Change in inflammatory biomarkers and adipose tissue in BRCA1/2+ breast cancer survivors following a year-long lifestyle modification program

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Abstract

Breast cancer survivors who carry a genetic mutation for one of the BRCA genes often undergo surgically-induced menopause a decade or more before the usual age of natural menopause. These women are at elevated risk for multiple negative health outcomes, including metabolic diseases, heart disease, and cancer recurrence. Effects of a 12-month commercially available web-based lifestyle program (Precision Nutrition) were tested on body composition and markers of inflammation in a randomized controlled trial. Participants (N=35) were BRCA1/2+, breast cancer survivors, and had completed surgically-induced menopause at age <45 years. DXA was used to quantify body composition. Fasting blood samples were used to assay insulin, IL1β, IL6, IL8, and TNFα. At baseline, we observed relationships between insulin, TNFα, and IL6, and between biomarkers and adiposity. Insulin and subcutaneous adipose tissue levels significantly decreased following the intervention compared to the change in the control group. Compared to baseline, TNFα and total adipose tissue levels decreased significantly in the intervention group. The percent change in insulin levels was moderately correlated with the percent change in subcutaneous adipose tissue ($r = 0.33$). Change in adiposity was not related to change in TNFα or IL6. Women in the intervention group decreased levels of subcutaneous, but not visceral, adipose tissue. The change in subcutaneous adipose tissue was the main driver of change in insulin levels for the women in the intervention group. However, the change in body composition achieved by the Precision Nutrition program was not sufficient to alter biomarker levels of inflammation.

Keywords: inflammation, insulin, biomarker, exercise, lifestyle modification, weight loss, BRCA1/2, breast cancer survivor, body composition, subcutaneous adipose tissue, visceral adipose tissue

Introduction

Chronic inflammation generates an excess of reactive oxygen and nitrogen species triggering DNA damage and malignancy (1,2). Chronic low-grade inflammation is also a risk factor for cardiovascular disease (3), metabolic diseases (4), and breast cancer recurrence and mortality (5,6). The concept of chronic inflammation, prolonged reactive oxygen species production, and activation of stress-linked pathways is considered central to the progression of many inflammatory diseases (7). The link between inflammatory processes, induction of reactive oxygen species, and resultant DNA damage is particularly important for individuals with defects in genes associated with DNA damage response, such as BRCA1/2.

The BRCA1 and BRCA2 proteins are involved in repairing DNA damage. Loss of BRCA function results in impaired ability to repair DNA double strand breaks. For women carrying BRCA1 or BRCA2 mutations, loss of BRCA function is not only linked to breast or ovarian cancer, but also other chronic diseases associated with chronic inflammation and metabolic dysregulation (8). Indeed, BRCA1 or BRCA2 mutation carriers have an elevated risk of overall mortality compared to non-carriers which is beyond the increased risk for cancer (9). Female BRCA1/2+ mutation carriers have a 5.7 year lower life expectancy compared to female non-carriers (9).

One source of chronic inflammation is obesity (10,11). Obesity and chronic inflammation are intrinsically linked to sedentary behavior, and together there is strong evidence for increased risk of cardiovascular disease, diabetes, and cancer recurrence risk in obese and or sedentary breast cancer survivors (6,12,13). Weight loss, aerobic exercise training, and resistance exercise training are well established with regard to decreases in inflammation and oxidative stress (14-18) as well as improved regulation of insulin (19,20). Breast cancer survivors carrying a BRCA1/2 mutation are at elevated risk for breast cancer recurrence or metabolic disorders. Thus, identifying scale-able interventions that will

decrease risk in this population is of even greater importance compared to the general population.

We conducted a randomized controlled lifestyle modification trial for breast cancer survivors who were BRCA1 or BRCA2 mutation carriers and had also elected to undergo a prophylactic salpingo-oophorectomy before age 45 (21). Participants were randomized to a waitlist control group or an intervention group which took part in a commercially available web-based weight loss program called Precision Nutrition Coaching, from Precision Nutrition Inc. This novel web-based behavioral intervention utilized best practices to improve dietary habits and increase levels of aerobic as well as resistance exercise (21,22). We assessed the effect of the Precision Nutrition lifestyle modification program on levels of insulin, inflammatory cytokines (IL1β, IL6, IL8, and TNFα), and body composition.

Methods

Detailed information on study recruitment, randomization, the intervention, and main results have been reported previously (21). Briefly, the eligibility criteria for the Project HOPE (Heart disease Osteoporosis Prevention and Efficacy) study was: aged 18-55, BRCA1/2+ breast cancer survivors who underwent prophylactic oophorectomy two or more years prior to study initiation and were age ≤ 45 at date of oophorectomy, breast cancer treatment was completed at least 4 months prior to study initiation, hormone replacement therapy was not used for at least 2 years prior to study initiation, physician clearance to participate in the weight loss and exercise program was received, had a BMI ≥ 23 kg/m^2, and were weight stable over the past year (e.g. no changes greater than 10%). All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of Pennsylvania and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Human Subjects Review Committee at the University of Pennsylvania and informed consent was obtained from all

subjects in writing prior to beginning study activities.

Intervention

Women were randomized into an intervention or control arm. The Precision Nutrition program included access (phone, email, or Skype) to a Precision Nutrition coach and completion of three daily activities: 1) exercises; 2) completing a nutritional/lifestyle habit, and 3) reading health related education material (21,22). The exercise component was completed at home or at a local gym and required 160 min/wk of exercise (3 days/week of progressive resistance exercise, 2 days/week of interval aerobic exercise, and 1 day/week of active recovery aerobic exercise). The habit and education components were linked and included activities and topics such as: eating lean protein with every meal and the importance of protein in your diet, eating a variety of vegetables and fruit, stopping eating when 80% full, and how your digestive system communicates with the brain. A new habit was introduced every 2 weeks and the reading material was updated daily. Women in the intervention group logged into their online portal daily to read the educational material, log completion of their habit, and view their personalized (by the Precision Nutrition Coach) progressive exercise program and log progress in the program. Control group participants were asked to maintain their usual daily activities.

Physical Measurements

All measurements took place on the campus of the University of Pennsylvania. Demographic characteristics (age, race, ethnicity, and education) as well as cancer treatment modalities were self-reported. Dual-energy x-ray absorptiometry (DXA) (Hologic, Bedford MA) was used to measure body composition. All scans were reviewed by a bio-nutritionist who was blinded to the study group. The DXA scanner was calibrated daily using a soft-tissue phantom. DXA was used to quantify appendicular skeletal muscle mass (ASMM), total adipose tissue (TAT), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT), using APEX v13.4 software. DXA-derived VAT has been validated

against CT-derived VAT (23). DXA scans were completed at baseline and follow up on the same instrument.

Biomarker Assays

Fasting blood draws (≥12 hrs) were collected at baseline and follow up. Blood was drawn in the morning (before 10am), patients were instructed to not perform any acute exercise in the 24 hours prior to the blood draw. Standard venipuncture procedures were followed and blood was drawn into EDTA or serum separator vacutainers. Tubes were centrifuged and plasma was aliquoted and stored at -80°C. Processing occurred within one hour of collection. All assays were conducted using freshly thawed samples and measured in duplicate. Baseline and follow up samples collected from the same women were assayed simultaneously at the end of the study and both intervention and control participants were distributed across the assay plates. Insulin was assayed from serum using an RIA kit (HI-14K, Millipore Sigma, Burlington, MA). The laboratory test was performed following the assay manual in the Radioimmunoassay and Biomarkers Core at the University of Pennsylvania. The mean intra-assay coefficient of variation was 1.78%. IL1β, IL6, IL8, TNFα were assayed from plasma on a commercial multiplex assay (U-plex K15053K, Meso Scale Discovery, Rockville, MD) following manufacturer's instructions by the Translational Core Laboratory of The Children's Hospital of Philadelphia. The mean intra-assay coefficient of variation was: IL1β, 5.1%; IL6, 4.1%; IL8, 2.7%, and TNFα, 3.5%.

Statistical Analysis

Statistical analyses were conducted using STATA version 14.1 (Stata Corp., College Station, TX), and SAS version 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics presented for demographics include counts and proportions for categorical variables and mean ± SD for continuous variables. Baseline differences between intervention groups were assessed using χ^2 statistics for categorical variables. Student's t-test was used to assess differences between groups at baseline for

continuous variables and repeated measures t-tests were used to assess differences within group for continuous variables. Pearson correlation coefficients were calculated between baseline biomarkers and baseline body composition, as well as, percent change in biomarkers and percent change in body composition variables. Changes in biomarkers and body composition were evaluated from baseline to follow up between the control and intervention group using a baseline adjusted linear regression model. All analyses were repeated after logarithmically transforming cytokine and body composition values to account for skewed distributions but are not shown as the results were unchanged. Significance level was set at $\alpha \leq 0.05$. The study was not designed or powered for biomarker outcomes. However, prior to conducting biomarker assays a power analysis was conducted for insulin levels. Given our sample size of 35 women, we estimated 72% power to detect a significant change in insulin levels, based on change found in a similar study (24).

Results

There were no significant differences between the intervention group and the control group at baseline with regard to demographics, biomarkers, or body composition. Our study population of breast cancer survivors was younger than the median age of US breast cancer diagnosis (62 years of age), white, and predominantly: non-Hispanic, recipients of a 4 year college degree or more, and had breast cancer treatment plans that included chemotherapy (Table 1). We have previously reported that both the control and intervention groups decreased caloric intake at follow up relative to their baseline caloric intake levels (21). However, only the intervention group significantly increased physical activity level which led to decreased fat mass and % body fat compared to a year earlier, while the control group lost lean body mass compared to a year earlier Overall adherence to the Precision Nutrition program was 74.8%. Detail on how adherence was calculated has been previously reported (21).

Given the above observed changes in body composition due to the intervention, and, given that our study population are BRCA1/2 mutation carriers and therefore at elevated risk of diseases associated with adipose tissue-linked chronic inflammation, we assessed effects of our intervention on biomarkers of chronic diseases linked to inflammation. In Table 2 we show correlations of baseline biomarker levels with each other and also baseline body composition. We observed a positive relationship between insulin levels and TNFα levels, as well as a positive relationship between TNFα levels and IL6 levels. The strongest association was observed between insulin levels and TAT levels. TNFα had a moderate association to TAT, with high levels of adiposity related to higher levels of TNFα. For both insulin and TNFα, the association between these biomarkers and adiposity appears to be most strongly associated with SAT levels as opposed to VAT levels in BRCA1/2+ breast cancer survivors.

The change in levels of biomarkers (Table 3) and body composition variables (Table 4) following the intervention was assessed. We observed a significant decrease in insulin levels in the intervention group compared to an increase in insulin levels in the control group. We did not observe any significant changes in cytokine levels in the intervention group compared to change in control levels. Within the control group, IL8 levels decreased significantly from baseline to follow up. Within the intervention group, TNFα levels significantly decreased from baseline to follow up. There was a trend in the intervention group for decreased TAT levels compared to the change in the control group. The decrease in TAT is driven by significant changes to SAT levels as the decrease in SAT in the intervention group compromises over 94% of the decrease in TAT. There were no changes in appendicular skeletal mass.

At baseline, we observed a relationship between insulin, TNFα, and IL6 levels. Additionally, strong correlations were observed between baseline biomarker levels and baseline SAT levels. Further,

we observed significant or near significant changes for these variables with regard to the main effect of the intervention. Therefore, we assessed the relationship between percent change in levels of insulin, TNFα, IL6, and percent change in volume of adipose tissue. Decreases in insulin levels were moderately correlated (r=0.34, P=0.06) with decreases in TAT, and moderately correlated (r=0.33, P=0.08) with decreases in SAT levels, but no correlation was found to VAT (r=0.29, P=0.11). A 10% change in TAT levels was associated with an 8.9% change in insulin levels (y = 0.8268x + 0.6643). No associations were observed between change in TAT, SAT, or VAT volume and change in TNFα or IL6 levels.

Discussion

BRCA1 or BRCA2 mutation carriers have a reduced life expectancy, even after excluding deaths due to cancers that have been shown to be related to these mutations (9). Thus, our study population of BRCA1/2+ breast cancer survivors is at elevated risk not only for death from breast cancer recurrence and other cancers, but also chronic metabolic diseases (8). As inflammation is a common mechanism in the etiology of chronic diseases, we examined biomarkers associated with inflammatory states (insulin, IL1β, IL6, IL8, and TNFα). Given the known link between inflammation and adiposity (10,11), we explored this relationship in our high risk study population. The goal of this post hoc analysis was to determine if a lifestyle modification program such as Precision Nutrition would be efficacious in decreasing biomarkers associated with inflammatory states, and if changes in inflammatory biomarkers would be related to changes in adiposity.

The intervention, Precision Nutrition, is a commercial product that incorporates the science of exercise physiology and implements best practices for health behavior changes (21,25,26). The online platform also allows the intervention to be administered remotely for a wide audience, and

systematically for consistent results. The major finding of our study is that we observed a main intervention effect for significantly decreased insulin and SAT levels. We also observed a within group effect of the intervention for significantly decreased TNFα and TAT levels. There is a paucity of research on the effects of a year-long lifestyle modification (diet and exercise) program on inflammatory biomarkers in breast cancer survivors. We have identified four randomized trials with multi-component interventions of at least 6 months in breast cancer survivors (non-BRCA1/2 mutation carriers) that also measured insulin levels (24,27-29). Befort et al. report a significant decrease in insulin levels which were accompanied by significant decreases in body weight and caloric intake (27). Campbell et al. and Greenlee et al. did not observe a main effect in their intervention for decreased insulin levels, however, they also did not observe a main effect for decreased caloric intake even though they observed a decrease in levels of body fat (28,29). Lastly, Rock et al. conducted a stratified analysis and reported decreased insulin levels in women who decreased their weight by 5% or more (24). To our knowledge, our study is the first to report on levels of inflammatory biomarkers following a 6+ month multi-component lifestyle modification program in breast cancer survivors.

In addition to the intervention effects, we also observed that while insulin and TNFα levels were associated with adiposity at baseline, only insulin levels changed concomitant to changes in adiposity following the Precision Nutrition intervention. Further, the change in adiposity was predominantly driven by decreases in subcutaneous adipose tissue levels. Greenlee et al. and Rock et al. also observed decreased insulin levels concomitant with changes in body composition. Greenlee et al. report decreased insulin levels with ≥ 2% fat loss (28), and Rock et al. report decreased insulin levels with ≥ 5% weight loss (24). We observed that a 10% change in TAT levels is associated with a 9% change in insulin levels.

Decreased levels of SAT achieved through the Precision Nutrition program may be particularly

important for breast cancer survivors. SAT includes breast white adipose tissue, and it has been previously reported that an inflammatory state in breast white adipose tissue occurs in the majority of women with elevated BMI and is associated with increased levels of aromatase, the rate-limiting enzyme for estrogen biosynthesis (30). Inflammation of white adipose tissue is also seen in women with normal BMI, and is associated with elevated levels of insulin (31).

The primary goals of the Project HOPE study were to examine changes in cardiovascular fitness and bone health in BRCA1/2+ breast cancer survivors who are at elevated risk of cardiovascular events and osteoporosis due to early age at surgically-induced menopause. The study was not powered to detect differences in biomarkers, and thus, the small sample size may impact our conclusions. While the sample size is a limitation, the sample was drawn from a national, rather than local, recruitment. Despite the wide recruitment, our sample was 100% Caucasian and thus is not representative and affects the ability to generalize our findings. Future directions for this study are two-fold: 1) compare responses in inflammatory biomarkers between BRCA1/2+ mutation carriers and non-carriers, and 2) assess local changes in breast tissue inflammation in order to better link systemic changes seen in biomarkers to local tissue adaptations. Both of these would be best explored in a study specifically powered to detect changes in inflammatory biomarkers. Further, it may be less important to focus on breast cancer survivors than to focus on BRCA1/2+ mutation carriers, regardless of cancer history.

In summary, we report that a yearlong, multi-component, commercially available, web-based, lifestyle modification program was successful in decreasing insulin levels and subcutaneous adipose tissue levels. While there were also decreases in TNFα levels, this change was not associated with changes in body composition. While the mechanisms linking adiposity to inflammation are still evolving, this connection may be particularly important for BRCA1/2+ breast cancer survivors. There is a two-fold

increase in risk for breast cancer recurrence and three-fold increase in risk of death in patients with the highest quartiles of fasting insulin levels (32). Thus, in a population genetically predisposed to increased risk of breast cancer and overall mortality, management of the insulin, adiposity, and inflammation axis may increase life expectancy.

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Table 1. Demographics and breast cancer treatment characteristics

Characteristics	Total Sample (n=35)	Control (n=16)	Intervention (n=19)	P-value
Age (y)	46.1 ± 4.0	47.2 ± 3.8	45.1 ± 4.0	0.12
BMI (kg/m^2)	29.9 ± 4.8	29.6 ± 5.1	30.2 ± 4.6	0.71
Race, no. (%)				
White	35 (100%)	16 (100%)	19 (100%)	--
Ethnicity, no. (%)				
Hispanic	2 (6%)	2 (13%)	0 (0%)	0.11
Non-Hispanic	33 (94%)	14 (87%)	19 (100%)	
Education, no. (%)				
High school or less	1 (3%)	0 (0%)	1 (5%)	0.65
Some college	2 (6%)	1 (6%)	1 (5%)	
College or more	32 (91%)	15 (94%)	17 (90%)	
Treatment, no. (%)				
Radiation	14 (40%)	7 (44%)	7 (37%)	0.46
Chemotherapy	29 (83%)	15 (94%)	14 (74%)	0.12
Immunotherapy	4 (14%)	2 (13%)	2 (10%)	0.85

Values are presented as mean ± SD.

Table 2. Correlations between biomarkers and body composition at baseline

	Insulin (µIU/ml)	IL1β (pg/ml)	IL6 (pg/ml)	IL8 (pg/ml)	TNFα (pg/ml)
Insulin (µIU/ml)					
IL1β (pg/ml)	-0.09, p=0.64				
IL6 (pg/ml)	0.27, p=0.15	0.06, p=0.75			
IL8 (pg/ml)	-0.01, p=0.99	0.05, p=0.77	-0.04, p=0.82		
TNFα (pg/ml)	**0.39, p=0.03**	-0.01, p=0.95	**0.39, p=0.02**	0.07, p=0.67	
ASMM (kg)	0.11, p=0.55	0.14, p=0.43	0.17, p=0.34	0.14, p=0.43	0.14, p=0.42
Total AT (cm^3)	**0.57, p<0.01**	0.08, p=0.65	0.22, p=0.20	0.22, p=0.21	**0.35 p=0.04**
Visceral AT (cm^3)	0.35, p=0.06	0.12, p=0.49	0.23, p=0.17	0.28, p=0.11	-0.03, p=0.87
Subcutaneous AT (cm^3)	**0.55, p<0.01**	0.06, p=0.75	0.19, p=0.27	0.17, p=0.33	**0.41, p=0.01**

Appendicular skeletal muscle mass (ASMM), adipose tissue (AT); Values are presented as Pearson correlation coefficient, r, and p value.

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Table 3. Change in biomarkers.

	Control			Intervention			
Variable	Baseline (mean ± SD)	Final (mean ± SD)	Change (LSM ± SE)	Baseline (mean ± SD)	Final (mean ± SD)	Change (LSM ± SE)	P-value
Insulin (uIU/ml)	12.28 ± 3.56	12.80 ± 5.02	0.52 ± 1.05	10.64 ± 4.19	8.76 ± 3.14	-1.88 ± 0.98	0.03
IL1β (pg/ml)	0.13 ± 0.06	0.09 ± 0.04	- 0.04 ± 0.11	0.25 ± 0.62	0.11 ± 0.04	-0.14 ± 0.10	0.43
IL6 (pg/ml)	1.43 ± 1.59	1.36 ± 1.01	-0.06 ± 0.27	1.01 ± 0.90	0.78 ± 0.57	-0.24 ± 0.25	0.09
IL8 (pg/ml)	5.34 ± 1.96	4.56 ± 1.20 [a]	-0.78 ± 0.38	5.08 ± 1.07	5.07 ± 1.49	0.07 ± 0.36	0.15
TNFα (pg/ml)	2.18 ± 0.52	2.08 ± 0.43	-0.11± 0.13	2.40 ± 1.18	2.16 ± 0.85 [a]	-0.30 ± 0.12	0.49

Values are presented as mean ± SD and least squares mean (LSM) ± SEM. [a]P < 0.05 within group, P-value baseline adjusted linear regression model

Table 4. Change in body composition.

	Control			Intervention			
Variable	Baseline (mean ± SD)	Final (mean ± SD)	Change (LSM ± SE)	Baseline (mean ± SD)	Final (mean ± SD)	Change (LSM ± SE)	p-value
ASMM (kg)	19.9 ± 3.5	19.9 ± 3.6	0.04 ± 0.2	20.8 ± 3.2	20.7 ± 3.1	-0.1 ± 0.2	0.72
Total AT (cm^3)	2795.0 ± 842.5	2798.0 ± 813.6	3.0 ± 68.7	2906.6 ± 691.6	2576.0 ± 785.2[a]	-196.5 ± 66.3	0.06
Visceral AT (cm^3)	543.0 ± 162.6	551.6 ± 148.6	8.5 ± 23.4	553.0 ± 217.8	480.9 ± 198.4	-13.6 ± 22.6	0.47
Subcutaneous AT (cm^3)	2252.0 ± 729.5	2246.4 ± 714.2	-5.5 ± 51.8	2353.7 ± 585.1	2095.1± 655.3[a]	-185.8 ± 49.9	0.03

Appendicular skeletal muscle mass (ASMM); Adipose tissue (AT); Values are presented as mean ± SD and least squares mean (LSM) ± SEM. [a]P < 0.01 within group, P-value baseline adjusted linear regression model

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Change in inflammatory biomarkers and adipose tissue in BRCA1/2+ breast cancer survivors following a year-long lifestyle modification program

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