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Randomized Trial Of Weight Loss On Ghrelin Levels Among Breast Cancer Survivors

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RANDOMIZED TRIAL OF WEIGHT LOSS ON GHRELIN LEVELS AMONG BREAST CANCER SURVIVORS

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Contents

Abstract

Purpose: Obesity is associated with increased breast cancer risk and mortality. Ghrelin is a hormone that participates in a negative feedback loop to regulate body weight. Understanding the impact of an individualized, sustainable, dietary and exercise weight-loss intervention on circulating ghrelin levels could provide insight on weight control mechanisms among overweight and obese breast cancer survivors.

Methods: The Lifestyle, Exercise, and Nutrition (LEAN) randomized controlled trial was a 6-month weight loss trial conducted to examine the effect of a weight loss intervention versus usual care on outcomes in 151 breast cancer survivors with BMI $\geq$ 25 kg/m^2. Fasting blood samples were collected at baseline and 6-months and ghrelin was measured using enzyme-linked immunosorbent assays (ELISA). Pearson correlation coefficients were used to examine baseline associations and general linear models and least square means were used to compare changes in ghrelin levels from baseline to 6-months between randomization groups.

Results: Ghrelin measurements from 128 women were analyzed. At baseline, there was a significant positive correlation between circulating ghrelin and age ($r=0.28$, $p<0.001$) and significant inverse correlations with weight ($r=-0.18$, $p=0.03$), lean body mass ($r=-0.18$, $p=0.02$), and leptin ($r=-0.18$, $p=0.03$). After adjusting for covariates, among the intervention group, ghrelin levels increased by 12.08% over 6-months versus a 31.05% decrease in the usual care arm ($p=0.04$). A non-significant greater weight loss was associated with increased ghrelin concentrations among the intervention group ($p_{trend} = 0.67$).

Conclusion: These findings support that greater weight loss, achieved through a sustainable diet and exercise intervention, is associated with increased circulating ghrelin levels in overweight or obese breast cancer survivors. Further research is warranted to explore whether this change might affect long-term maintenance of weight loss as well as to further understand the role ghrelin may play in breast cancer recurrence and mortality.

Introduction

In 2017, the American Cancer Society estimated there were 3,560,570 breast cancer survivors living in the United States with this number expected to grow to 4,571,210 breast cancer survivors by 2026 [1]. The increase in female breast cancer survival rates is attributable to widespread mammography use and improvements in treatments [1]. As the population of breast cancer survivors expands, it becomes increasingly important to understand the specific needs associated with cancer survivorship.

Weight gain among women with breast cancer is relatively common during the first year of diagnosis, with weight gain commonly ranging from 2.0 to 6.0 kg [2]. Over 65% of breast cancer survivors are overweight or obese [3]. Factors related to post-diagnosis weight gain include chemotherapy, postmenopausal status, decreased physical activity and increased total caloric intake [4-6]. Obesity and post-treatment gain in adipose tissue places breast cancer survivors at an increased risk for recurrence and mortality [7].

Ghrelin, often referred to as the "hunger hormone", is a 28-amino acid peptide hormone that plays an important role in regulating appetite [8]. Ghrelin was first isolated in 1999 in rat gastric mucosa [9,10]. It has since been identified that over 90 percent of ghrelin in the human body is found in the stomach and duodenum [11, 12]. Ghrelin is primarily produced in the gastric fundus by endocrine cells and stimulates pituitary Growth Hormone (GH) secretion through the GH secretagogue receptor [11]. Ghrelin binds to specific hypothalamic receptors to initiate different signaling that leads to an increase in appetite and food intake [11, 13] (Figure 1). Ghrelin levels fluctuate naturally throughout the day, with higher levels before food intake and during the night and lower levels following food consumption [14]. Studies have identified that plasma ghrelin levels are downregulated in patients with a BMI $\geq$ 30 kg/m^2 compared to

individuals with a BMI < 25 kg/m^2 with a linear inverse correlation between circulating ghrelin levels and body mass index [8, 14, 15]. The mechanism that explains this relationship remains unclear, however, it was proposed that lower plasma ghrelin concentrations observed in obesity represent a physiological adaptation to the positive energy balance associated with obesity [15].

Unlike other gut-derived, meal-patterning peptides, ghrelin has been shown to play a role in long-term body weight regulation [9]. Administration of ghrelin to animals caused an increase in food intake and decreased energy expenditure, leading to weight gain, while blockage of ghrelin signaling led to a decrease in food intake and body weight [9, 18]. These findings suggest that ghrelin could participate in a negative feedback loop that regulates body weight. The response of circulating ghrelin to weight loss has been examined primarily in the setting of surgical weight loss interventions and to a lesser extent in lifestyle interventions [8, 13-17].

Scientists have been trying to explain the success of bariatric surgery in regards to weight loss by evaluating changes in ghrelin concentrations that are detected following various surgical procedures. Currently, the published literature reporting theses effects is inconsistent. Some studies have indicated that only procedures isolating or removing the fundus of the stomach (e.g. Roux-en-Y gastric bypass (RYGBP) or Sleeve gastrectomy) show a decrease in serum ghrelin levels and this decrease is associated with a decrease in body mass index (BMI) [19]. It is hypothesized that complete removal of the gastric fundus is associated with suppression of ghrelin because the majority of the ghrelin-producing cells are resected [14]. For example, Cummings et al. studied individuals who underwent RYGBP and showed that ghrelin levels no longer oscillated in relation to meals and were lower than both the normal weight controls with an average BMI = 27.4 kg/m^2 and the matched obese controls with an average BMI = 40.0 kg/m^2, regardless of the amount of weight lost [16]. This supports the notion that gastric

restriction which causes early satiety may suppress ghrelin and therefore may be one mechanism in which gastric bypass induces weight loss [19]. A recent meta-analysis of sixteen studies looked at the differences between short term (measuring time within or equal to 3 months) and long-term (measuring time exceeding 3 months) effects of RYGB surgery on ghrelin and weight loss [20]. This paper reported that ghrelin levels after RYGB surgery were significantly lower than pre-surgery levels in the short-term while ghrelin levels were markedly higher than pre-surgery levels in the long-term [20]. Further investigation is warranted to fully understand the role that ghrelin plays on long-term weight loss and maintenance after bariatric surgery.

Exploring the role circulating ghrelin levels play in lifestyle weight loss interventions is especially challenging given the lack of randomized controlled trials that exist today [8]. One prospective randomized controlled intervention trial conducted in 173 postmenopausal women with a BMI $\geq$ 25 kg/m^2, found that circulating ghrelin levels significantly increased by 5.3% during the 12-month exercise intervention ($p<0.05$ compared to baseline). Individuals randomized to usual care (stretching) showed a non-statistically significant 2.2% increase in ghrelin levels over 12-months. Women randomized to the 12-month exercise intervention showed a 17.7% increase in ghrelin levels in response to moderate weight loss (average 3kg loss) without an average reduction in food intake ($p<0.001$ compared to baseline; $p<0.01$ compared with exercisers without weight loss at 12 months). Comparatively, women randomized to usual care (stretching) showed a non-statistically significant 10.7% increase in ghrelin levels over 12-months in response to moderate weight loss (average 3kg loss) [21]. Thus, weight loss may play more of a role than exercise in increasing ghrelin levels. A more recent randomized controlled trial published by Mason et al. examined the independent and combined effects of a 12-month dietary weight loss and/or aerobic exercise intervention on total ghrelin levels in 398 post-

menopausal women with a BMI ≥ 25 kg/m^2 [17]. The usual care group showed a 1.8% increase in ghrelin over the 12-month intervention. Using the usual care group as the reference, total fasting ghrelin levels increased significantly in the combined diet and exercise arm (7.4%, p=0.008) but not in either the diet only (6.5%, p=0.07) or exercise only (1.0%, p=0.53) arms [17]. This study also indicated that among women randomized to the diet and exercise arm, when stratified by percent weight loss (≤0%, <5%, 5-10% or ≥10%) had a 3% decrease, a 3.6% increase, 8.4% increase and a 9.2% increase in ghrelin levels, respectively, over the 12-month intervention (ptrend=0.0041).

Ghrelin has been studied in relation to many cancer types including colorectal, head and neck, liver, non-Hodgkin lymphoma, pancreatic, thyroid, ovarian, prostate and breast cancers [29]. A review written in 2017 by Au et al. summarized the current understanding of the role ghrelin plays in hormone dependent cancers such as breast cancer [30]. While few studies have examined circulating ghrelin levels in women with breast cancer or with a history of breast cancer, most have been focused on the effects of therapies such as chemotherapy rather than relating blood levels with cancer risk [30]. In the review it is suggested that while studies published to date have presented conflicting results regarding the effects of ghrelin on breast cancer with some showing pro-proliferative effects and others indicating inhibitory effects, the ability for ghrelin to reduce estrogen and breast cancer growth may support its use as therapeutics for certain cancer types in the future [30].

Given the results of the two randomized controlled trials examining the relationship between lifestyle interventions and circulating ghrelin, the growing body of evidence regarding the association between ghrelin and hormone dependent cancers and the known risks associated with weight gain among breast cancer survivors, there is a need to study the link between

lifestyle interventions on serum ghrelin levels among this particular population. The Lifestyle, Exercise and Nutrition (LEAN) study examined the effects of a behavioral, dietary, and physical activity intervention through in-person and telephone counseling sessions, among breast cancer survivors. This randomized trial found an average 6.4% (in-person) and 5.4% (telephone) reduction in body weight for women in the lifestyle intervention arms compared to a 2.0% decrease in the usual care group ($p<0.05$ for in-person and telephone vs. controls) [3]. The purpose of our analysis was to examine the effect of the LEAN weight loss intervention versus usual care on ghrelin levels among breast cancer survivors with a body mass index (BMI) ≥ 25 kg/m^2. To our knowledge, this is the first study to examine the effects of a lifestyle weight-loss intervention on changes in circulating ghrelin levels among a population of breast cancer survivors. Understanding the impact of an individualized, sustainable dietary and exercise weight-loss intervention on circulating ghrelin levels could provide information on mechanisms associated with changes in body composition related to diet and exercise. Participants randomized to the weight loss intervention were hypothesized to have increased serum ghrelin levels after 6 months as compared to those randomized to usual care and the magnitude of increase would be proportional to the amount of weight lost.

Methods

The Lifestyle Exercise and Nutrition (LEAN) study was a Phase III randomized-controlled weight loss trial evaluating the effectiveness of in-person or telephone-based weight loss counseling versus usual care on changes in body composition, physical activity, diet, and serum biomarkers over 6 months in 100 breast cancer survivors. The detailed protocol and primary results for the LEAN study have been published previously [3]. Based on the initial results, 51 additional participants were recruited and randomized to intervention or usual care to

increase the sample size for this study (total n=151). The Yale School of Medicine Human Investigation Committee approved all procedures, including written informed consent.

Participants and Recruitment

Eligible participants included breast cancer survivors with a BMI $\geq$ 25.0 kg/m^2, diagnosed with stage 0-III breast cancer who had completed chemotherapy and/or radiation therapy at least 3 months before enrollment. Women had to be capable of exercising (i.e. not be in a wheelchair or use a cane), agree to be randomly assigned, provide informed consent to participate, communicate in English and be accessible by telephone. Women were excluded from the study if they were pregnant or intending to become pregnant in the next year, had experienced (past 6 months) stroke or myocardial infarction, or had severe uncontrolled mental illness. Breast cancer survivors were recruited between June 1, 2011 and February 1, 2016. Participants were identified through medical oncology clinics, self-referred via study brochures in the Breast Center at Smilow Cancer Hospital at Yale-New Haven Hospital and the Yale Cancer Center Survivorship Clinic. Details surrounding the eligibility criteria, recruitment and study design have been described in previously published literature [3, 25].

Outcome Measures

Demographic and Medical History

Medical record review and questionnaires were used to determine disease stage, surgery, adjuvant therapy, endocrine therapy, self-reported weight, and comorbidities at baseline and 6 months.

Body Composition Measures

Height and weight were measured at baseline and 6 months and rounded to the nearest 0.1. All measurements were made by the same staff members and were performed and recorded twice in succession and then averaged for the analyses. Dual-energy x-ray absorptiometry scans were performed to assess body fat, lean body mass (LBM), and bone mineral density (BMD) at baseline and 6 months with a Hologic 4500 scanner. All scans were evaluated by a Radiologic Technician Certified in Bone Density who was blinded to randomization group.

Ghrelin Analysis

A fasting (≥ 12 h) blood draw was performed at baseline and 6 months. All serum samples were stored at -80 degree Celsius until assayed. Total ghrelin levels were measured by ELISA (enzyme-linked immunosorbent assays). The plasma samples from each individual were duplicated and analyzed using commercial ELISA kits for ghrelin (BMS2192, ThermoFisher Scientific, Waltham, MA). The absorbance was measured at the wavelength of 450 nm with the reference wavelength of 620 nm for correction using a 96-well BioTek Synergy HT microplate spectrophotometerreader (BioTek, Winooski, VT). All ghrelin samples were measured with coefficients of variation for all samples of 1.69%. Description of other serum biomarker analyses have been previously described [3]. Serum concentrations of insulin, leptin, and adiponectin were measured using radioimmunoassay kits; and C-reactive protein (CRP) was measured using an automated chemistry analyzer. Baseline and 6-month specimens were assayed simultaneously at the end of the study, and participants from the intervention and the usual care arms were included in each batch of assays. Laboratory technicians were blinded to intervention assignment.

Weight Loss Intervention

The lifestyle intervention for the weight loss group was designed using a combination of behavioral therapy, reducing caloric intake and increasing physical activity. The program was modified from the Diabetes Prevention Program, updated with 2010 US Dietary Guidelines and adapted to the breast cancer survivor population using the American Institute for Cancer Research/World Cancer Research Fund and American Cancer Society nutrition and physical activity guidelines [23, 24]. All counseling sessions provided to the participants was conducted by a Registered Dietitian who is a certified Specialist in Oncology Nutrition, trained in exercise physiology and behavior modification counseling.

The 6-month weight loss intervention included participants receiving individual counseling sessions once a week for the first month, every two weeks for months two and three, followed by once a month for months four, five and six. The 11 sessions, each 30 minutes in duration, provided individualized information on nutrition, exercise and social-cognitive theory-based behavior strategies.

The dietary counseling instructed participants to reduce energy intake to a range of 1,200 to 2,000 kcal/day based upon baseline weight and to incur an energy deficiency of 500 kcal/day. This reduction was promoted by maintaining a predominantly plant-based diet with education on portion size, tracking fat grams, reducing simple sugars, increasing fiber and incorporating mindful eating techniques. The physical activity program was home based, with the goal of 150 minutes per week of moderate-intensity activity, such as brisk walking. Each participant was provided a pedometer and was coached to increase their daily step count to 10,000 steps per day in addition to reducing sedentary behaviors.

Usual Care

Study participants assigned to the usual care group were provided the American Institute for Cancer Research nutrition and physical activity brochures and were referred to the Yale Cancer Center Survivorship Clinic, which offers a two-session weight management program. At the conclusion of their 6-month participation in the usual care group, they were offered the same 11 session in-person or telephone counseling sessions that the intervention group had received along with all other LEAN materials.

Statistical Analysis

Descriptive statistics were used to describe the population at baseline, stratified by treatment group. Baseline characteristics included age, postmenopausal status, ethnicity, level of education, time from diagnosis to study enrollment, body weight, BMI, disease stage, adjuvant treatment, and current endocrine therapy. Comparisons of baseline characteristics by randomization group were performed using t-tests for continuous variables and chi-square tests or Fischer's exact test for categorical variables. Of the 151 LEAN participants, 149 had baseline serum ghrelin measurements. Those who were discontinued or lost to follow-up were dropped from the analysis (n=14), as well as an additional 7 participants who were missing follow-up blood draws, resulting in the sample size included in this analysis of 128 (84.8%) women.

Pearson correlation coefficients were used to examine baseline associations. General linear models were performed using SAS PROC GLM procedure to compare the change in ghrelin and weight by randomization group. Least square means and standard errors were calculated. Age, baseline ghrelin, and baseline BMI were included in the adjusted model, as both age and baseline BMI were significantly different between randomization groups at baseline. A sensitivity analysis was performed excluding those with a fasting status of less than 8 hours (n=7).

Post hoc analysis examined ghrelin levels stratified by tertiles of percent of weight lost (≤0% weight gain or no weight loss, <4.15% weight loss, 4.15-9.5% weight loss and >9.5% weight loss). Potential covariates were added to the models for exploratory analysis including age, baseline ghrelin, and baseline BMI.

All analyses were performed using SAS software version 9.4 (Cary, NC). A two-sided type I error rate of 0.05 was used throughout the data analysis.

Results

Study Population and Recruitment

Baseline characteristics

Full recruitment details are illustrated in Fig. 2 and baseline characteristics are reported in Table 1. Of the 975 women assessed for eligibility, 151 women were randomized, 149 had baseline blood samples (Intervention=91, Usual Care=58) and 128 had 6-month blood samples (Intervention = 76, Usual Care = 52). Mean age of participants at baseline was 58.0 ± 7.8 years (mean ± SD, unless otherwise noted) and women were on average 2.9 ± 2.5 years out from diagnosis at the time of enrollment in LEAN. Women were predominately post-menopausal (83.2%), non-Hispanic white (89.1%), and highly educated, with 61% holding at least a college degree. Most women had been diagnosed with Stage I or II breast cancer (49.7% and 24.2% respectively), with 16.8% diagnosed as Stage 0 (ductal carcinoma in situ: DCIS). A majority of women had received adjuvant treatment from chemotherapy and/or radiation (88.6%) and reported some form of previous or current endocrine therapy with tamoxifen and/or aromatase inhibitors (64%).

Age, baseline body weight, baseline BMI and BMI category were found to be statistically significantly different between the intervention versus usual care groups, ($p \leq 0.05$). Individuals

randomized to the intervention were on average 59.0 ± 7.3 years old whereas those randomized to usual care were on average 56.3 ± 8.4 years old (p=0.04). Those randomized to intervention were found to have a baseline mean body weight of 85.0 ± 16.9 kg whereas baseline mean body weight in the usual care group was 92.3 ± 18.1 kg (p=0.01). Mean baseline BMI was 32.2 ± 6.0 kg/m^2 in the intervention group and 34.6 ± 6.7 kg/m^2 in the usual care group (p=0.03). There were no other statistically significant differences in baseline characteristics between randomization groups.

Adherence to Intervention

Over half (60.2%) of participants randomized to intervention attended all 11 weight loss counseling sessions, and 80.6% attended at least 80% of the counseling sessions making for high overall adherence across the LEAN study [3].

Baseline Associations

Baseline associations between circulating ghrelin and a variety of body composition and serum biomarkers are described in Table 2. At baseline, there was a significant positive correlation between circulating ghrelin level and age ($r = 0.28$, $p<0.001$). Circulating ghrelin levels at baseline was significantly inversely correlated with weight ($r = -0.18$, $p=0.03$), lean body mass ($r = -0.18$, $p=0.02$), and leptin ($r = -0.18$, $p=0.03$). Baseline ghrelin levels was not significantly associated with BMI, totally body fat, insulin, adiponectin or CRP.

Changes in Body Weight

The women randomized to the intervention arm lost 5.91% of their body weight after the 6-month intervention while those randomized to usual care lost 0.21% of their body weight. After adjusting for covariates, the women randomized to the intervention arm lost 5.73% of their

body weight after the 6-month intervention while those randomized to usual care lost 0.41% of their body weight.

Changes in Serum Ghrelin

Mean baseline ghrelin levels by randomization group and change between baseline and 6-months can be found in Table 3a and Table 3b. At baseline, ghrelin levels were not statistically significantly different between the usual care (1417.10 pg/mL ± 293.30) and intervention arms (1922.44 pg/mL ± 242.61) (p=0.19). After the 6-month intervention ghrelin levels differed significantly between study arms (Usual care=1066.99 pg/mL ± 239.20, Intervention= 2042.57 pg/mL ± 289.17, p=0.01). After adjusting for covariates, ghrelin levels at 6-months differed significantly between study arms (Usual care= 1165.31 pg/mL ± 288.63, Intervention= 1975.30 pg/mL ± 237.65, p=0.03). The change in serum ghrelin level from baseline to 6-months between the usual care (-493.30 pg/mL ± 258.70) and intervention arms (218.10 pg/mL ± 212.96) were statistically significantly different (p=0.04). Serum ghrelin levels decreased by 31.05% among the usual care and increased by 12.08% among the intervention group over 6-months. A sensitivity analysis was performed excluding those with a fasting status of less than 8 hours (n=7) however the results did not change significantly.

Association Between Weight Loss and Ghrelin Within the Intervention Group

Table 4 presents mean change in circulating plasma ghrelin levels in the intervention group only, stratified by tertiles of percent weight lost (≤0% weight gain or no weight loss, <4.15% loss, 4.15-9.5% loss and ≥9.5% loss). Very few women in the intervention group gained weight or did not lose weight over 6-months (n=8). The 23 women who lost <4.15% of body weight showed a decrease in ghrelin levels of -310.36 pg/mL ± 461.29 (-15.85%). The 23

women who lost 4.15-9.5% of their body weight over the 6-month intervention showed an increase in ghrelin levels of 482.52 pg/mL ± 455.92 (24.54%). The 22 women who lost greater than or equal to 9.5% of their body weight over the 6-month intervention showed an increase in ghrelin levels of 494.60 pg/mL ± 480.88 (27.49%). Larger increases in ghrelin were associated with more weight loss in the intervention study arm (ptrend = 0.67).

Discussion

Studies examining the role of circulating ghrelin levels on weight loss and weight maintenance have been primarily conducted in the setting of surgical interventions, and less so in the setting of lifestyle interventions [8,13-17]. A review published in 2011 on lifestyle factors and ghrelin implied the need for future research to focus on teasing apart the confounding effects of diet, weight loss and exercise [28]. Literature reviewing various bariatric surgery techniques have suggested that Roux-en-Y gastric bypass, a technique where the ghrelin producing cells are completely removed, can result in a decrease in ghrelin levels that is associated with a decrease in BMI [19]. It has also been proposed that since bariatric surgeries alter the gastrointestinal anatomy and induces early satiety, suppression of ghrelin may be one mechanism contributing to the success of weight loss from bariatric surgeries [16]. Comparatively, two lifestyle interventions, including caloric restriction and/or increased physical activity, have shown an inverse correlation between ghrelin levels and weight loss [17, 21]. Since obesity, weight loss and breast cancer are all interrelated, we sought to understand the association between weight loss and ghrelin levels in patients who had breast cancer. To our knowledge, this is the first study to examine the effect of a diet and exercise-based weight loss intervention on ghrelin levels in a population of breast cancer survivors.

The six-month LEAN intervention led to a 12.08% increase in circulating ghrelin levels among the intervention group. This result was consistent with the findings of two randomized controlled lifestyle intervention trials [17, 21]. A trial conducted by Mason et al. studied changes in ghrelin levels among postmenopausal women with a BMI $\geq 25 kg/m^2$ randomized to dietary weight loss, moderate-to-vigorous intensity aerobic exercise, combined diet and exercise or control [17]. This study observed a 7.4% increase in circulating ghrelin after 12-months among the diet and exercise intervention arm compared to a non-significant 6.5% increase among the diet only arm and a 1.0% increase among the exercise only arm [17]. Similarly, a study performed in 2005 by Foster-Schubert et al. that randomized 173 postmenopausal women with a BMI $\geq 25 kg/m^2$ to either moderate intensity aerobic exercise intervention or stretching control program, found similar results [21]. This study observed that women randomized to the exercise arm had a 5.3% increase in ghrelin levels over 12-months [21]. While we did not hypothesize a decrease in ghrelin levels among the usual care group in our study (-31.05%), Foster-Schubert et al. similarly reported a -0.6% decrease in ghrelin after 3-months followed by a 2.2% increase in ghrelin after 12-months in the stretching (control arms). Therefore, if our study follow-up was longer than 6-months we may have also seen this shift in direction.

Similar to other studies, we found negative correlations between ghrelin and body weight ($r=-0.18$, $p=0.03$), BMI ($r=-0.14$, $p=0.08$), leptin ($r=-0.18$, $p=0.03$) and lean body mass ($r=-0.18$, $p=0.02$). These are consistent with the findings from Foster Schubert et al. who reported negative correlations between ghrelin and body weight ($r=-.29$, $p<0.0001$), BMI ($r=-0.29$, $p<0.0001$), lean body mass ($r=-0.24$, $p=0.001$) and leptin ($r=-0.14$, $p=0.08$). Tschop et al. also reported similar results with ghrelin levels negatively correlated with BMI ($r=-0.5$, $p<0.01$) and leptin ($r=-0.39$, $p<0.05$). Our results, along with previous studies, support the notion that ghrelin levels are

downregulated in individuals with a BMI $\geq$ 25kg/m^2 and that the hormone leptin and ghrelin work inversely to regulate appetite and satiety.

Among the diet and exercise intervention arm, Mason et al. found a -3.0% decrease in ghrelin levels among those who did not lose any weight, a 3.6% increase in ghrelin levels among those who lost less than 5% of their body weight, an 8.4% increase in ghrelin levels among women who lost 5-10% of their body weight and a 9.2% increase in ghrelin levels among those achieving a weight loss of greater than 10% [17]. Our study results were consistent with these findings. Individuals in the intervention arm who gained or did not lose weight showed a -35.17% decrease in ghrelin levels, among those who lost less than 4.15% of their body weight showed a -15.85% decrease in ghrelin levels, among those who lost 4.15-9.5% of their body weight showed a 24.54% increase in ghrelin levels and among those achieving a weight loss of greater than 9.5%. showed a 27.49% increase in ghrelin levels. Similarly, Foster-Schubert et al. found that ghrelin levels increased commensurately with the amount of weight lost over the 12-month intervention. Individuals who lost no weight (<0.5kg) showed a 0.49% increase in ghrelin, those who experienced mild weight loss (0.5kg-3.0kg) showed a 6.7% increase in ghrelin and women who lost moderate weight (>3.0kg) showed a 17.7% increase in ghrelin [21].

These results, taken together, indicate that ghrelin plays a role in the adaptive responses to weight loss. However, no study to our knowledge has prospectively examined changes in weight and changes in ghrelin long-term. This magnifies the need to understand the impact of physical activity- and diet-induced weight loss on ghrelin levels as well as the mechanisms by which body composition impacts ghrelin levels in the body.

It has been proposed that individuals with obesity and lower plasma ghrelin concentrations represent a physiological adaptation to the positive energy balance associated

with obesity [15]. Another hypothesis suggests that individuals with obesity, may have increased sensitivity to ghrelin and therefore require less ghrelin to stimulate hunger [26]. Individuals with obesity have sufficient energy stores that may suppress ghrelin secretion and therefore turn off the drive to eat that is usually generated by ghrelin [26]. Based on the current scientific literature, the mechanisms by which weight loss leads to an increase in circulating ghrelin levels is also not fully understood. It remains relatively unclear which aspects of body composition are related to the systems that regulate ghrelin in addition to how changes in these body composition components communicate to the ghrelin producing cells [21]. Future studies should further investigate which body composition factors play the biggest role in regulating ghrelin levels.

A potential limitation of our findings is that this intervention is limited to a 6-month duration, and therefore, long-term weight loss maintenance was not captured beyond 6-months.. Further longitudinal research and long-term follow-up assessments of weight and biomarkers is warranted.

The results of this study should also be viewed in the context that participants were predominately non-Hispanic white and highly educated, which may limit the generalizability of our findings. Also, while 151 women were enrolled in the LEAN study, 14 were discontinued or lost to follow-up and 7 were missing follow-up blood draws. This reduced the sample size available for analysis to n=128. However, only a few randomized weight loss trials in breast cancer survivors have been published, the majority of which have smaller sample sizes.

Strengths of this study include a low attrition rate and high adherence to the LEAN intervention was high. As explained in previous LEAN results, significantly greater weight loss was seen in women who completed all counseling sessions compared to those who missed sessions [3]. Another strength of this study was the high rates of individuals who provided

fasting serum measurements. Overnight fasting correlates well with 24-hour profiles of ghrelin [27]. Of the 256 blood draws included in this analysis (128 participants with baseline and 6-month blood draws) 249 were documented as having fasted ≥ 8 hours (97.3%). Removing individuals who fasted for <8 hours did not significantly alter our results.

Multiple studies have provided evidence that maintaining a healthy weight through reduced caloric intake and increased physical activity can help prevent various health outcomes including cancer recurrence, second primary cancers and other chronic diseases [3-7]. Since obesity, weight loss and breast cancer are all interrelated, understanding the association between weight loss and ghrelin levels in women diagnosed with breast cancer becomes increasingly important.

In summary, we show for the first time that ghrelin levels increased in breast cancer survivors undergoing a 6-month diet and physical activity, intervention. This observation is consistent with previous findings examining populations of postmenopausal women with obesity and supports the notion that future research should be performed to determine the long-term effect of changes in ghrelin on weight maintenance. Future studies should explore the role ghrelin plays, whether pro- or anti-proliferative, on hormone dependent cancers to identify how changes in ghrelin levels may impact cancer risk and mortality.

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Appendix

Figure 1. Physiologic effects of ghrelin

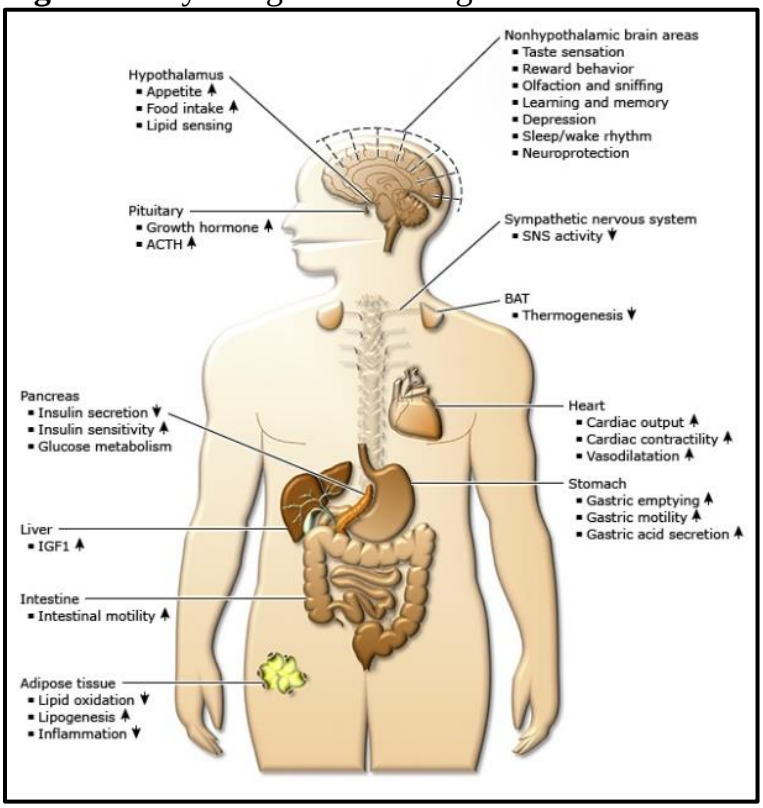

Figure 2. Consort Diagram

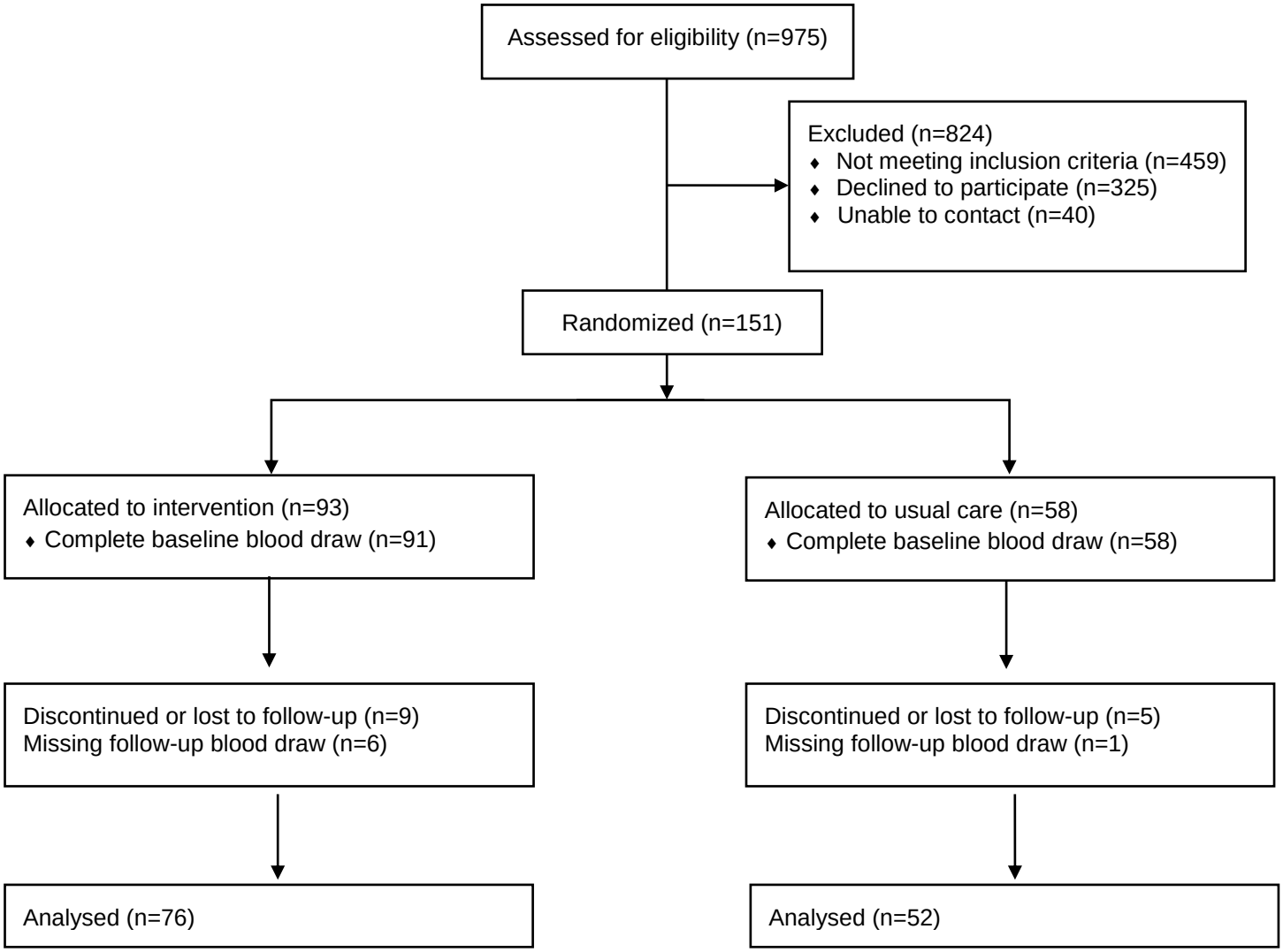

Table 1. LEAN 1 and LEAN 2 Study Participants Characteristics

Characteristic	Total n=149	Intervention n=91	Usual Care n=58	*p* value
Age, years, mean (SD), range (n=149)	58.0 ± 7.8 32-73	59.0 ± 7.3 44-73	56.3 ± 8.4 32-72	0.04
Postmenopausal, n (%) (n=149)	124 (83.2)	77 (84.6)	47 (81.0)	0.57
Race/Ethnicity, n (%) (n=149)				0.53
White (non-Hispanic)	131 (87.9)	82 (90.1)	49 (84.5)	
Black or African American	10 (6.7)	5 (5.5)	5 (8.6)	
Hispanic	6 (4.0)	3 (3.3)	3 (5.2)	
Other	1 (0.7)	0 (0.0)	1 (1.7)	
Declined to report	1 (0.7)	1 (1.1)	0 (0.0)	
Education, n (%) (n=149)				0.17
High school degree	19 (12.8)	10 (11.0)	9 (15.5)	
Some college degree	39 (26.2)	22 (24.2)	17 (29.3)	
College degree	38 (25.5)	29 (31.9)	9 (15.5)	
Graduate degree	53 (35.6)	30 (33.0)	23 (39.7)	
Time from diagnosis to LEAN enrollment, years, mean (SD) (n=147)	2.9 ± 2.5	2.7 ± 2.0	3.2 ± 3.1	0.25
Body weight, kg, mean (SD) (n=149)	87.8 ± 17.7	85.0 ± 16.9	92.3 ± 18.1	0.01
Percent body fat (SD) (n=149)	43.2 ± 4.9	43.3 ± 4.5	42.9 ± 5.5	0.63
Baseline BMI, kg/m^2, mean (SD) (n=149)	33.2 ± 6.4	32.2 ± 6.0	34.6 ± 6.7	0.03
BMI (kg/m^2) (n=149)				0.02
Overweight BMI <30	61 (40.9)	44 (48.4)	17 (29.3)	
Obese BMI ≥ 30	88 (59.1)	47 (51.7)	41 (70.7)	
Disease stage, n (%) (n=149)				0.81
DCIS (stage 0)	25 (16.8)	13 (14.3)	12 (20.7)	
Stage I	74 (49.7)	46 (50.6)	28 (48.3)	
Stage II	36 (24.2)	22 (24.2)	14 (24.1)	
Stage III	11 (7.4)	8 (8.8)	3 (5.2)	
Unknown	3 (2.0)	2 (2.2)	1 (1.7)	
Adjuvant treatment after surgery, n (%) (n=149)				0.38
None	17 (11.4)	8 (8.8)	9 (15.5)	
Radiation only	57 (38.3)	34 (37.4)	23 (39.7)	
Chemotherapy only	23 (15.4)	17 (18.7)	6 (10.3)	
Radiation and chemotherapy	52 (34.9)	32 (35.2)	20 (34.5)	
Current endocrine therapy, n (%) (n=128)				0.07
Aromatase inhibitors (AI's) only	30 (23.4)	21 (26.3)	9 (18.8)	
Tamoxifen	43 (33.6)	20 (25.0)	23 (47.9)	
Both	9 (7.0)	6 (7.5)	3 (6.3)	
None	46 (35.9)	33 (41.3)	13 (27.1)	

Table 2. Unadjusted baseline Pearson correlation coefficients of ghrelin levels with age, measurements of body composition and serum biomarkers in all study participants (n=149)

	Correlation	***p-value***
Age	0.28	0.001
Weight (kg)	-0.18	0.03
BMI (kg/m^2)	-0.14	0.08
Total Body fat (kg)	-0.13	0.11
Lean Body Mass (kg)	-0.18	0.02
Leptin (ng/mL)	-0.18	0.03
Insulin (μU/mL)	-0.13	0.11
Adiponectin (μg/mg)	0.05	0.54
C-Reactive Protein (mg/L)	-0.04	0.60

Table 3a. Unadjusted baseline, 6-month, and change in ghrelin levels by Intervention group (N=76) Versus Usual Care group (n=52)				
	Baseline, Mean (SE)	6 Months, Mean (SE)	Change Over 6 Months, Mean (SE)	% Change
Ghrelin (pg/mL)				
Usual Care	1417.10 (293.30)	1066.99 (239.20)	-350.12 (300.25)	-24.70
Intervention	1922.44 (242.61)	2042.57 (289.17)	120.13 (248.36)	6.25
p-value	0.19	0.01	0.23	
Weight (kg)				
Usual Care	90.34 (2.42)	90.15 (2.53)	-0.19 (0.55)	-0.21
Intervention	84.80 (2.00)	79.79 (2.10)	-5.01 (0.46)	-5.91
p-value	0.08	0.002	<0.001	

Table 3b. Adjusted baseline, 6-month, and change in ghrelin by Intervention group (N=76) Versus Usual Care group (n=52)				
	Baseline, Mean (SE)	6 Months, Mean (SE)	Change Over 6 Months, Mean (SE)	% Change
Ghrelin (pg/mL)				
Usual Care	1588.98 (285.72)[a]	1165.31 (288.63)[a]	-493.30 (258.70)[b]	-31.05
Intervention	1804.84 (235.26)[a]	1975.30 (237.65)[a]	218.10 (212.96)[b]	12.08
p-value	0.57	0.03	0.04	
Weight (kg)				
Usual Care	89.89 (2.43)[c]	89.52 (2.53)[c]	-0.37 (0.54)[c]	-0.41
Intervention	85.10 (2.00)[c]	80.22 (2.08) [c]	-4.88 (0.45)[c]	-5.73
p-value	0.13	0.01	<0.001	

[a] adjusted for age, baseline BMI
[b] adjusted for age, baseline BMI and baseline ghrelin
[c] adjusted for age

Table 4. 6-Month change in ghrelin levels, stratified by % weight loss among those in the intervention group

	Ghrelin (pg/mL)			
		Baseline[a]	**Change[b]**	
	N	Mean (SE)	Mean (SE)	%
Intervention				
≤0 % loss	8	2029.96 (830.35)	-713.87 (792.41)	-35.17
< 4.15 % loss	23	1958.48 (483.41)	-310.36 (461.29)	-15.85
4.15-9.5 % loss	23	1966.58 (477.77)	482.52 (455.92)	24.54
≥ 9.5 % loss	22	1799.51 (503.75)	494.60 (480.88)	27.49
Ptrend		0.99	0.67	

[a] adjusted for age, baseline BMI
[b] adjusted for age, baseline BMI and baseline ghrelin