	94.36	(±11.37)	96.97	(±13.06)	0.48
Radiotherapy	1 (3.8%)		2 (8.3%)		0.56
Chemotherapy	3 (11.5%)		5 (20.8%)		0.38
Hormonal therapy	19 (73.0%)		17 (70.8%)		0.88
Aromatase inhibitors	17 (65.3%)		13 (54.1%)		
Tamoxifen	2 (7.6%)		4 (16.6%)		
Alcohol consumers[a]	11 (31.4%)		8 (33.3%)		0.98
Smokers (current)	9 (31.4%)		5 (14.2%)		0.06
Vitamin C (mg/mL)	6.57	(±1.74)	3.93	(±1.26)	<0.002
CoQ10 (mg/L)	1.45	(±0.23)	1.24	(±0.33)	0.033
Vitamin A (retinol-mg/L)	0.46	(±0.14)	0.38	(±0.17)	0.12
a-Tocopherol (mg/L)	8.13	(±1.534)	7.31	(±1.815)	0.13
Blood glucose (mg/dL)	91.03	(±9.96)	105.95	(±21.04)	<0.002
Total cholesterol (mg/dL)	203.83	(±44.56)	209.15	(±36.36)	0.62
LDL-cholesterol (mg/dL)	123.18	(±46.73)	130.78	(±34.39)	0.56
HDL-cholesterol (mg/dL)	66.52	(±17.56)	57.36	(±13.83)	0.08
TG (mg/dL)	89.00	(±61.13)	86.79	(±43.74)	0.86
MET-mins	910.44	(±879.34)	541.62	(±262.60)	0.08
Drop out	9 (25.7%)		11 (31.4%)		0.79
MD score	7.65	(±0.68)	4.44	(±1.04)	<0.002
Saturated fat (mg/day)	19.38	(±2.49)	22.80	(±9.40)	0.021
Monosaturated fat (mg/day)	25.04	(±3.48)	26.46	(±11.73)	0.35
Polyunsaturated fat (mg/day)	16.56	(±0.03)	18.98	(±8.71)	<0.002
Vitamin A RAE (mcg/day)	1077.76	(±164.14)	747.47	(±245.94)	<0.002
Vitamin C (mg/day)	447.13	(±28.36)	162.42	(±28.48)	<0.002
a-Tocopherol (mg/day)	23.16	(±0.41)	31.23	(±11.45)	<0.002

Comparisons between the two study groups were performed using the Student's t test and the Mann–Whitney test for continuous variables, and Fisher's exact test for dichotomous variables After the Bonferroni corrections the statistical significance was set at $P < 0.002$. Data are presented as n (%) or mean (SD)

RAE retinol active equivalents

[a] Participants who consume an average monthly volume of alcohol

Table 2 Comparison of characteristics of the intervention group from the beginning to the end of the study

	Beginning ($n = 26$)	Standard deviation	3 months ($n = 26$)	Standard deviation	6 months ($n = 26$)	Standard deviation	*P* value
Intervention group							
Weight (kg)	76.46	(±13.98)	73.02	(±13.83)	72.69	(±13.83)	<0.002
Body fat mass (kg)	32.38	(±9.66)	28.71	(±9.83)	27.91	(±9.65)	<0.002
% Body fat mass	41.70	(±6.79)	38.44	(±7.09)	37.57	(±6.95)	<0.002
Waist circumference (cm)	95.75	(±9.28)	95.00	(±11.24)	94.36	(±11.370)	<0.002
BMI (kg/m^2)	29.20	(±3.63)	27.66	(±4.82)	27.56	(±4.69)	<0.002
>18.5	0		0		0		
18.5–24.9	6 (23.7%)		9 (34.6%)		12 (46.1%)		
25–29.9	10 (38.4%)		9 (34.6%)		7 (26.9%)		
≥30	10 (38.4%)		8 (22.9%)		7 (26.9%)		
Alcohol consumers[a]	10 (38.4%)		8 (22.9%)		11 (31.4%)		0.35
Blood glucose (mg/dL)	102.93	(±13.47)	102.70	(±13.91)	93.50	(±7.61)	0.11
Total cholesterol (mg/dL)	190.33	(±46.00)	185.79	(±47.16)	184.78	(±26.95)	0.85
LDL-cholesterol (mg/dL)	113.66	(±35.22)	112.25	(±47.50)	102.87	(±31.97)	0.55
HDL-cholesterol (mg/dL)	61.87	(±24.72)	63.70	(±16.32)	70.16	(±20.02)	<0.002
TG (mg/dL)	90.00	(±35.41)	90.50	(±40.95)	76.37	(±36.17)	0.37
METS	610.57	(±433.52)	1025.07	(±779.48)	1041.07	(±899.90)	0.030
Vitamin C (mg/mL)	5.02	(±2.02)	–	–	6.54	(±1.76)	<0.002
CoQ10 (mg/L)	1.05	(±0.79)	–	–	1.45	(±0.23)	0.05
Vitamin A (retinol-mg/L)	0.32	(±0.12)	–	–	0.47	(±0.11)	0.08
a-Tocopherol (mg/L)	6.04	(±1.75)	–	–	8.22	(±1.49)	0.21
MD score	4.66	(±0.78)	–	–	7.65	(±0.68)	0.044
Saturated fat (mg/day)	19.15	(±3.49)	–	–	19.38	(±2.49)	0.76
Monosaturated fat (mg/day)	22.73	(±7.19)	–	–	25.04	(±3.48)	0.06
Polyunsaturated fat (mg/day)	16.05	(±8.95)	–	–	16.56	(±0.030)	0.97
Vitamin A RAE (mcg/day)	549.30	(±304.71)	–	–	1077.76	(±164.14)	0.06
Vitamin C (mg/day)	243.21	(±52.75)	–	–	447.13	(±28.36)	0.010
a-Tocopherol (mg/day)	22.01	(±16.24)	–	–	23.16	(±0.41)	0.53

Data represented as *n* (%) or mean (SD). An one way Anova with repeated measures (for the variables measured at 3 different time intervals) and a paired *t* test and a Wilcoxon test (for the variables with 2 measurements) were performed to conduct intragroup comparisons. After the Bonferroni corrections the statistical significance was set at $P < 0.002$

RAE retinol active equivalents

[a] Participants who consume an average monthly volume of alcohol

and the participants' BMI ($\chi^2 = 12.549$, $P < 0.001$). The greatest level of compliance was observed in normal weighted patients (BMI 18.5–24.9 kg/m^2) [56].

Clinical data

The volunteers suffered from invasive BC stages I–IIIA, they had undergone a surgery removal of the malignancy before recruitment, while some were on radiotherapy ($n = 33$), chemotherapy ($n = 38$) or hormone therapy ($n = 34$) during the intervention, according to the Supplementary Table 1. All volunteers had been on a diet (at least once) in the past. Their nationality was Greek and they were residents of Attica. At the beginning of the study, 18 volunteers of the control group and 16 of the intervention group were postmenopausal before cancer diagnosis ($P = 0.63$), but by the end of the study all volunteers were postmenopausal women. They did not follow any special diet (e.g., vegetarian or special dietary preferences or aversions), except for a tendency to avoid the low-fat dairy products which was observed in 65% of patients.

Antioxidant biomarkers assessment

Comparisons between the two groups at the beginning did not show any significant difference between the levels of serum vitamin A, a-tocopherol and CoQ10 levels (Supplementary Table 1). However, at the end of the study a significant difference was observed between the two groups regarding serum vitamin C levels (Table 1). This difference

Table 3 Comparison of characteristics of the control group from the beginning to the end of the study

	Beginning ($n = 24$)	Standard deviation	3 months ($n = 24$)	Standard deviation	6 months ($n = 24$)	Standard deviation	*P* value
Control group							
Weight (kg)	70.37	(±15.13)	71.35	(±15.96)	72.53	(±15.61)	<0.002
Body fat mass (kg)	26. 96	(±11.96)	26.76	(±11.42)	28.02	(±11.19)	<0.002
% Body fat mass	36.81	(±9.28)	36.03	(±9.04)	36.84	(±6.95)	0.16
Waist circumference (cm)	94.71	(±12.57)	95.35	(±12.90)	96. 97	(±13.06)	0.07
BMI (kg/m^2)	27.09	(±5.56)	27.48	(±5.93)	27.73	(±5.70)	0.045
>18.5	1 (4.1%)		1 (4.1%)		0 (0%)		
18.5–24.9	11 (45.8%)		7 (29.1%)		9 (37.5%)		
25–29.9	4 (16.6%)		8(33.3%)		8 (33.3%)		
≥30	8 (33.3%)		8 (33.3%		7 (29.1%)		
Blood glucose (mg/dL)	94.22	(±8.13)	91.66	(±20.35)	108.36	(±7.61)	0.11
Total cholesterol (mg/dL)	185.42	(±37.11)	198.55	(±33.04)	205.37	(±34.95)	<0.002
LDL-cholesterol (mg/dL)	108.37	(±27.51)	115.87	(±25.41)	119.00	(±28.80)	0.21
HDL-cholesterol (mg/dL)	63.87	(±13.82)	58.75	(±13.25)	70.16	(±20.02)	0.34
TG (mg/dL)	81.75	(±25.75)	100.87	(±47.68)	92.63	(±35.15)	0.40
Alcohol consumers[a]	10 (41.7%)		5 (20.8%)		8 (33.3%)		0.17
METS	720.18	(±491.72)	538.06	(±287.45)	541.60	(±562.60)	0.032
Vitamin C (mg/mL)	3.12	(±1.54)	–	–	4.04	(±1.27)	0.06
CoQ10 (mg/L)	1.22	(±0.28)	–	–	1.25	(±0.34)	0.57
Vitamin A (retinol-mg/L)	0.36	(±0.13)	–	–	0.46	(±0.14)	0.55
a-Tocopherol (mg/L)	6.55	(±1.52)	–	–	7.27	(±1.83)	0.09
MD score	4.64	(±0.79)	–	–	4.44	(±1.04)	<0.002
Saturated fat (mg/day)	19.45	(±7.32)	–	–	22.80	(9.40)	0.010
Monosaturated fat (mg/day)	22.07	(±10.48)	–	–	26.46	(11.73)	0.034
Polyunsaturated fat (mg/day)	16.16	(±11.15)	–	–	18.98	(8.71)	0.07
Vitamin A RAE (mcg/day)	698.31	(±441.46)	–	–	747.47	(245.94)	<0.002
Vitamin C (mg/day)	183.01	(±35.47)	–	–	162.42	(28.48)	<0.002
a-Tocopherol (mg/day)	27.35	(±15.91)	–	–	31.23	(11.45)	0.036

Data represented as *n* (%) or mean (SD). An one way Anova with repeated measures (for the variables measured at 3 different time intervals) and a paired *t* test and a Wilcoxon test (for the variables with 2 measurements) were performed to conduct intragroup comparisons. After the Bonferroni corrections the statistical significance was set at $P < 0.002$

RAE retinol active equivalents

[a] Participants who consume an average monthly volume of alcohol

remained statistically significant after an analysis of covariates, in which the levels of vitamin C were estimated [after the adjustment of vitamin C at the beginning of the study, the smoking habits and the BMI of the participants] (Table 4). Intragroup comparisons for the intervention group showed that the levels of serum vitamin C and CoQ10 were elevated during the intervention. The increase of CoQ10 did not remain significant after the Bonferroni correction (Table 2). Values for vitamin C of the control group assessed for the aforementioned time intervals did not follow the same trend (Table 3). For the control group, the levels of serum vitamin C and CoQ10 at the end of the study correlated positively and significantly ($r_p = 0.482$, $P = 0.023$). In the intervention group, serum levels of serum vitamin C and CoQ10 were increased significantly during the intervention by 30.3 and 38%, respectively.

Biochemical data

Comparisons between the two groups at the beginning showed that serum levels of blood glucose, TC, LDL-C, HDL-C and TG did not differ significantly. According to Table 1 the intervention group had lower BG (glycemic) levels at the end of the study ($P < 0.002$). During the intervention period HDL-C increased significantly in the same group ($P < 0.002$) (Table 2). Values of the rest of the biochemical parameters did not differ significantly between the two groups. Intragroup comparisons for the

Table 4 Analysis of covariates (ANCOVA) between the two groups at the end of the study

	F test	*P* value
Blood glucose levels adjusted for BMI and estimated weekly MET-mins	7.73	0.01
Vitamin C levels adjusted for BMI, smoking, vitamin C levels at the beginning of the study	6.99	0.01

Statistical significance was set at $P < 0.05$

control group showed that total serum cholesterol increased significantly.

Anthropometric and physical activity data

At the beginning, the two groups differed significantly regarding physical activity levels, BFM and PBFM (Supplementary Table 1), without showing the same trend at the end of the study (Table 1). For the intervention group BW, BMI, WC, BFM and PBFM decreased significantly. METs-min increased in the same group during the intervention period. Moreover, a significant negative correlation between physical activity levels and body fat percentage was observed at the end of the study ($r_p = -0.442$, $P = 0.02$) for the same group. BW, BFM and BMI of the control group increased significantly during the 6-month study, while the same trend was reversed for the METs-min.

[Bonferroni corrections have been applied for all variables (Tables 1, 2, 3)]. For the intervention group (Table 2) regarding the variables, weight, body fat mass, % body fat mass, waist circumference, BMI, HDL-cholesterol and serum vitamin C, the levels remained statistical significant. For the control group regarding the variables, weight, body fat mass, total cholesterol, MD score, vitamin C and A dietary consumption the levels remained statistical significant (Table 3).

Nutritional intake data

Comparisons between the two groups showed at the discharge that the intervention group had a significantly higher MD score and dietetic intake of vitamin A, vitamin C (Table 1). Furthermore, the control group consumed greater amount of polyunsaturated fats, and alpha-tocopherol ($P < 0.01$) and less amount of vitamin C and vitamin A ($P < 0.01$). Intragroup comparisons have shown that dietetic intake of vitamin C and A increased significantly for the control group. Regarding MD score, it was also significantly increased by 2.99 points in the intervention group and it exceeded the threshold of 6 in a scale from 0 to 9, which is indicative of one adhering more closely to the MD [35]. The adherence of the intervention group to the MD increased from 4.66 to 7.65 [and exceeded the threshold of 6 (Table 2), which is aligned with the third aim of this study].

For the intervention group, beginning serum vitamin A levels were associated with dietary intake levels of vitamin A during the same period ($r_p = 0.57$, $P < 0.01$), but not at the end of the study. CoQ10 levels at the discharge were significantly correlated with the intake of monounsaturated fats during the same period ($r_s = 0.61$, $P = 0.03$). Moreover, for intervention group, BMI was significantly negatively associated with MD score ($r_p = -0.362$, $P = 0.03$) as well as with serum a-tocopherol levels at the beginning ($r_p = -0.446$, $P = 0.01$). Still, there was no link between the dietary intake in the intervention group, as recorded in the diaries and FFQ MD (more information about FD and FFQ is available in Supplementary material). For the control group, the levels of vitamin C and CoQ10 in serum correlated positively and significantly.

Discussion

This randomized clinical trial showed that an individualized nutritional intervention based on MD and enriched with olive oil can significantly reduce the BMI, BW, WC, BFM and PBFM of overweight BC survivors. In the intervention group MD score increased which has been connected with BC risk. Characteristically, the EPIC study showed that an increase of the MD score by two points can reduce the risk of primary BC by up to 22% in postmenopausal women [35] and prevent approximately the 10% of all new BC incidences [35]. BC survivors could also benefit from a dietary pattern close to MD due to the fact that they have higher risk of second primary BC incidence [5–7].

Considering the first and the second target of our trial (% levels for PBFM and 10% loss of body weight in 6 months, respectively) for the intervention group, at the beginning of the study only two women had normal PBFM as given in [48], while the rest of our participants ($n = 24$) excited the recommended levels. At the end of the study, five women had normal PBFM in the same group. Moreover, those with increased baseline PBFM managed to decrease their weight by 5.3% after 6 months. Despite the fact that the first and the second objective of our study were not entirely accomplished, the intervention group managed to move towards

lower body weight in contrast to the women of the control group which increased their average body weight (Table 3).

During our trial, the dietary intake of vitamin C in the control group, significantly increased by approximately 204 mg/day, while a similar, modest, but non-significant trend was observed for vitamins E. In fact, a meta-analysis of Harris et al. [57], showed that an increase in dietary intake of vitamin C by 100 mg/day can reduce the risk of mortality from BC by 15%. Despite the fact that the intervention received personalized and intensified dietary treatment, the two groups were statistically different regarding their serum levels of vitamin E, vitamin A and CoQ10. These observations could be possibly explained by: (1) the greater and statistically significant increase of serum levels of vitamin A and E of the control group, (2) the enhanced dietetic consumption in SF, polyunsaturated fats, monounsaturated fats of the control group, (3) the study's withdrawal rate, (4) the type of intervention, as both groups received diet instructions, undergone anthropometry and were communicating with the registered dietitians. It is noteworthy, that the control group had not received individualized and quantified diet, and as such they consumed ad libitum amounts of fatty products, which could explain the aforementioned observations.

This study is in agreement with other studies illustrating the antioxidant potential of the ingredients of MD, such as fruits, vegetables and olive oil [58, 59]. Antioxidants from dietary intake, including vitamin A, vitamin E and vitamin C, have a significant role in the alleviation of oxidative stress, which could modify the risk of BC, as described in Ref. [27]. This risk is also integrated with body weight, body fat mass and aspects concerning patients' lifestyle, e.g., physical activity [60]. According to the American Cancer Institute, a higher BMI has been reported as a cause of BC for postmenopausal women and at the same time, overweight and obesity are associated with a higher risk of secondary tumors and all cause mortality rate [13, 61]. Characteristically, recurrence risk increases by 14% for every increase in BMI by five points [62]. In our study, participants of the intervention group, managed to reduce their BW significantly, and especially their BFM, even if weight gain is observed in the vast majority of BC survivors following anticancer treatment, including chemotherapy [8]. Furthermore, they managed to moderately increase their physical activity, which is associated with lower risk for second cancer, sarcopenic obesity and mortality [11].

In addition, participants of the intervention group improved their biochemical profile and indicators of metabolic syndrome, especially their WC and fasting BG levels in comparison with the control group. These findings are aligned with those of the PREDIMED study, which showed a reduction of diabetes incidence in individuals following a Mediterranean-type diet enriched with olive oil [40]. It is noteworthy that meta-analyses have shown that a poor glycemic control is associated with oxidative stress, primary BC and CVD risk [63–65].

Various results of this study piqued our interest. To begin with, despite the fact that this intervention was conducted in Greece, a country in the Mediterranean region, the volunteers had initially moderate or low adherence with MD, which was improved in the intervention group at the end of the study, indicating that there is much room left for dietetic improvements in this population. Another challenging issue was the absence of correlation between dietary intake of patients of both groups and the measured levels of serum antioxidants. One possible explanation is the deviation between the recorded and the actual food intake of patients, which could possibly modify our study's results.

The main limitations of the study stem from the fact, that a lifestyle intervention cannot be double blind. As a result, the same researchers, who contact the intervention group, contact also with the control group. For example the research team of DIANA-5 trial [60] report that due to ethical and practical reasons, a "contamination" of the control group with more detailed information and recommendations to improve their lifestyle and dietary habits is possible. That was the main reason in the present study, which led researchers to choose an impersonal communication via phone calls with the control group.

In addition, due to the small sample size of the study, the existence of dropout and the multiple comparisons between the two groups, the overall power of the analysis is expected to be low (more information is available in Supplementary material).

Conclusion

In conclusion, this study is a unique randomized clinical trial in postmenopausal women, who managed through an individualized dietary intervention based on MD to reduce their BW and body composition and at the same time to improve their blood glucose and antioxidant profile within a 6 months period. Due to the fact that these parameters are associated with (1) an increased risk of BC recurrence, (2) other comorbidities including CVD and (3) total mortality rates, BC survivors could be benefit from adhering to a dietary pattern based on traditional MD. Future studies could investigate the effect of the MD in other countries of the Mediterranean region. Moreover, additional studies should examine the perceptions of cancer patients regarding (a) their ability to comply to the MD in countries outside the Mediterranean region and (b) identify their expectations of a dietary intervention promoting the MD in their countries.

Acknowledgements The authors would like to express their gratitude to all the volunteers who participated in the study. The authors would like also to express their very great appreciation to (1) Nikolaos Skourlis for his help in the Supplementary material and to (2) Anna Maria Maragaki for her help concerning data collection.

Compliance with ethical standards

Conflict of interest M. Skouroliakou, D. Grosomanidis, P. Massara, C. Kostara, P. Papandreou, D. Ntountaniotis and G. Xepapadakis reported no conflict of interest related to the study.

References

1. Siegel R, Naishadham D, Jemal A (2013) Cancer statistics. CA Cancer J Clin 63(1):11–30. doi:10.3322/caac.21166
2. Chu KC, Tarone RE, Kessler LG, Ries LA, Hankey BF, Miller BA, Edwards BK (1996) Recent trends in US breast cancer incidence, survival, and mortality rates. J Natl Cancer Inst 88(21):1571–1579
3. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 49(6):1374–1403. doi:10.1016/j.ejca.2012.12.027
4. UK CR (2011). http://www.cancerresearchuk.org/health-professional/cancer-statistics/survival. Accessed 20 May 2016
5. Dong C, Hemminki K (2001) Second primary neoplasms in 633,964 cancer patients in Sweden, 1958–1996. Int J Cancer 93(2):155–161. doi:10.1002/ijc.1317
6. Soerjomataram I, Louwman WJ, Lemmens VE, de Vries E, Klokman WJ, Coebergh JW (2005) Risks of second primary breast and urogenital cancer following female breast cancer in the south of The Netherlands, 1972–2001. Eur J Cancer 41(15):2331–2337. doi:10.1016/j.ejca.2005.01.029
7. Rubino C, Arriagada R, Delaloge S, Le MG (2010) Relation of risk of contralateral breast cancer to the interval since the first primary tumour. Br J Cancer 102(1):213–219. doi:10.1038/sj.bjc.660543
8. Makari-Judson G, Braun B, Jerry DJ, Mertens WC (2014) Weight gain following breast cancer diagnosis: Implication and proposed mechanisms. World J Clin Oncol 5(3):272–282. doi:10.5306/wjco.v5.i3.272
9. Haque R, Prout M, Geiger AM, Kamineni A, Thwin SS, Avila C, Silliman RA, Quinn V, Yood MU (2014) Comorbidities and cardiovascular disease risk in older breast cancer survivors. Am J Manag Care 20(1):86–92
10. Parkin DM, Boyd L, Walker LC (2011) 16. The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010. Br J Cancer 105(Suppl 2):S77–S81. doi:10.1038/bjc.2011.489
11. Rock CL, Doyle C, Demark-Wahnefried W, Meyerhardt J, Courneya KS, Schwartz AL, Bandera EV, Hamilton KK, Grant B, McCullough M, Byers T, Gansler T (2012) Nutrition and physical activity guidelines for cancer survivors. CA Cancer J Clin 62(4):243–274. doi:10.3322/caac.21142
12. Ibrahim EM, Al-Homaidh A (2011) Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies. Med Oncol 28(3):753–765. doi:10.1007/s12032-010-9536-x
13. Sheppard VB, Hicks J, Makambi K, Hurtado-de-Mendoza A, Demark-Wahnefried W, Adams-Campbell L (2016) The feasibility and acceptability of a diet and exercise trial in overweight and obese black breast cancer survivors: The Stepping STONE study. Contemp Clin Trials 46:106–113. doi:10.1016/j.cct.2015.12.005
14. Nichols HB, Trentham-Dietz A, Egan KM, Titus-Ernstoff L, Holmes MD, Bersch AJ, Holick CN, Hampton JM, Stampfe MJ, Willet WC, Newcomb PA (2009) Body mass index before and after breast cancer diagnosis: associations with all-cause, breast cancer, and cardiovascular disease mortality. Cancer Epidemiol Biomark Prev 18(5):1403–1409. doi:10.1158/1055-9965.EPI-08-1094
15. Goodwin PJ, Boyd NF (1990) Body size and breast cancer prognosis: a critical review of the evidence. Breast Cancer Res Treat 16(3):205–214
16. Protani M, Coory M, Martin JH (2010) Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. Breast Cancer Res Treat 123(3):627–635. doi:10.1007/s10549-010-0990-0
17. Vera-Ramirez L, Sanchez-Rovira P, Ramirez-Tortosa MC, Ramirez-Tortosa CL, Granados-Principal S, Lorente JA, Quiles JL (2011) Free radicals in breast carcinogenesis, breast cancer progression and cancer stem cells. Biological bases to develop oxidative-based therapies. Crit Rev Oncol Hematol 80(3):347–368. doi:10.1016/j.critrevonc.2011.01.004
18. Nourazarian AR, Kangari P, Salmaninejad A (2014) Roles of oxidative stress in the development and progression of breast cancer. Asian Pac J Cancer Prev 15(12):4745–4751
19. Allen RG, Tresini M (2000) Oxidative stress and gene regulation. Free Radic Biol Med 28(3):463–499
20. Sangeetha P, Das UN, Koratkar R, Suryaprabha P (1990) Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic Biol Med 8(1):15–19
21. Michels KB, Mohllajee AP, Roset-Bahmanyar E, Beehler GP, Moysich KB (2007) Diet and breast cancer: a review of the prospective observational studies. Cancer 109(Suppl 12):2712–2749
22. Knekt P, Aromaa A, Maatela J, Aaran R-K, Nikkari T, Hakama M, Hakulinen T, Peto R, Teppo L (1990) Serum vitamin A and subsequent risk of cancer: cancer incidence follow-up of the Finnish Mobile Clinic Health Examination Survey. Am J Epidemiol 132(5):857–870. doi:10.1093/oxfordjournals.aje.a115728
23. Gerber M, Segala C (1992) Aging and cancer: plasma antioxidants and lipid peroxidation in young and aged breast cancer patients. EXS 62:235–246
24. Folkers K, Osterborg A, Nylander M, Morita M, Mellstedt H (1997) Activities of vitamin Q10 in animal models and a serious deficiency in patients with cancer. Biochem Biophys Res Commun 234(2):296–299. doi:10.1006/bbrc.1997.6522
25. Cooney RV, Dai Q, Gao YT, Chow WH, Franke AA, Shu XO, Li H, Ji B, Cai Q, Chai W, Zheng W (2011) Low plasma coenzyme Q(10) levels and breast cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev 20(6):1124–1130. doi:10.1158/1055-9965.EPI-10-1261
26. Bakker MF, Peeters PH, Klaasen VM, Bueno-de-Mesquita HB, Jansen EH, Ros MM, Travier N, Olsen A, Tjonneland A, Overvad K, Rinaldi S, Romieu I, Brennan P, Boutron-Ruault MC, Perquier F, Cadeau C, Boeing H, Aleksandrova K, Kaaks R, Kuhn T, Trichopoulou A, Lagiou P, Trichopoulos D, Vineis P, Krogh V, Panico S, Masala G, Tumino R, Weiderpass E, Skeie G, Lund E, Quiros JR, Ardanaz E, Navarro C, Amiano P, Sanchez MJ, Buckland G, Ericson U, Sonestedt E, Johansson M, Sund M, Travis RC, Key TJ, Khaw KT, Wareham N, Riboli E, van Gils CH (2016) Plasma carotenoids, vitamin C, tocopherols, and retinol and the risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition cohort. Am J Clin Nutr 103(2):454–464. doi:10.3945/ajcn.114.101659
27. Shah FD, Patel JB, Shukla SN, Shah PM, Patel PS (2009) Evaluation of plasma non-enzymatic antioxidants in breast cancer etiology. Asian Pac J Cancer Prev 10(1):91–96

28. Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K (2001) Which plasma antioxidants are most related to fruit and vegetable consumption? Am J Epidemiol 154(12):1113–1118
29. Albuquerque RC, Baltar VT, Marchioni DM (2014) Breast cancer and dietary patterns: a systematic review. Nutr Rev 72(1):1–17. doi:10.1111/nure.12083
30. Trichopoulos D, Lagiou P (2004) Mediterranean diet and cardiovascular epidemiology. Eur J Epidemiol 19(1):7–8
31. Trichopoulou A (2004) Traditional Mediterranean diet and longevity in the elderly: a review. Public Health Nutr 7(7):943–947. doi:10.1079/PHN2004558
32. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH et al (1986) The diet and 15-year death rate in the seven countries study. Am J Epidemiol 124(6):903–915
33. Toledo E, Salas-Salvado J, Donat-Vargas C, Buil-Cosiales P, Estruch R, Ros E, Corella D, Fito M, Hu FB, Aros F, Gomez-Gracia E, Romaguera D, Ortega-Calvo M, Serra-Majem L, Pinto X, Schroder H, Basora J, Sorli JV, Bullo M, Serra-Mir M, Martinez-Gonzalez MA (2015) Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED Trial: a randomized clinical trial. JAMA Intern Med 175(11):1752–1760. doi:10.1001/jamainternmed.2015.4838
34. Mourouti N, Kontogianni MD, Papavagelis C, Plytzanopoulou P, Vassilakou T, Malamos N, Linos A, Panagiotakos DB (2014) Adherence to the Mediterranean diet is associated with lower likelihood of breast cancer: a case–control study. Nutr Cancer 66(5):810–817. doi:10.1080/01635581.2014.916319
35. Trichopoulou A, Bamia C, Lagiou P, Trichopoulos D (2010) Conformity to traditional Mediterranean diet and breast cancer risk in the Greek EPIC (European Prospective Investigation into Cancer and Nutrition) cohort. Am J Clin Nutr 92(3):620–625. doi:10.3945/ajcn.2010.29619
36. Schwingshackl L, Hoffmann G (2016) Does a Mediterranean-type diet reduce cancer risk? Curr Nutr Rep 5:9–17. doi:10.1007/s13668-015-0141-7141
37. Couto E, Boffetta P, Lagiou P, Ferrari P, Buckland G, Overvad K, Dahm CC, Tjonneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Cottet V, Trichopoulos D, Naska A, Benetou V, Kaaks R, Rohrmann S, Boeing H, von Ruesten A, Panico S, Pala V, Vineis P, Palli D, Tumino R, May A, Peeters PH, Bueno-de-Mesquita HB, Buchner FL, Lund E, Skeie G, Engeset D, Gonzalez CA, Navarro C, Rodriguez L, Sanchez MJ, Amiano P, Barricarte A, Hallmans G, Johansson I, Manjer J, Wirfart E, Allen NE, Crowe F, Khaw KT, Wareham N, Moskal A, Slimani N, Jenab M, Romaguera D, Mouw T, Norat T, Riboli E, Trichopoulou A (2011) Mediterranean dietary pattern and cancer risk in the EPIC cohort. Br J Cancer 104(9):1493–1499. doi:10.1038/bjc.2011.106
38. Pitsavos C, Panagiotakos DB, Tzima N, Chrysohoou C, Economou M, Zampelas A, Stefanadis C (2005) Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: the ATTICA study. Am J Clin Nutr 82(3):694–699
39. Dilis V, Trichopoulou A (2010) Antioxidant intakes and food sources in Greek adults. J Nutr 140(7):1274–1279. doi:10.3945/jn.110.121848
40. Salas-Salvado J, Bullo M, Babio N, Martinez-Gonzalez MA, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Aros F, Ruiz-Gutierrez V, Ros E (2011) Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes Care 34(1):14–19. doi:10.2337/dc10-1288
41. Penalvo JL, Oliva B, Sotos-Prieto M, Uzhova I, Moreno-Franco B, Leon-Latre M, Ordovas JM (2015) Greater adherence to a Mediterranean dietary pattern is associated with improved plasma lipid profile: the Aragon Health Workers Study cohort. Rev Esp Cardiol (Engl Ed) 68(4):290–297. doi:10.1016/j.rec.2014.09.019
42. Bland KCE (2009) The breast—comprehensive management of benign and malignant diseases, 4th edn. Saunders, Philadelphia
43. Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, Gapstur S, Patel AV, Andrews K, Gansler T (2012) American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin 62(1):30–67. doi:10.3322/caac.20140
44. Harasym J, Oledzki R (2014) Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. Nutrition 30(5):511–517. doi:10.1016/j.nut.2013.08.019
45. Flower G, Fritz H, Balneaves LG, Verma S, Skidmore B, Fernandes R, Kennedy D, Cooley K, Wong R, Sagar S, Fergusson D, Seely D (2014) Flax and breast cancer: a systematic review. Integr Cancer Ther 13(3):181–192. doi:10.1177/1534735413502076
46. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87(23):1767–1776
47. Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y (2000) Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. Am J Clin Nutr 72(3):694–701
48. NHLBI NHLI Target Levels for Weight Loss (2016) http://www.nhlbi.nih.gov/health-pro/guidelines/current/obesity-guidelines/e_textbook/txgd/4311.htm. Accessed 20 June 2017
49. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, Medina FX, Battino M, Belahsen R, Miranda G, Serra-Majem L (2011) Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 14(12A):2274–2284. doi:10.1017/S1368980011002515
50. Mourouti N, Papavagelis C, Psaltopoulou T, Aravantinos G, Samantas E, Filopoulos E, Manousou A, Plytzanopoulou P, Vassilakou T, Malamos N, Panagiotakos DB (2013) Aims, design and methods of a case-control study for the assessment of the role of dietary habits, eating behaviors and environmental factors, on the development of breast cancer. Maturitas 74(1):31–36. doi:10.1016/j.maturitas.2012.10.004
51. Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D (1995) Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 61(6 Suppl):1402S–1406S
52. Trichopoulou A, Georga K (2004) Composition tables of foods and Greek dishes, 3rd Edn. Parisianou Publications, Athens, GA
53. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348(26):2599–2608. doi:10.1056/NEJMoa025039348/26/2599
54. Papathanasiou G, Georgoudis G, Papandreou M, Spyropoulos P, Georgakopoulos D, Kalfakakou V, Evangelou A (2009) Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. Hellenic J Cardiol 50(4):283–294
55. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5(6):649–655
56. Centers for Disease Control and prevention (CDC, 2015) About Adult BMI. https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html. Accessed 20 June 2017
57. Harris HR, Orsini N, Wolk A (2014) Vitamin C and survival among women with breast cancer: a meta-analysis. Eur J Cancer 50(7):1223–1231. doi:10.1016/j.ejca.2014.02.013

58. Potentas E, Witkowska AM, Zujko ME (2015) Mediterranean diet for breast cancer prevention and treatment in postmenopausal women. Prz Menopauzalny 14(4):247–253. doi:10.5114/pm.2015.5638126384
59. Wise J (2015) Reduced breast cancer risk seen with Mediterranean diet and added olive oil. BMJ 351:h4911
60. Villarini A, Pasanisi P, Traina A, Mano MP, Bonanni B, Panico S, Scipioni C, Galasso R, Paduos A, Simeoni M, Bellotti E, Barbero M, Macellari G, Venturelli E, Raimondi M, Bruno E, Gargano G, Fornaciari G, Morelli D, Seregni E, Krogh V, Berrino F (2012) Lifestyle and breast cancer recurrences: the DIANA-5 trial. Tumori 98(1):1–18. doi:10.1700/1053.11494
61. Demark-Wahnefried W, Platz EA, Ligibel JA, Blair CK, Courneya KS, Meyerhardt JA, Ganz PA, Rock CL, Schmitz KH, Wadden T, Philip EJ, Wolfe B, Gapstur SM, Ballard-Barbash R, McTiernan A, Minasian L, Nebeling L, Goodwin PJ (2012) The role of obesity in cancer survival and recurrence. Cancer Epidemiol Biomarkers Prev 21(8):1244–1259. doi:10.1158/1055-9965.EPI-12-0485
62. Druesne-Pecollo N, Touvier M, Barrandon E, Chan DS, Norat T, Zelek L, Hercberg S, Latino-Martel P (2012) Excess body weight and second primary cancer risk after breast cancer: a systematic review and meta-analysis of prospective studies. Breast Cancer Res Treat 135(3):647–654. doi:10.1007/s10549-012-2187-1
63. De Bruijn KM, Arends LR, Hansen BE, Leeflang S, Ruiter R, van Eijck CH (2013) Systematic review and meta-analysis of the association between diabetes mellitus and incidence and mortality in breast and colorectal cancer. Br J Surg 100(11):1421–1429. doi:10.1002/bjs.9229
64. Boyle P, Boniol M, Koechlin A, Robertson C, Valentini F, Coppens K, Fairley LL, Zheng T, Zhang Y, Pasterk M, Smans M, Curado MP, Mullie P, Gandini S, Bota M, Bolli GB, Rosenstock J, Autier P (2012) Diabetes and breast cancer risk: a meta-analysis. Br J Cancer 107(9):1608–1617. doi:10.1038/bjc.2012.414
65. Tabatabaei-Malazy O, Fakhrzadeh H, Sharifi F, Mirarefin M, Arzaghi SM, Badamchizadeh Z, Alizadeh Khoee M, Larijani B (2015) Effect of metabolic control on oxidative stress, subclinical atherosclerosis and peripheral artery disease in diabetic patients. J Diabetes Metab Disord 14:84. doi:10.1186/s40200-015-0215-5215