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Carbohydrate Intake and Outcomes among Postmenopausal Breast Cancer Survivors

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy

in

Public Health (Health Behavior)

by

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2013

The Dissertation of Jennifer Ann Emond is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

To my Mother, who taught me the value of honesty; in honor of my Father, who taught me the value of hard work; to my husband, who blessed me with encouragement at every step; and to my daughter, who has shown me there is no joy in persevering without meaning.

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LIST OF ABBREVIATIONS

95% CI:	95% Confidence interval
BMI:	Body mass index
CHO:	Carbohydrates
ER:	Estrogen receptor
GH:	Growth hormone
HR:	Hazard ratio
IGF-1:	Insulin-like-growth-factor-1
IGF-1R:	Insulin-like-growth-factor-1 receptor
IHC:	Immunohistochemical
IGFBP:	Insulin-like-growth-factor binding protein
MCC:	Moores Cancer Center
MET:	Metabolic equivalent
MHW:	Metabolic equivalent (MET) hours per week
OR:	Odds ratio
PR:	Progesterone receptor
TB333:	Breast cancer tissue, positive control
UCSD:	University of California, San Diego
WHEL:	Women's Health Eating and Living

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2. **Emond JA**, Patterson RE, Jardack PM, Arab L. Validating associations between sugar-sweetened beverage intake and adiposity among African-American and White adults in a doubly labeled water study. Experimental Biology Annual Meeting 2012. San Diego, CA. April 21-25, 2012.

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Publications

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1. **Emond JA**, Ayala GX. Nutrition for health promotion. In: Madanat H, Ayala GX, Arredondo E, eds. *Introduction to Health Promotion and Behavioral Science in Public Health*. 1st ed. Clifton Park, NY: Cengage Learning, Inc. 2012 (In press).

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Refereed

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31. **Emond JA**, Patterson RE, Jardack PM, Arab L. Using doubly labeled water to validate associations between sugar-sweetened beverage intake and body mass among White and African-American adults. *Int J Obes*. Accepted July 19, 2013.

ABSTRACT OF THE DISSERTATION

Carbohydrate Intake and Outcomes among Postmenopausal Breast Cancer Survivors

by

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Background: Converging lines of research suggest that activation of the insulin/insulin-like-growth-factor axis may impact prognosis among breast cancer survivors. Carbohydrate intake can stimulate the insulin/insulin-like-growth-factor axis by elevating blood glucose concentrations, and insulin-like-growth-factor-1 receptor (IGF-1R) activation in breast cancers triggers proliferative signaling. This dissertation examined the influence of carbohydrate intake on breast cancer recurrence and all-cause mortality among postmenopausal breast cancer survivors, and whether the odds of recurrence was modified by IGF-1R expression in the primary cancer.

Methods: Secondary analysis of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial. Baseline and one-year dietary intake was assessed

using 24-hour dietary recalls. One-year change in carbohydrate intake was quantified as tertiles (grams/day) and change in approximately one serving of high or low quality carbohydrate-based foods; quality considered the impact on blood glucose concentrations. Samples of primary breast cancer tissue (N=265) were stained in a nested, case-control, immunohistochemical study to test the interaction between a decreased carbohydrate intake and IGF-1R expression on odds of recurrence. Finally, the impact of carbohydrate quality (high vs. low) on outcomes was assessed among the full cohort.

Results: Dietary changes began a median 24 months post-diagnosis. Over a median 7 years, there were N=247 (11.7%) recurrences. Risk of recurrence significantly increased over tertiles of change in carbohydrate intake (p=0.055). A decreased carbohydrate intake (< -26 grams/day) reduced the risk of recurrence by 30% (HR: 0.7; 95%CI:0.5-1.0). Risk reduction significantly differed (p=0.110) by IGF-1R expression in the primary breast cancer: a decreased carbohydrate intake reduced the odds of recurrence by 30% (conditional OR: 0.7;95%CI:0.2-1.7) among participants who had IGF-1R negative cancers and 80% (conditional OR: 0.2;95%CI:0.03-0.3) among participants who had IGF-1R positive cancers. Among the full cohort, only a decreased intake of low quality carbohydrates (e.g., refined grains, sweets, starchy vegetables) was protective, and only among participants who did not decrease their intake of high quality carbohydrates (e.g., fruits, non-starchy vegetables, whole grains, dairy).

Conclusions: Results support that modifications in carbohydrate intake may impact prognosis among postmenopausal breast cancer survivors via the insulin/insulin-

like-growth-factor axis. Importantly, carbohydrate quality may be more important that quantity relative to prognosis.

INTRODUCTION

Invasive breast cancer affects over 230,000 women in the United States each year (1). Improvements in detection and treatments have decreased breast cancer mortality since 1998, and the current 5-year survival rate is nearly 90% (1). That translates to more than 2.9 million breast cancer survivors in the United States today (1); a substantial pool of women who retain feelings of vulnerability for many years after completing treatment (2).

Lifestyle behaviors have been shown to influence prognosis among breast cancer survivors (3, 4), and many breast cancer survivors are concerned about the links between diet and cancer recurrence (5). Dietary advice for breast cancer survivors has often emphasized a dietary pattern low in total fat intake (6, 7). That advice was motivated largely from early animal studies and ecological studies comparing per capita consumption of fat to population level breast cancer rates (8, 9). While those studies focused on incident breast cancer, similar dietary advice was recommended for breast cancer survivors (6, 10). However, two randomized dietary trials have examined the effectiveness of a plant-based, dietary pattern low in total fat on prognosis among breast cancer survivors, and the evidence does not suggest an association between total fat intake and breast cancer recurrence or mortality in the absence of weight loss (11, 12).

Current research interest is focusing on the role that dietary carbohydrate intake may have on prognosis among breast cancer survivors (13, 14). However, there is a paucity of data on carbohydrate intake and prognosis among breast cancer survivors.

Thus, data are insufficient for dietary recommendations related to carbohydrate intake. As more women are living as breast cancer survivors, a better understand of how carbohydrate intake may impact prognosis among breast cancer survivors is needed.

Insulin Regulation and Breast Cancer Growth

The growing interest in the potential role that carbohydrate intake may have on breast cancer growth in part stems from the similarities observed between obesity, metabolic disorders, and breast cancer risk (15-17). Obesity is a risk factor for developing insulin resistance, hyperinsulinemia, and type-2 diabetes mellitus (herein referred to as diabetes). Among postmenopausal women, obesity is also a risk factor for incident breast cancer (18, 19), and excessive weight is also a risk factor for poor prognosis among breast cancer survivors (20). When considering diabetes, the rates of incident breast cancer are higher among diabetic women compared to women without this disorder (21, 22), suggesting that characteristics of diabetes contribute to breast tissue metastasis. Further, breast cancer survivors with diabetes have a worse prognosis than breast cancer survivors without diabetes (20, 23). Such data again suggest that characteristics of diabetes may impact breast cancer progression. Epidemiological studies among diabetics offer more insight: diabetic women taking exogenous insulin have a greater risk of developing breast cancer compared to diabetic women not taking exogenous insulin (24), suggesting that an increased circulating concentration of insulin contributes to breast cancer progression. Furthermore, treatment with metformin, a non-insulin drug that helps regulate blood glucose concentrations, may lower the risk of incident breast cancer among diabetics (25, 26).

Diabetics and pre-diabetics are encouraged to follow a dietary pattern that helps manage their blood glucose concentrations (27, 28). For example, the American Diabetes Association promotes a set of Diabetes Exchanges Lists (27, 30) to help diabetics and pre-diabetics manage the quantity and quality of carbohydrate intake. That management of carbohydrates is meant to maintain a stable blood glucose concentration. Considering the similarities between obesity, metabolic disorders, and breast cancer risk, it is possible that similar treatments to manage blood glucose concentrations among diabetics may also be effective at improving prognosis among breast cancer survivors. Indeed, randomized trials are currently testing the effectiveness of metformin on breast cancer prognosis (29) or biomakers of breast cancer prognosis (30) among breast cancer survivors. It is worthwhile to examine the impact that carbohydrate quantity and quality may have on prognosis among breast cancer survivors.

Dietary Carbohydrates

Dietary carbohydrates include several subtypes of carbohydrates (31). Those subtypes include simple monosaccharide and disaccharide sugars, and complex carbohydrates. Monosaccharides are single sugar units of glucose, fructose, or galactose. Glucose is the most common monosaccharide, fructose is a natural fruit sugar, and galactose is a rare monosaccharide. Disaccharides are carbohydrates based on two monosaccarides and include sucrose (glucose + fructose), maltose (glucose+glucose), and lactose (glucose + galactose). Finally, complex carbohydrates are starchy polysaccharides of multiple (often hundreds) of glucose units linked together. The typical adult woman in America consumes 224 grams of carbohydrates per day (32), the

majority of which in the form of starch (33). As carbohydrates are metabolized, bonds between monosaccharide units are broken down, freeing up individual glucose units for absorption into the blood stream. That process occurs mainly in the small intestine (31). Many tissues within the body, including skeletal and adipose tissues, utilize the free glucose in the blood as energy (31). Fructose differs from glucose in that the liver is the primarily location of fructose metabolism (34).

To note, dietary fiber, a non-starchy polysaccharide, is also considered a carbohydrate according to the United States Department of Agriculture (33). However, dietary fiber is indigestible. Dietary fiber slows the absorption of glucose into the blood stream within the small intestine by two likely mechanisms: by forming a gel that slows and possibly prevents absorption of glucose into the blood stream, and by inactivating a required enzyme needed to breakdown the bonds of starchy polymers (35). Thus, dietary fiber can attenuate the rise in blood glucose concentrations after consuming foods or beverages that contain simple or complex carbohydrates. Dietary fiber is a component of many nutrient-dense carbohydrate-based foods, such as fruits, non-starchy vegetables, legumes, and whole grains. To note, for the purposes of this dissertation, carbohydrates refer to monosaccharides, disaccharides, and starchy polysaccharides, and dietary fiber is treated as distinct from digestible carbohydrates. Finally, functional fiber is fiber added to foods or beverages as a supplement (33), and this dissertation does not consider functional fiber when rating carbohydrate-based foods on quality.

Proposed Mechanisms of Action for Carbohydrate Intake on Prognosis

Figure 0.1 presents the framework that relates an increased carbohydrate intake to breast cancer recurrence via activation of the insulin/insulin-like-growth-factor axis as proposed in this dissertation.

Among humans with normal metabolic functioning, digestion of carbohydrates results in elevated blood glucose concentrations, which then stimulates the secretion of insulin from beta cells of the pancreas (36). Insulin stimulates the liver to store excess glucose as glyogen, and likewise inhibits the creation of glucose from stored glycogen molecules (i.e., gluconeogenosis). Insulin is also needed for cells within tissues, mainly muscle and adipose, to uptake glucose to use as an energy source (31).

Cancer cells utilize glucose as a fuel source in a process commonly referred to as the Warburg effect (37). However, that process of converting glucose into energy is inefficient, and therefore the rate of glucose metabolism in cancer cells is high. In fact, positron emission tomography (PET) is used to image cancer tissue in the body based on the rapid metabolism of radioactively labeled sugar in cancer cells. Thus, it is possible that elevated circulating glucose may encourage breast cancer growth. A secondary analysis of a dietary intervention trial (N=3,088) (12) found a positive association between elevated HbA1C concentrations at baseline, a marker of average blood glucose concentrations over the previous 3 months, and all-cause mortality among breast cancer survivors (23). In that study 80% of deaths were breast cancer related.

Insulin is also a known mitogen (38). Breast cancers cells express receptors for insulin, and elevated fasting insulin has been associated with poor prognosis among breast cancer survivors in several studies (39-41). Further, insulin promotes an increase in circulating insulin-like-growth-factor-1 (IGF-1), another hormone that stimulates

breast cancer growth (38). Insulin promotes elevations in IGF-1 in at least two ways. Insulin secretion stimulates the synthesis and expression of growth hormone receptors in the liver (42), which in turn increases circulating IGF-1 by limiting the availability of growth hormone, an IGF-1 inhibitor. Insulin also increases the bioavailability of IGF-1 by inhibiting the production of IGF binding proteins in the liver (42, 43). Elevated IGF-1 concentrations are associated with an increased risk of several cancers, including breast cancer (44). Studies examining the association between IGF-1 concentrations and prognosis among breast cancer survivors, however, have been mixed (45-48).

The IGF-1 Receptor

The IGF-1 receptor (IGF-1R) is a transmembrane, tyrosine kinase receptor consisting of two pairs of heterodimers. The IGF-1R has two extracellular alpha subunits and two beta subunits that span the cell membrane. Binding of the IGF-1 ligand by the receptor results in phosphorylation of three intracellular tyrosine residues on the beta subunit, resulting in receptor activation.

IGF-1 is a growth hormone that is needed for normal cell development, and the IGF-1R is expressed in many healthy tissues including healthy breast tissue. However, impairment in the IGF-1R signaling process initiates downstream proliferative and pro-survival signaling via the Ras and AKT pathways (48), which promotes cancer growth. Breast cancers have been found to over-express the IGF-1R (49) relative to normal breast tissue, and IGF-1R expression in primary breast cancer tissue has been positively associated with treatment resistance including resistance to tamoxifen (45, 50, 51), chemotherapies (48), and radiation therapy (52). One case-control study showed an

increased risk of early recurrence (within 4 years of diagnosis) with greater IGF-1R expression levels where cancers with higher IGF-1R expression levels displayed greater radioresistance (52). Therefore, any microscopic breast cancer tissue that remains after completion of treatment may be particularly sensitive to stimulation from circulating IGF-1.

Dissertation Chapters

The aims of this dissertation addressed three novel approaches to examining the potential associations between carbohydrate intake and breast cancer prognosis under the framework presented in Figure 0.1.

Chapter 1 examined the associations between a change in the net quantity of carbohydrate intake over one year and breast cancer recurrence as well as all-cause mortality among a cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial. Quantity of carbohydrate intake was based on grams of carbohydrates per day. Chapter 1 was a secondary data analysis of the Women's Health Eating and Living (WHEL) dietary intervention trial, a dietary intervention trial that enrolled N=3,088 breast cancer survivors and followed them over a median 7 years. As part of the WHEL trial, dietary changes occurred a median 24 months post-diagnosis, and significant dietary changes were achieved over the first year of study enrollment for considerable portion of participants. At this time, the work presented in Chapter 1 appears to be the first study to examine a net change in carbohydrate intake post-diagnosis and prognosis among breast cancer survivors.

Chapter 2 presents the results from a primary, immunohistochemical analysis where N=265 samples of primary breast cancer tissue were stained to detect the presence of the IGF-1R. Participants for Chapter 2 were selected from the sample of N=2,111 postmenopausal breast cancer survivors included in Chapter 1. Chapter 2 was a nested case-control study that compared the associations between a change in carbohydrate intake with expression of the IGF-1R in the primary cancer tissue on the odds of a breast cancer recurrence. At this time, the work presented in Chapter 2 appears to be the first study that has considered the potential moderating affect of IGF-1R expression in the primary breast cancer tissue on recurrence with respect to dietary intake of carbohydrates.

Finally, Chapter 3 presents the findings from a secondary analysis also based on the subset of N=2,111 postmenopausal breast cancer survivors enrolled in the WHEL trial who were included in the analysis for Chapter 1. While Chapter 1 examined quantity of carbohydrates on the macronutrient level, Chapter 3 examined carbohydrate intake based on a common serving size of carbohydrate-based foods and beverages. Foods and beverages were also classified according to quality based on the American Diabetes Association's Dietary Exchange Lists. Results from Chapter 3 are useful to translate the findings based on carbohydrate intake measured at the macronutrient level to concrete dietary advice that is more useful when considering modifiable dietary behaviors.

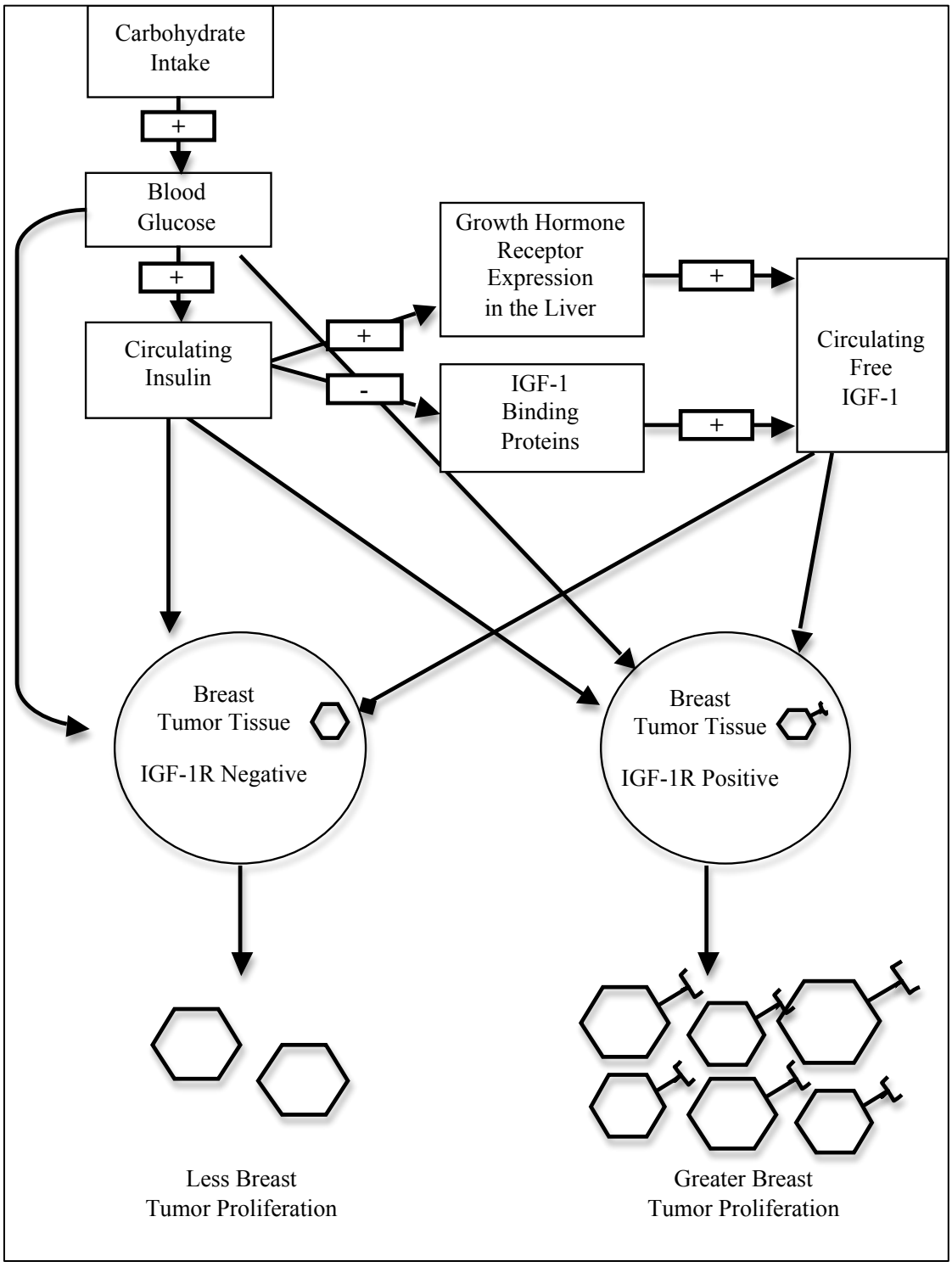

Figure 0.1. Framework of biological mechanisms that relates an increased carbohydrate intake to breast cancer recurrence via activation of the insulin-insulin-like-growth-factor axis as proposed in this dissertation.

IGF-1: Insulin-like-growth factor 1; IGF-1R: Insulin-like-growth factor 1 receptor.

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CHAPTER 1

Influence of a Post-Diagnosis Change in Carbohydrate Intake on Prognosis Among Postmenopausal Breast Cancer Survivors

Abstract

Background: Dietary advice for breast cancer survivors has generally focused on total fat intake. Current evidence suggests a potential influence of carbohydrate intake on breast cancer growth.

Methods: Secondary analysis of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trail. Participants were a median 24 months post-diagnosis. Dietary intake was assessed with multiple 24-hour dietary recalls. Considerable dietary change occurred within 6 months of enrollment; carbohydrate intake was not an intervention target. Delayed entry, Proportional Hazards models fit time to breast cancer recurrence or all-cause mortality on tertiles of one-year change in carbohydrate intake (grams/day). Total carbohydrates and carbohydrate subtypes were examined.

Results: Over a median 7.3 years, there were N=247 (11.7%) recurrences and N=166 (7.9%) deaths from any cause. Baseline total caloric, carbohydrate, or dietary fiber intake were not associated with recurrence. There was a significant, linear trend with the risk of recurrence over increasing tertiles of change in total carbohydrates ($p=0.055$). Specifically, a net decrease in carbohydrate intake ($\leq$ -26.7 grams/day) reduced the risk of recurrence by 30% (HR: 0.68; 95%CI: 0.46-1.01) compared to minimal change in intake. Significant linear trends ($p<0.10$) were observed for changes in fructose and maltose intakes. Results for all-cause mortality were similar to results for recurrence.

Conclusions: Results suggest dietary modifications related to carbohydrate intake within a few years post-diagnosis may impact prognosis among postmenopausal breast cancer survivors.

Impact: Dietary trials targeting both quantity and quality of carbohydrate intake are needed to clarify dietary recommendations for postmenopausal breast cancer survivors.

Introduction

Breast cancer is the most common cancer among women in the United States (1). Five-year survival rates are nearly 90% (1), meaning a substantial number of women, currently 2.9 million, are living as breast cancer survivors in the United States (1). Breast cancer survivors are at in increased risk of developing a second cancer (recurrence or new primary) and certain chronic diseases such as type II diabetes and cardiovascular disease (as reviewed in Rock 2012). There is evidence that breast cancer survivors can adapt healthful lifestyle behaviors in an effort to manage their health (2-6). However, a better understanding is needed regarding how changes in modifiable lifestyle behaviors after a breast cancer diagnosis may relate to prognosis.

Breast cancer survivors are motivated to make dietary changes in an effort to improve prognosis (7,8). Initial dietary recommendations for breast cancer survivors promoted a dietary pattern low in total fat (9-11). However, results from early dietary intervention trials among breast cancer survivors have not conclusively supported that a low-fat dietary pattern improves prognosis in the absence of weight loss (12, 13). Evidence is accumulating for the role that carbohydrate intake may have on breast cancer risk and prognosis (12, 14, 15). Carbohydrate intake results in postprandial increases in blood glucose and insulin concentrations. Both glucose and insulin directly stimulate breast cancer proliferation, (16-18) and elevated circulating concentrations of glucose activate the insulin/insulin-like-growth factor axis, which may play a role in breast cancer

progression (18,19). However, data are currently insufficient to warrant specific dietary recommendations regarding carbohydrate intake for breast cancer survivors.

Few studies have compared post-diagnosis carbohydrate intake at the macronutrient level to prognosis among breast cancer survivors (20-23), and no significant associations have been reported. However, carbohydrates include monosaccharides (glucose, fructose, galactose), disaccharides (sucrose, lactose, maltose), and polysaccharides (starch), and these different carbohydrate subtypes differ in the potential impact their metabolism has on postprandial blood glucose concentrations (24, 25). Previous studies have reported positive associations between incident breast cancer and carbohydrate intake limited to certain carbohydrate subtypes (26, 27). It is therefore worthwhile to examine how prognosis among breast cancer survivors might be influenced by changes carbohydrate intake also based on different carbohydrate subtypes.

This study compared post-diagnosis changes in carbohydrate intake and prognosis among a subset of breast cancer survivors enrolled as part of a dietary intervention trial. Analyses included total carbohydrate intake and the intake of seven carbohydrate subtypes.

Methods

Participants

Data were from the multi-site, Women's Healthy Eating and Living (WHEL) dietary intervention trial (12). During 1995-2000, the WHEL trial enrolled 3,088 breast cancer survivors diagnosed with early stage breast cancer within the prior four years. Half of the women were randomized to a dietary intervention with the following daily

targets: 5 vegetable and 3 fruit servings, 16 ounces of vegetable juice, 30 grams of fiber and 15-20% of total energy intake from fat. The WHEL trial did not have a specific carbohydrate target and was not a weight loss trial. Women in the intervention arm made significant dietary changes towards trial targets by month 6; dietary changes over one year were confirmed with changes in plasma carotenoid concentrations from a subset of women (28). An additional breast cancer event was the primary endpoint for the WHEL trial; events were defined as local, regional or distant invasive metastasis or new primary breast cancer. Two oncologists and one study pathologist reviewed participant medical records to confirm any reported additional event. As the majority of events were recurrences (85%), the outcome of an additional event is herein referred to as a recurrence. All-cause mortality was a secondary outcome of the WHEL trial and 83% of all deaths were breast cancer related. Death status and date of death was confirmed by searching the National Death Index using participant Social Security number, name and date of birth. The WHEL primary analysis did not find a significant association between the dietary intervention and recurrence or all-cause mortality rates (12). As such, the sample is treated as a cohort for this analysis.

This study hypothesized that a change in carbohydrate intake may influence prognosis based on the impact carbohydrate metabolism has on postprandial glucose and the resultant activation of the insulin/insulin-like-growth-factor axis. Endogenous sex hormones have been shown to influence insulin sensitivity over the menstrual cycle (29), and postmenopausal women exhibit higher levels of insulin resistance (30). Therefore, this study was limited to WHEL participants who were postmenopausal at the time of study enrollment (e.g., not having menses for 12 months prior to student enrollment).

Data were further limited to women who were recurrence free for 1.5 years after enrollment into the WHEL trial. Each study site's International Review Board approved of the WHEL study, and all women provided written informed consent.

Dietary Assessment

Dietary intake was assessed using telephone-based, 24-hour dietary recalls. Trained counselors completed four calls over a three-week period at each time point. Counselors used a multi-pass protocol during data collection. Dietary intake data were collected and analyzed using Nutrition Data System (NDS) for Research software version 4.03 (1994-2006) developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Dietary intake data from the baseline and year one assessments were used for this analysis.

Daily intake of carbohydrates (grams) was computed using data in the NDS database; both total carbohydrate and carbohydrate subtypes were examined (i.e., glucose, fructose, galactose, sucrose, lactose, maltose and starch). Carbohydrate subtypes included both naturally occurring and added sugars. For items where high fructose corn syrup was used as an added sugar, the NDS database deconstructed high fructose corn syrup into the related glucose and fructose moieties (Personal communication with NDS help desk, Jan 31, 2013).

Daily intakes were computed as the mean intake over the multiple 24-dietary recalls at baseline or year one; change in intake was computed as the mean year one intake minus the mean baseline intake. The WHEL study was not a weight loss study. Participants were not encouraged to follow a reduce calorie diet and total carbohydrate

intake was not an intervention target. As such, the mean one-year change in total carbohydrate intake for the overall sample was roughly zero. Thus, one-year change in carbohydrate intake was categorized into tertiles with the intent of ranking participants relative to a minimal change in total carbohydrate intake. Tertiles were labeled as a decreased intake (i.e., tertile one), minimal change in intake (i.e., tertile two), or an increased intake (i.e., tertile three).

Food groups were used to qualitatively describe the foods and beverages that contributed to changes in total carbohydrate intake. Food groups were based on the American Diabetes Association's Dietary Exchange Lists (31). The Exchange Lists classify foods and beverages by the expected impact their metabolism would have blood glucose concentrations while also considering the nutritional content of foods and beverages.

Outcomes

Primary outcome was time to a breast cancer recurrence; all-cause mortality was a secondary outcome. For each outcome, participants who were non-events were censored at the time of last contact or at the WHEL trial end date (June 1, 2006) as appropriate.

Additional Measures

Demographic, lifestyle, clinical and treatment characteristics were collected at baseline. Weight and height were measured at trial sites. Physical activity level was assessed using the Women's Health Initiative Personal Habits Questionnaire, and activity

levels were categorized as inactive, mild to moderately active, active, and very active based on MET hours per week (MHW) as previously reported (32).

Data Analyses

Baseline characteristics were summarized by tertiles of one-year change in total carbohydrate intake using Chi-Square tests, T-tests or ANOVA as appropriate. The contribution to total carbohydrate intake by food group was computed for baseline intake per the methods of Block (33). The primary analysis modeled time to breast cancer recurrence on tertiles of one-year change using a delayed entry, Cox Proportional Hazard model. Model covariates included baseline carbohydrate intake and baseline characteristics related to recurrence status ($p<0.100$) or tertiles of change in total carbohydrate intake ($p<0.100$). Models were also adjusted for trial site, baseline and one-year change in total energy intake, baseline and one-year change in fiber intake, and baseline alcohol intake. The primary analysis was repeated for each carbohydrate subtype as the main predictor using the same set of covariates for parsimony. Finally, the primary analysis was repeated using all-cause mortality as the outcome. All analyses were run using the R Language and Environment for Statistical Computing, version 2.15.2 (http://www.R-project.org).

Results

The final sample consisted of 2,111 postmenopausal breast cancer survivors who remained recurrence-free up to 18 months after study enrollment. Participants enrolled a median 24 months after their primary diagnosis. Median follow-up was 7.3 years with

n=247 (11.7%) additional breast cancer events, 83.4% of which were recurrences. Table 1.1 presents select baseline characteristics by recurrence status. Mean age at enrollment was 56 years (SD 7.6), and the majority of the participants (87%) were White non-Hispanic. Except for age at enrollment, none of the baseline demographic or lifestyle characteristics examined, including dietary intake, was significantly related to the likelihood of a breast cancer recurrence. On average, participants consumed 235 grams of total carbohydrates (SD 62) and 21 grams of fiber (SD 8) at baseline. Starch contributed the most to total carbohydrate intake at baseline, at 94 grams/day.

Overall, primary breast cancers were mostly earlier stage and well to moderately defined (Table 1.1); most participants (74%) were taking tamoxifen at study enrollment. Table 1.2 presents the contribution to total carbohydrate intake at baseline by carbohydrate-based food groups. The 10 food groups presented in Table 1.2 accounted for 98% of total carbohydrate intake at baseline; three other food groups based on fat, protein and alcohol provided 2% of dietary carbohydrates. Refined grains and sweets and desserts contributed the most to total carbohydrate intake at baseline, at roughly 50% of total carbohydrate intake combined.

Overall mean change in carbohydrate intake was -1.96 grams/day (SD 62.9). To rank change in total carbohydrate intake, one-year change in carbohydrate intake was categorized into tertiles. Participants in the lowest tertile decreased their total carbohydrates intake by at least 26 grams/day, and participants in the upper tertile increased their total carbohydrates intake by at least 22 grams/day. A minimal change in intake was defined as tertile 2, reflecting a net change in intake between -26.7 and +22.2 grams/day. Table 1.3 displays the distribution of select baseline characteristics by tertiles

of change in total carbohydrates. Compared to participants who increased their total carbohydrate intake, participants who decreased their intake over the first year of the WHEL study were significantly more likely to be younger, overweight or obese, and have lower levels of physical activity. Primary cancer characteristics and treatment characteristics were balanced by tertiles of change in total carbohydrate intake (data not shown), except for antiestrogen use: rates of tamoxifen use were significantly greater over increasing tertiles of change (70.3%, 75.5%, 77.5%, respectively; Chi-Square p-value=0.006).

Figure 1.1 presents the mean change in carbohydrate intake (grams/day) for each food group. Results in Figure 1.1 are presented stratified by tertiles of change in total carbohydrates; all comparisons of mean change per food group across tertiles of change were statistically significant at $p<0.001$. As illustrated in Figure 1.1, participants who decreased their total carbohydrate intake were more likely to decrease their intakes of refined grains and sweets and desserts while making minimal changes within the other carbohydrate-based food groups. Conversely, participants who increased their total carbohydrate intake were more likely to make changes related to fruits and 100% fruit juices, whole grains, and non-starchy vegetables.

Table 1.4 presents the distribution of one-year change in each carbohydrate subtype, along with the unadjusted recurrence rates over tertiles of change. Unadjusted recurrence rates appeared to increase over increasing tertiles of change in total carbohydrates, although results were not statistically significant at the $p<0.050$ level (Chi-Square p-value=0.091). Results from the fully adjusted model for change in total carbohydrate intake and breast cancer recurrence are presented in Figure 1.2. In the fully

adjusted model (Figure 1.2), there was a significant trend in increasing hazard ratios over increasing tertiles of change for total carbohydrate intake (p=0.055), although point estimates were not statistically significant at the p<0.050 level. However, it appeared that there was a reduced risk of recurrence among participants who decreased total carbohydrate intake (HR: 0.68; 95% CI: 0.46 – 1.01; p=0.056) compared to participants who made minimal change to their carbohydrate intake. Among participants who increased total carbohydrate intake, the risk of recurrence appeared elevated (HR: 1.25; 95% CI: 0.87 – 1.80; p=0.230) as compared to participants who made minimal change to their carbohydrate intake. There were no violations of the proportional hazards assumption overall (global Grambsch and Therneau test, p=0.270) or within tertiles of carbohydrate change.

When considering each carbohydrate subtype, results suggested an increasing risk of recurrence over increasing tertiles of change in fructose (p for trend=0.075) and maltose (p for trend=0.030) intake (Figure 1.2). There were no significant trends (all p>0.100) or significant point estimates (all p>0.050) for any of the adjusted models fitting time to recurrence on tertiles of change in glucose, galactose, sucrose, lactose or starch intake.

There were n=166 confirmed deaths during the study period, of which 121 (72.9%) were breast cancer related. There was not a statistically significant association between tertiles of change in total carbohydrate intake and all-cause mortality, although the direction and magnitude of the results were consistent with the findings based on recurrence. Specifically, the adjusted hazard ratio for a decreased carbohydrate intake versus minimal change in intake was 0.64 (95% CI: 0.40 – 1.04; p=0.074), and the

adjusted hazard ratio for an increased carbohydrate intake versus minimal change in intake was 1.20 (95% CI: 0.77 – 1.87; p=0.421; p-for trend=0.111).

Discussion

These results suggest that a change in total carbohydrate intake in the first years after a breast cancer diagnosis has the potential to impact the risk of recurrence among postmenopausal breast cancer survivors. Specifically, compared to a minimal change in carbohydrate intake over one year, a decreased carbohydrate intake was associated with a roughly 30% decreased risk of breast cancer recurrence, an effect size that was borderline statistically significant in this sample. Furthermore, an increased carbohydrate intake appeared to be associated with a roughly 25% increased risk of recurrence. While point estimates did not reach statistical significance at the $p<0.05$ level, there was a significant, linear trend over increasing tertiles of change in carbohydrate intake with the risk of recurrence. Results highlight the need for additional studies to examine how a change in carbohydrate intake may impact prognosis among postmenopausal breast cancer survivors.

This study also compared changes in the intake of carbohydrate subtypes with breast cancer prognosis. Significant, linear trends in the risk of a breast cancer recurrence were observed over increasing tertiles of change for fructose and maltose intakes. While the metabolism of fructose does not directly impact blood glucose concentrations (34, 35), increased fructose intakes are positively associated with the development of components of metabolic syndrome, (35, 36) including insulin resistance (35-37), conditions which are predictors of poor prognosis among breast cancer survivors (9).

Fructose may also directly stimulate breast cancer growth (38). However, caution is warranted when interpreting the findings for fructose. For example, while fructose is often used as part of added sugars in refined grains, snacks and beverages (36), many fruits, vegetables, and 100% juices may be high in fructose yet also provide fiber, vitamins, minerals, and other phytochemicals believed to reduce risk of many chronic diseases (39). Additionally, the dietary fiber that is part of fruits and vegetables can slow or reduce the absorption of glucose into the blood stream (25, 40).

For example, a cross-sectional study of N=1,999 women enrolled in the Nurses' Health Study (37) compared fructose intake as well as the intakes of foods high in fructose to circulating c-peptide concentrations, a marker of activated insulin. While a positive association was found between increased fructose intakes and c-peptide concentrations, results were limited to sugar-sweetened beverages where fructose was included as an added sugar. The consumption frequency of other top food sources of fructose was not associated with circulating c-peptide concentrations (e.g., 100% orange juice, fresh apples), or was associated with a decreased circulating c-peptide concentration (e.g., raisins) (37). Therefore, our current findings are not sufficient to support dietary recommendations to limit the intake of fruits, vegetables and whole grains among postmenopausal breast cancer survivors in an effort to minimize fructose or total carbohydrate intake. Finally, while the results for maltose appear novel, these results are limited due to the low levels of dietary intake.

The metabolism of carbohydrates may impact prognosis by increasing circulating concentrations of known breast cancer mitogens. Carbohydrate metabolism increases postprandial blood glucose concentrations, which ultimately results in activation of the

insulin/insulin-like-growth-factor axis (41). Elevated concentrations of blood glucose (42) and insulin (43-45) and markers of insulin resistance (43) predict poor prognosis among breast cancer survivors. Importantly, such associations between markers of insulin resistance and poor prognosis among breast cancer survivors may be confined to a five year time frame after the primary diagnosis (43), suggesting that the few years after completing treatment for breast cancer may be a critical time period for when dietary changes could impact prognosis among postmenopausal breast cancer survivors.

This study did not find an association between carbohydrate intake at baseline and prognosis, consistent with previous studies that compared usual dietary intake at one time point to prognosis among breast cancer survivors (20-23). However, in this study, dietary changes made after study enrollment are likely confounding any potential associations between usual (e.g., pre-enrollment) dietary intake and prognosis. This appears to be the first study to compare a change in carbohydrate intake on the macronutrient level and prognosis among breast cancer survivors.

A limitation of this study is the reliance on self-reported dietary intake, which has considerable sources of random and systematic error (46, 47). Notably, bias in self-reported intake is greater among overweight individuals (47). However, these results showed that the participants with the lowest rates of breast cancer recurrence, namely those who decreased their intake of total carbohydrates, were also more likely to be overweight and less active at baseline. Excess weight and low levels of physical activity are characteristics that are inconsistent with an improved prognosis (4, 9). Thus, it is unlikely that reporting bias or even regression to the mean are accounting for the observed effects between changes in carbohydrate intake and prognosis, and that reported

effect sizes may be attenuated. Importantly, this study did not measure potential mediating factors including one-year changes in blood glucose concentrations or insulin sensitivity, and therefore we are not able to directly test the hypothesis that changes in carbohydrate intake were mediated by blood glucose or activation of the insulin/insulin-like-growth-factor axis.

Strengths of this study include the use of 24-hour dietary recalls, a method that while not free of reporting bias (46), is more accurate in measuring the intake of specific macronutrients than food frequency questionnaires (47). Importantly, participants in the WHEL trial made considerable dietary change within 6 months of enrollment, and changes were sustained over the course of the trial (12). While the WHEL dietary intervention did not impact prognosis (12), these results suggest that with additional tailoring of the dietary intervention targets, that a dietary modification program based on telephone counseling may help postmenopausal breast cancer survivors make lifestyle modifications that could improve prognosis.

In summary, results from this study suggest that a change in carbohydrate intake may impact prognosis among postmenopausal breast cancer survivors when dietary changes are made within the few years after a diagnosis. Furthermore, associations between changes in carbohydrate intake and prognosis may differ based on carbohydrate subtype. Results need to be confirmed from other studies among postmenopausal breast cancer survivors, and additional studies are needed to measure potential mediating factors include blood glucose concentrations, insulin sensitivity and insulin resistance.

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Table 1.1 Baseline Characteristics by Breast Cancer Recurrence Status among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1]

	Non-Events N=1864 (88.3%)	**Events N=247 (11.7%)**	**Chi-Square p-value**
Demographics			
Age			
<45 years	102 (5.5%)	25 (10.1%)	0.019
45-54 years	790 (42.4%)	90 (36.4%)	
55-59 years	400 (21.5%)	57 (23.1%)	
>=60 years	572 (30.7%)	75 (30.4%)	
White, non-Latina	1605 (86.1%)	215 (87.0%)	0.761
College graduate	1014 (54.5%)	122 (49.4%)	0.157
Baseline lifestyle			
BMI			
<25	785 (42.1%)	93 (37.7%)	0.376
25-29.9	586 (31.4%)	81 (32.8%)	
>=30	493 (26.5%)	73 (29.6%)	
Total energy intake, kcals per day, quartiles[2]			
<1423	461 (24.7%)	69 (27.9%)	0.498
1424 – 1670	463 (24.8%)	64 (25.9%)	
1671 – 1956	466 (25.0%)	61 (24.7%)	
>1956	474 (25.4%)	53 (21.5%)	
Total carbohydrate intake, grams per day, quartiles[2]			
<= 194	493 (26.5%)	74 (30.0%)	0.256
195-233	462 (24.8%)	69 (27.9%)	
234-275	475 (25.5%)	52 (21.1%)	
>275	434 (23.3%)	52 (21.1%)	

Table continued next page

Table 1.1. Baseline Characteristics by Breast Cancer Recurrence Status among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1] Continued.

	Non-Events N=1864 (88.3%)	**Events N=247 (11.7%)**	**Chi-Square p-value**
Total dietary fiber intake, grams per day, quartiles[2]			
<16	468 (25.1%)	64 (25.9%)	0.450
16-20	451 (24.2%)	69 (27.9%)	
21-25	473 (25.4%)	61 (24.7%)	
>25	472 (25.3%)	53 (21.5%)	
Physical activity level			
Inactive: <3.3 MHW	438 (24.1%)	62 (26.2%)	0.531
Moderate: 3.3 - <10 MHW	426 (23.4%)	60 (25.3%)	
Active: 10 - <20 MHW	441 (24.3%)	59 (24.9%)	
High: ≥20 MHW	510 (28.1%)	56 (23.6%)	
Primary cancer clinical characteristics			
Stage[3]			
I	774 (41.5%)	51 (20.7%)	<0.001
II	1016 (54.5%)	172 (69.6%)	
IIIA	74 (4.0%)	24 (9.7%)	
Tumor differentiation			
Well-moderate	1104 (59.2%)	122 (49.4%)	0.013
Poor	595 (31.9%)	97 (39.3%)	
Unspecified	165 (8.9%)	28 (11.3%)	
Tumor size			
>2cm	695 (37.4%)	146 (59.1%)	<0.001
Number of positive nodes			
0	1114 (59.8%)	95 (38.5%)	<0.001
1-3	545 (29.3%)	76 (30.8%)	
>3	204 (11.0%)	76 (30.8%)	

Table continued next page

Table 1.1. Baseline Characteristics by Breast Cancer Recurrence Status among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1] Continued.

	Non-Events N=1864 (88.3%)	**Events N=247 (11.7%)**	**Chi-Square p-value**
ER Positive	1418 (77.2%)	186 (76.2%)	0.810
PR Positive	1246 (68.5%)	166 (68.0%)	0.951
Treatments			
Surgery			
Lumpectomy	902 (48.4%)	102 (41.3%)	0.102
Mastectomy	961 (51.6%)	145 (58.7%)	
Chemotherapy	1249 (67.0%)	196 (79.4%)	<0.001
Radiation therapy	1115 (59.9%)	162 (65.6%)	0.096
Ever tamoxifen use[4]	1389 (74.5%)	182 (73.7%)	0.838

[1] Survivors also remained recurrence-free 1.5 years after enrollment.
[2] Dietary intake assessed with multiple 24-hour dietary recalls.
[3]American Joint Committee on Cancer staging, version IV
[4] N=199 reported any anti-estrogen use, N=194 (97.5%) of which was tamoxifen.

Table 1.2. Percent Contribution of Each Food Group to Total Carbohydrate Intake at Baseline, Grams per Day, among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1]

Food Group	**% Total carbohydrate intake from food group and % within subgroups**	
Grains: Refined	26.2%	
Cereals and grains (e.g., pasta, rice)		48.4%
Breads		29.2%
Crackers, chips, savory snacks		15.0%
Ready-to-eat cereal		5.1%
Granola, breakfast, diet or energy bars		1.2%
Sweets and desserts	23.2%	
Sugar-sweetened beverages		31.3%
Added sugar		27.1%
Cookies, cakes, muffins		13.3%
Candy and chocolate		12.4%
Frozen treats		10.0%
Flavored yogurt with added sugars		4.5%
Other		1.3%
Fruits and fruit juice	18.6%	
Fresh fruit		66.2%
100 % Fruit juice		22.3%
Dried fruit		7.1%
Cooked fruit		4.1%
Grains: Whole	10.3%	
Ready-to-eat cereal		38.5%
Breads		35.3%
Cereals and grains (e.g., pasta, rice)		26.1%
Crackers, chips, savory snacks		0.1%

Table continued next page

Table 1.2. Percent Contribution of Each Food Group to Total Carbohydrate Intake at Baseline, Grams per Day, among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1] Continued.

Food Group		**% Total carbohydrate intake from food group and % within subgroups**	
Non-starchy vegetable		5.4%	
	Tomatoes, tomato sauce, tomato salsa		23.0%
	Carrots		21.7%
	Alliums		10.7%
	Broccoli		6.3%
	Other[3]		38.3%
Starchy vegetable		5.2%	
	White potato		75.4%
	Corn		14.5%
	Sweet potato or yam		7.3%
	Winter squash		2.2%
	Other		0.6%
Dairy		4.0%	
	Milk as beverage		84.4%
	Cheese, cream, butter, non-beverage milk		15.6%
Spreads and condiments		2.6%	
	Condiments		35.8%
	Jams, jellies, preserves		30.6%
	Salad dressings and mayonnaise		24.6%
	Imitation dairy[3]		9.0%
Beans, peas, lentils		2.3%	
Vegetable Juice		0.2%	

[1] Survivors also remained recurrence-free 1.5 years after enrollment.
[2] The 10 carbohydrate-based food groups accounted for 98.0% of total carbohydrate intake at baseline; 2.0% of carbohydrate intake was provided by fat-, protein- and alcohol-based food groups.
[3] Other non-starchy vegetables includes 20 items.
[4] Imitation dairy includes non-dairy processed cheese and cheese spreads, margarines and non-dairy creamers.

Table 1.3. Select Characteristics by Tertiles of One-Year Change in Total Carbohydrate Intake among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1]

	One-Year Change in Total Carbohydrate Intake			
	Decrease ≤ -26.7 g/day (N=696)	**Minimal change (-26.7, +22.2) g/day (N=721)**	**Increase >22.2 g/day (N=694)**	**p-value[2]**
Baseline Demographic Characteristics				
Age, mean (SD)	55.1 (7.7)	56.4 (7.5)	56.5 (7.7)	0.001
College graduate, N (%)	349 (50.1%)	398 (55.2%)	389 (56.1%)	0.057
Baseline Lifestyle Characteristics				
BMI, N (%)				
<25 kg/m^2	247 (35.5%)	316 (43.8%)	315 (45.4%)	0.003
25-29.9 kg/m^2	242 (34.8%)	217 (30.1%)	208 (30.0%)	
≥30 kg/m^2	207 (29.7%)	188 (26.1%)	171 (24.6%)	
Activity Level[3], N (%)				
Inactive	176 (26.1%)	162 (23.3%)	162 (23.8%)	0.009
Mild-moderate	169 (25.1%)	172 (24.9%)	144 (21.1%)	
Active	163 (24.2%)	145 (20.8%)	192 (28.2%)	
Very active	166 (24.6%)	216 (31.0%)	184 (27.0%)	
Total carbohydrate intake, mean (SD)				
Baseline	269 (61)	228 (57)	208 (53)	<0.001
Month 12	200 (56)	225 (57)	274 (61)	<0.001

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Table 1.3. Select Characteristics by Tertiles of One-Year Change in Total Carbohydrate Intake among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1] Continued.

	One-Year Change in Total Carbohydrate Intake			
	Decrease ≤ -26.7 g/day (N=696)	**Minimal change (-26.7, +22.2) g/day (N=721)**	**Increase >22.2 g/day (N=694)**	**p-value[2]**
Change in carbohydrate intake (g/day), median (IQR)				
	-60.1 (-86.6, -40.8)	-2.8 (-14.3, +9.6)	+57.5 (+39.0, 84.8)	--

g/day: grams per day.
[1] Survivors also remained recurrence-free 1.5 years after enrollment.
[2] p-value from ANOVA F-test for means and Chi-Square tests for categorical measures.
[3] Activity levels defined as inactive: <3.3 MHW, mild-moderate: 3.3-9.9 MHW, active: 10-19.9 MHW, and very active: 20+ MHW.

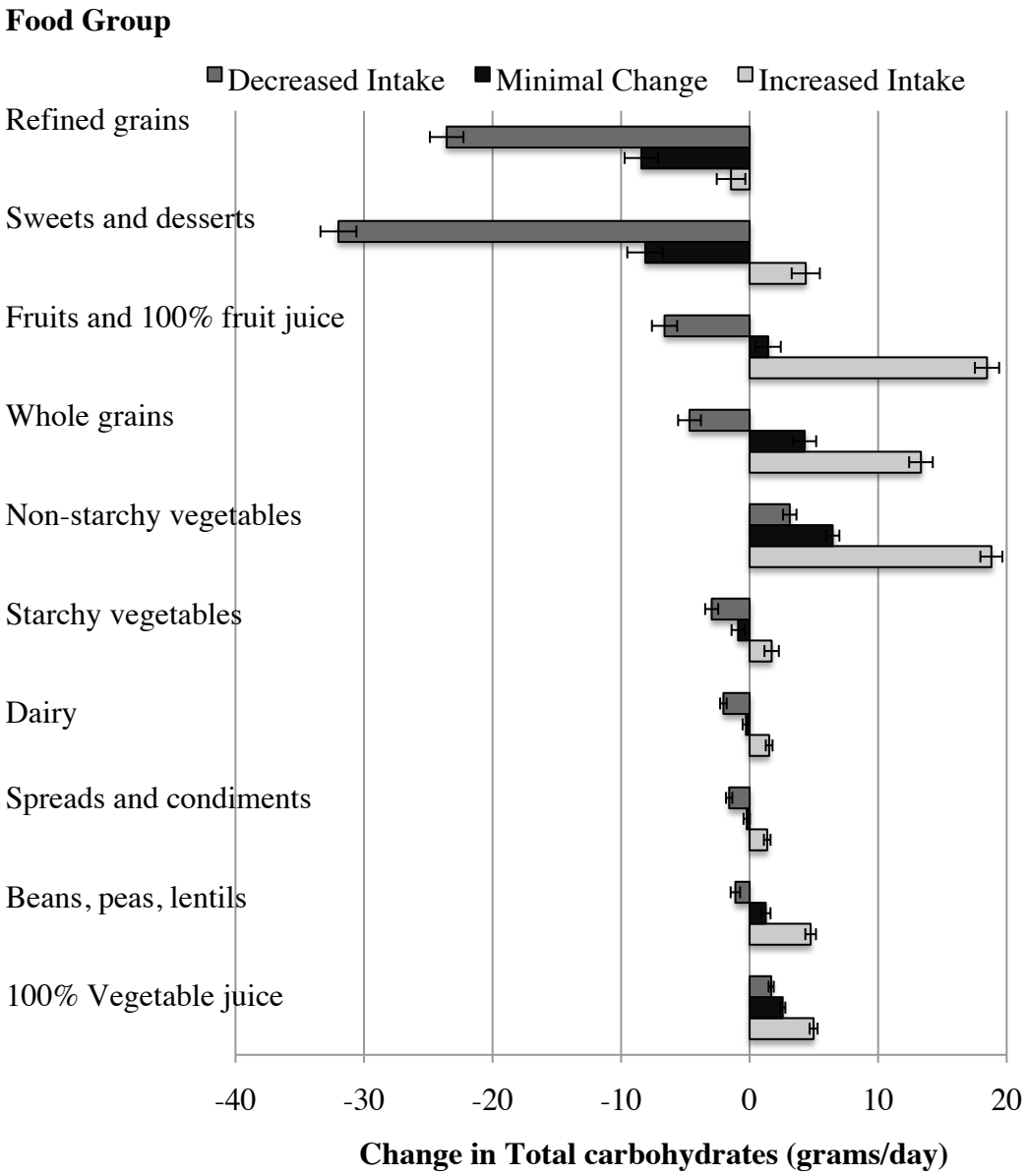

Figure 1.1 Change in Carbohydrate Intake (Grams/Day) per Each Carbohydrate-Based Food Group By Tertiles Of Overall Change in Carbohydrate Intake among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1,2,3]

[1]Decreased intake of total carbohydrates: tertile 1 of one-year net change (i.e. < -27 grams/day).
[2]Mean change per food group presented with standard error of mean.
[3]All between-group differences significant at $p<0.001$; ANOVA methods comparing unadjusted mean change.

Table 1.4. Unadjusted Breast Cancer Recurrence Rate by Distribution of One-Year Change in Total Carbohydrates and Carbohydrate Subtype Intake among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1]

	Tertiles of Change	**N**	**Median g/day**	**Additional Events, % (N)**
Total Carbohydrates[2]				
Decrease	≤ -26.7 g/day	696	-60.1	9.8% (68)
Minimal change	-26.7 to 22.2 g/day	721	-2.8	11.8% (85)
Increase	>22.2 g/day	694	57.5	13.5% (94)
Carbohydrate subtypes[2]				
Glucose				
Decrease	≤ -3.5 g/day	693	-9.8	10.1% (70)
Minimal change	-3.5 to 5.4 g/day	720	0.8	13.8% (99)
Increase	>5.4 g/day	697	11.0	11.2% (78)
Fructose				
Decrease	≤-3 g/day	695	-8.8	10.5% (73)
Minimal change	-3 to 6.9 g/day	698	1.9	11.9% (83)
Increase	>6.9 g/day	718	13.1	12.7% (91)
Galactose				
Decrease	≤-0.1 g/day	648	-0.3	10.3% (67)
Minimal change	-0.1 to 0.1 g/day	842	0.0	13.1% (110)
Increase	>0.1 g/day	621	0.3	11.3% (70)
Sucrose				
Decrease	≤-9.8 g/day	708	-20.6	11.9% (84)
Minimal change	-9.8 to 6.1 g/day	708	-2.6	11.2% (79)
Increase	>6.1 g/day	695	15.0	12.1% (84)

Table continued next page

Table 1.4. Unadjusted Breast Cancer Recurrence Rate by Distribution of One-Year Change in Total Carbohydrates and Carbohydrate Subtype Intake among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1] Continued.

	Tertiles of Change	**N**	**Median g/day**	**Additional Events, % (N)**
Lactose				
Decrease	≤-2.6 g/day	696	-6.1	12.2% (85)
Minimal change	-2.6 to 1.7 g/day	703	-0.4	11.0% (77)
Increase	>1.7 g/day	712	5.1	11.9% (85)
Maltose				
Decrease	≤-0.8 g/day	697	-2.2	9.6% (67)
Minimal change	-0.8 to 0.9 g/day	690	0.0	11.9% (82)
Increase	>0.9 g/day	723	2.4	13.6% (98)
Starch[2]				
Decrease	≤-19.1 g/day	684	-35.3	9.4% (64)
Minimal change	-19.1 to 4.8 g/day	725	-6.6	13.5% (98)
Increase	>4.8 g/day	702	18.5	12.1% (85)

[1]Survivors also remained recurrence-free 1.5 years after enrollment.
[2]All unadjusted event rates non-significant at the $p<0.050$ level per Chi-Square test, except for starch: $p=0.048$.

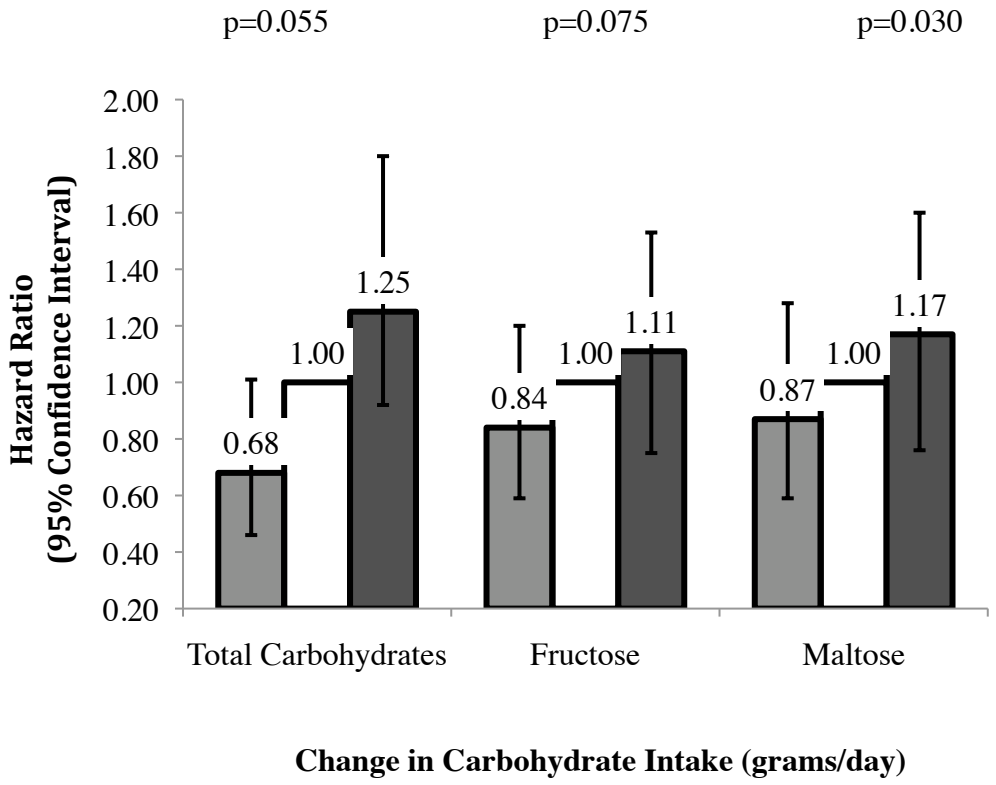

Figure 1.2. Hazard Ratios for Time to Breast Cancer Recurrence by Tertiles of Change in Total Carbohydrates, Fructose, or Maltose (Grams/Day) among N=2,111 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1,2,3]

[1] Survivors also remained recurrence-free 1.5 years after enrollment.
[2] Models adjusted for site, baseline measures (age, education, BMI, physical activity level, hot flash status), clinical/treatment measures of primary cancer (stage, grade, number of positive nodes, tumor size, chemo- and radiation therapy, ever use of anti-estrogen therapy) and dietary measures (baseline total energy, alcohol and fiber intake, and one-year change in total energy and fiber intake).
[3] p-value is for test of trend over point estimates: linear regression model fitting hazard ratio on median of change per tertile.

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CHAPTER 2

Associations between a Change in Carbohydrate Intake and Breast Cancer Recurrence by Expression of the Insulin-Like-Growth-Factor-1 Receptor in Primary Cancer Tissue

Abstract

Background: Carbohydrate metabolism activates the insulin/insulin-like-growth-factor (IGF) axis; IGF-1 receptor (IGF-1R) activation in breast cancer tissue triggers proliferation. We hypothesized that a reduced carbohydrate intake would improve prognosis among breast cancer survivors with primary cancers expressing IGF-1R.

Methods: Nested case-control study of N=265 postmenopausal breast cancer survivors enrolled in a dietary intervention trial. The primary exposure was change in carbohydrate intake (grams/day) over the first year of study enrollment, categorized as tertiles. Cases, defined as breast cancer recurrence, were matched to controls and counter-matched on a decreased carbohydrate intake (i.e., tertile 1) compared to stable/increased intake (i.e., tertiles 2,3). Primary breast cancers were stained (IHC) for IGF-1R. Weighted conditional logistic regression models fit the odds of recurrence on dietary change and IGF-1R status.

Results: Half of primary cancers were IGF-1R positive. IGF-1R expression significantly increased the odds of recurrence (OR: 1.7; 95%CI: 1.2-2.5). A decreased carbohydrate intake significantly decreased the odds (OR: 0.5; 95% CI: 0.2-0.8) of recurrence. The protective effect of a decreased carbohydrate intake was significantly greater among participants who had IGF-1R positive cancers (p=0.110). Among participants with IGF-1R negative cancers, a decreased carbohydrate intake reduced the odds of recurrence by 30% (OR: 0.7; 95%CI: 0.2-1.7). Among participants with IGF-1R positive cancers, a decreased carbohydrate intake reduced the odds by 80% (OR: 0.2; 95%CI: 0.03-0.3).

Conclusions: A reduced carbohydrate intake may improve prognosis among postmenopausal breast cancer survivors by limiting IGF-1R activation.

Impact: Results support a role of the insulin/insulin-like-growth-factor axis on prognosis among postmenopausal breast cancer survivors.

Introduction

A growing body of evidence supports positive associations between impaired insulin regulation and poor prognosis among breast cancer survivors (1-7). Insulin is a mitogen that can stimulate breast cancer proliferation (8). In addition, elevations in circulating insulin lead to hepatic production of insulin-like growth hormone-1 (IGF-1) (9), another mitogen that can trigger proliferative and pro-survival pathways in cancerous cells (9). Given that the metabolism of carbohydrates stimulates the insulin/IGF-1 axis (10), it is worthwhile to explore how dietary changes related to carbohydrate intake might impact prognosis among breast cancer survivors.

Breast cancers are known to over express the insulin-like growth factor-1 receptor (IGF-1R) (9, 11), and receptor expression has been associated with poor prognosis, (12-15) possibly due to treatment resistance and treatment evasion. Specifically, expression of IGF-IR has been positively associated with resistance to chemotherapies (12), radiation therapies (13, 14) and even trastuzumab (16, 17). Breast cancers may also up-regulate expression of the IGF-1R when treated with hormonal therapies such as tamoxifen (12, 15). Several clinical trials are currently underway to test the efficacy of monoclonal antibodies targeting the IGF-1R as part of usual breast cancer treatment (18).

Given that IGF-1R expression may be an indicator of resistant cancer tissue, it is possible that limiting activation of the IGF-1R may play a role in improving prognosis.

In this nested case control analysis of postmenopausal breast cancer survivors, we addressed how the association between a post-diagnosis change carbohydrate intake and breast cancer recurrence is modified by expression of the IGF-1R in the primary breast cancer.

Methods

Parent Study

This study is a nested, case control analysis among postmenopausal breast cancer survivors enrolled in the Women's Healthy Eating and Living (WHEL) dietary intervention trial (19). The WHEL study was a dietary intervention trial that enrolled N=3,088 breast cancer survivors diagnosed with early stage breast cancer. Women enrolled between 1995-2000, from 6 months to 4 years after their primary diagnosis. At baseline, half of the participants were assigned to a dietary pattern high in fruits, vegetables, fiber and low in total fat while half were given a printed copy of the USDA dietary guidelines. Total carbohydrate intake was not a target of the WHEL trial, and the WHEL trial did not target weight loss. The primary analysis did not find a significant association between intervention arm assignment and recurrence or mortality (19). This current study therefore treats participants in the WHEL study as a cohort.

For this analysis, participants were selected from a cohort of N=2,111 postmenopausal breast cancer survivors who had complete dietary intake data at baseline and year one, and who also remained recurrence free for 18 months after the baseline

assessment. Participants were limited to postmenopausal breast cancer survivors as insulin sensitivity differs by menopausal status (20), and endogenous sex hormones among premenopausal women can impact insulin sensitivity (21).

Tissue Samples

Tissue samples of the primary breast cancer were achieved at the WHEL coordinating center (22). Local hospitals provided paraffin-embedded blocks of representative cancer tissue to the Coordinating Center, and the Histology Core Shared Resources Laboratory at the University of California, San Diego (UCSD) Moores Cancer Center prepared ten unstained slides from each block. The study pathologist reviewed each slide to confirm that tissue samples were consistent with pathology reports; slides were preserved in paraffin wax and stored at room temperature at the Coordinating Center (22). All women in the WHEL study provided written, informed consent for all aspects of the WHEL study including tissue acquisition. The Institutional Review Board for UCSD approved of the WHEL study, and all WHEL study sites received IRB approval.

Dietary Intake

Dietary intake data from the baseline and year one assessments of the WHEL study were used in this analysis. Dietary intake was assessed with multiple, 24-hour dietary recalls at each time point. Dietary intake data were collected and analyzed using Nutrition Data System (NDS) for Research software version 4.03 (1994-2006) developed

by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Complete methods for the dietary assessments have been published (19).

For this analysis, total carbohydrate intake (grams) was extracted from the NDS database, and the average daily intakes at baseline and at year one were computed. Change in intake over the first year of study enrollment was computed as the year one minus the baseline average daily intake. Using data from the full cohort of eligible postmenopausal women (N=2,111), change in carbohydrate intake was categorized as tertiles of change over the first year of enrollment (i.e., tertile 1: < -26.8 grams/day, tertile 2: -26.8 to +22.2 grams/day, tertile 3: > +22.2 grams/day). A decreased intake was defined as the lowest tertile. A preliminary analysis by our group showed a positive trend over increasing tertiles of change in carbohydrate intake with an increased risk of recurrence (test of linear trend: p=0.055) (*Please see Chapter 1 of this dissertation for those results*), with the point estimate for a decreased intake nearing statistical significance (adjusted HR: 0.68; 95% CI: 0.46-1.01; p=0.056). Therefore, to improve power for this analysis, change in carbohydrate intake was defined as a decreased intake (i.e., tertile 1 of change) versus stable/increased intake (i.e., tertiles 2 and 3 of change).

Case control design

Cases were defined as a confirmed breast cancer recurrence over the WHEL study period. Recurrence included local, regional or distant invasive metastasis or new primary breast cancer. From the full cohort of postmenopausal breast cancer survivors eligible for this study (N=2,111), there were N=247 breast cancer recurrences, and primary cancer tissue was available for N=91. Two controls for each case were selected from eligible

participants who remained recurrence free one year after the index case's date of recurrence. Controls were matched to cases based on stage of primary cancer (exact match based on American Joint Committee on Cancer staging, version IV), age at diagnosis (within 5 years) and time from diagnosis to enrollment into the WHEL trial (within one year). Less than 5% of controls were matched based on relaxed criteria for age at diagnosis (within 10 years) or time from diagnosis to enrollment (within 2 years).

The primary hypothesis of this study postulated that the protective effect of a net decrease in carbohydrate intake would be most pronounced among participants with IGF-1R expression in the primary cancer. Therefore, controls were counter-matched (23) on change in total carbohydrate intake (grams/day) over the first year of enrollment into the WHEL study. Counter-matching increases the power to detect an interaction between two independent predictors (24). Cases and controls were counter-matched based on a decreased carbohydrate intake versus stable/increased carbohydrate intake. For each case, both matched controls were counter-matched on change in carbohydrate intake.

Immunohistochemical analysis

Immunohistochemical (IHC) analysis was used to quantify expression of total IGF-1R. Analyses were completed at the Histology Core Shared Resources Laboratory at the UCSD Moores Cancer Center, and the WHEL study pathologist supervised the analysis. Primary antibody was mouse monoclonal, clone 24-31, purified without BSA and Azide and supplied at 20 μg/mL (Thermo Scientific #MS-641-PABX; Freemont, CA). Antibody epitope was the amino acid sequence 283-440 on the alpha-subunit of the IGF-1R. Staining methods began with a standard IHC protocol, and a series of

optimization steps were completed to ensure that the integrity of tissue samples was sufficient for analyses.

Slides were first deparaffinized with a sequence of incubations in Xylene and decreasing concentrations of ethanol. Endogenous peroxidases were blocked with 0.3% H_2O_2 in PBS (Fisher-Scientific H325-100) for 20 minutes at room temperature, followed by three buffer rinses with PBS. Endogenous collagen binding was blocked using 1% BSA/PBST for 5 minutes followed by tapping-off of liquid without washing. Antigen retrieval was performed with Proteinase K treatment; the primary antibody was overlaid overnight, followed by treatment with the secondary antibody and color development. The IGF-1R antibody was diluted 1:100 with 1% BSA (Sigma #A 4503-50G). Sections were tested twice with reagents with two overnight incubations. The Dako EnVision+ System, HRP (Dako K4001) was used as the secondary reagent. Labeled polymer (prediluted) was applied for one hour at room temperature. Slides were rinsed three times in PBS. Chromagen (AEC substrate kit for peroxidase, Vector Labs SK4200) was applied for 40 minutes at room temperature, followed by three buffer rinses with PBS. Samples were mounted in aqueous mounting medium (Vectamount H-5501) and stored at room temperature. Mouse IgG (Dako #N1698) was used as a negative control against the primary antibody; mouse anti-Vimentin (Dako #N1521) was used as a positive control. In-house tissue samples of breast cancer were used as positive controls for breast cancer tissue.

Scoring of IGF-1R expression

A pathologist affiliated with the UCSD School of Medicine scored all slides. The study pathologist was blind to both case and control status as well as blind to all matching and counter matching criteria. Slides were scored based on total membrane staining and intensity: 0 (no staining for membrane staining in <10% of cancer cells); 1+ (faint membrane staining in ≥10% of cancer cells); 2+ (weak to moderate complete membrane staining ≥10% of cancer cells; 3+ (strong complete membrane staining in >30% of cancer cells). Methods are analogous to those used to score HER2 expression. Cancers with no evidence of staining were considered negative, and cancers with positive staining (1, 2, 3) were considered positive. Eight samples were unreadable due to poor tissue integrity (all samples from controls), resulting in a final sample size of N=265 participants (N=91 cases and N=174 controls).

Statistical Analyses

Primary cancer characteristics, treatment history and baseline demographics were compared by case status and also by IGF-1R status (i.e., negative versus positive). Bivariate analyses were completed with un-weighted Chi-Square or T-Tests. The primary aim of this analysis was to determine how expression of the IGF-1R in primary breast cancer tissue modified the main effect of a decreased carbohydrate intake on recurrence. Weighted conditional logistic regression modeling was used to address that primary aim, fitting case status (i.e., breast cancer recurrence) on the cross product of a decreased carbohydrate intake and IGF-1R status. Weighting in the conditional logistic regression models accounted for the counter-matching design (25). The regression model was adjusted for a-prior covariates related to dietary carbohydrate intake (i.e.,

carbohydrate intake at baseline, total caloric intake at baseline and change over one-year, and total dietary fiber intake at baseline and change over one-year) and for covariates that were not balanced by IGF-1R status ($p<0.100$).

As described above, two controls were selected for each case, forming a triplet eligible for analysis. Because each control selected per case was counter-matched on change in carbohydrate intake, only one control from each triplet could be used in the conditional logistic regression analysis when incorporating appropriate conditional weighting (25). Therefore, for the primary analysis, one control from each triplet was randomly selected. That resulted in a sample of one control matched and counter-matched to each case, for a final sample size of N=182. An adjusted conditional logistic regression model was run on that sample and point estimates were saved for analysis. That process of randomly selecting one control from each triplet to create one dataset (N=182) for the primary analysis was repeated 5,000 times; results were used to empirically estimate the expected distribution of point estimates. The final odds ratios presented were computed by exponentiation of the mean of the model coefficients over the 5,000 runs and 95% confidence intervals were computed by exponentiation of the 2.5^{th} and 97.5^{th} percentiles of the model coefficients over the 5,000 runs. P-values do not follow a normal distribution and are not presented. However, the median p-value (along with the inter-quartile range) is presented for the results from Likelihood Ratio Tests used to assess the significance of the overall interaction between change in carbohydrate intake and IGF-1R status. Specifically, for each conditional logistic regression model, a Likelihood Ratio Test compared two nested models, one with the main effects of a change in carbohydrate intake and IGF-1R status, and one with main effects along with

an interaction term. All analyses were computed using the R Language and Environment for Statistical Computing, version 2.15.2 (http://www.R-project.org).

Results

Table 2.1 presents demographic and baseline lifestyle characteristics overall and by case status. Median time from diagnosis to enrollment was 21.7 months with no difference by case status (Rank-sum p=0.563). Mean age at diagnosis was 56.8 (SD 6.3) years, with the majority of the sample being 55 years or older. Most participants (84%) were White, non-Latina. There were no differences in baseline BMI or physical activity levels by case status, with the majority of the sample (37%) being obese. Table 2.2 presents clinical characteristics of the primary breast cancer and treatments received overall and by case status. Consistent with the full WHEL study sample (Pierce 2007), primary cancers were mostly earlier stage and well to moderately differentiated. Roughly half of all primary cancers were > 2 cm in diameter, and most were positive for the estrogen or progesterone receptors. Cases and controls were well balanced on demographic, lifestyle and primary cancer characteristics. There was only one significant difference between cases and controls: cases were more likely to have had node positive cancers. Regardless, cases and controls were well balanced on surgical and adjuvant therapies.

Figure 2.1 presents representative images of tissue samples from the IHC analysis. One-half (50.2%) of primary cancers stained positive for IGF-1R. The majority of tissues that stained positive for IGF-1R were given a score of 1 (N=87, 65.4%), with fewer given a score of 2 (N=40, 30.1%) or 3 (N=6, 4.5%). Table 2.3

presents the distribution of IGF-1R status for demographic and baseline lifestyle characteristics, as well as clinical and treatment characteristics that were not balanced ($p<0.100$) by IGF-1R status. White, non-Latina women were less likely to have a primary cancer that was IGF-1R positive ($p=0.074$). Notably, 11 of the 12 African-American participants and 11 of the 19 Latina participants had primary breast cancers that were IGF-1R positive. Thus, African-American and Latina participants were considerably more likely to have had a primary cancer that expressed the IGF-1R. When considering clinical characteristics of the primary cancer and treatments received, only two differences were found by IGF-1R status. Cancers that were IGF-1R positive were more likely to be positive for the progesterone receptor ($p=0.004$), and participants with IGF-1R positive cancers were less likely to have received adjuvant chemotherapy ($p=0.007$).

Mean change in carbohydrate intake over the first year of study enrollment did not differ by IGF-1R status (-31 grams/day, SD 59, T-test p-value=0.963). Figure 2.2 presents the mean change in total carbohydrate intake, stratified by a decreased carbohydrate intake and IGF-1R status. There were no statistical differences in net change in carbohydrate intake by IGF-1R status within each stratum.

In separate conditional logistic regression models, both of the main effects for a change in carbohydrate intake and expression of the IGF-1R were significantly associated with breast cancer recurrence. Specifically, a decreased carbohydrate intake over one year after study enrollment was associated with a reduced likelihood of recurrence compared to a stable/increased intake (OR: 0.5; 95% CI: 0.2-0.8); that model was adjusted for carbohydrate intake, total energy, and fiber intake at baseline, as well as

changes in intakes for total energy and fiber over one year. Separately, a primary cancer positive for IGF-1R was associated with an increased likelihood of recurrence (OR: 1.7; 95% CI: 1.2-2.5); that model was adjusted for race/ethnicity, number of positive nodes, PR status of primary cancer, and chemotherapy treatment.

To address the primary analysis, conditional logistic regression was used to fit recurrence status on the cross-product of change in carbohydrate intake and IGF-1R status, adjusted for covariates (Table 2.4). Results from the Likelihood Ratio Test suggest a significant interaction between a decreased carbohydrate intake and IGF-1R status with prognosis (p-value=0.110). Specifically, the protective effect of a decreased carbohydrate intake was more pronounced among participants whose primary cancer was IGF-1R positive. Among participants with IGF-1R negative cancers (Table 2.4), a decreased carbohydrate intake reduced the odds of recurrence by 30% (OR: 0.7; 95% CI: 0.2-1.7). In comparison, among participants with IGF-1R positive cancers (Table 4), a decreased carbohydrate intake reduced the odds of recurrence by 80% (OR: 0.2; 95% CI: 0.03-0.3).

Discussion

In this study of postmenopausal breast cancer survivors, expression of the IGF-1 receptor significantly increased the likelihood of a breast cancer recurrence. However, a decreased intake of carbohydrates was protective against a breast cancer recurrence, and that protective effect was significantly greater among participants whose primary cancer expressed the IGF-1R. Specifically, among participants whose primary cancer was IGF-1R negative, a decreased carbohydrate intake reduced the odds of recurrence by 30%. In

comparison, among participants whose primary cancer was IGF-1R positive, a decreased carbohydrate intake reduced the odds of recurrence by 80%.

Consistent with several case-control studies (26-29), our findings demonstrate that expression of the IGF-1R is predictive of a poor prognosis. Our study also reported a significant, positive association between IGF-1R expression and expression of the progesterone receptor, as did one previous study (28). Our study did not find any significant associations with IGF-1R status and stage or grade of the primary breast cancer, which is inconsistent with previous studies which have reported that IGF-1R expression is down-regulated in more severe cancers (27, 28). However, associations between IGF-1R expression and breast cancer characteristics or subtypes have not been consistent across studies (14, 15, 26-29, 30-32).

These results report that minority participants, specifically African-American and Latina postmenopausal breast cancer survivors, were more likely to have a primary cancer that was positive for the IGF-1R. Those findings are in alignment with a previous study (33) which found increased protein expression of the IGF-1R (via Western blot analyses) and increased levels of activated IGF-1R (via ELISA methods) in primary breast cancer tissue from African American breast cancer survivors compared to tissue from White, non-Latina breast cancer survivors. African American and Latina breast cancer survivors have a worse prognosis than White, non-Latina breast cancer survivors (34). Racial and ethnic disparities in prognosis relate to screening, disease severity, treatment access, and treatment response (35); however, differences in lifestyle behaviors also account for a proportion of the observed disparities (36). Results from our current study suggest that African-American and Latina postmenopausal breast cancer survivors

may particularly benefit from a reduced intake of carbohydrates based on the likelihood of IGF-1R expression in the primary breast cancer.

The protective effect of a decreased carbohydrate intake observed in this study was not limited to women with primary breast cancers that were IGF-1R positive. It is possible that a reduced intake of carbohydrates may relate to a reduced risk of recurrence by limiting the availability of glucose as an energy source for malignant cells (37), by reducing circulating insulin, a mitogen of breast cancer growth, (9) or by reducing levels of systemic inflammation (38). Additionally IGF-1 (and to a lesser extent insulin) are also ligands for the insulin/IGF-1R hybrid receptors, receptors that also trigger breast cancer proliferation (9, 15, 39). Our antibody was not specific for the insulin or IGF-1R hybrid receptors. Therefore, it is possible that a decreased carbohydrate intake influenced breast cancer progression by limiting activation of receptors other than the IGF-1R. Regardless, these results clearly demonstrate a benefit of reduced carbohydrate intake within the few years after a breast cancer diagnosis among postmenopausal survivors.

A strength of this study includes the specific counter-matching design, an approach useful for detecting interactions with nested designs (24). Strengths also include the careful dietary assessment methods used in the parent study, and the considerable variability in change in carbohydrate intake observed in the parent randomized trial. As a limitation, we are not able to address the impact of potential effect modifiers such as anti-estrogen treatments (40, 41), or estrogen receptor status of the primary cancer (42) without losing a considerable proportion of matched case/control pairs for analysis. Additionally, we only investigated a decreased intake of carbohydrates in comparison to a stable intake or increased intake of carbohydrates combined. We

cannot make any inference specifically related to an increased carbohydrate intake. However, our results highlight the potential impact post-diagnosis dietary modifications may have on prognosis with respect to the IGF-1R.

In this study, we found that a decreased carbohydrate intake, as compared to a stable/increased intake, may reduce the risk of recurrence among postmenopausal breast cancer survivors. Importantly, such a decreased intake may have a significantly more profound effect for postmenopausal breast cancer survivors whose primary cancer was IGF-1R positive. The results from this study contribute to the growing evidence linking the insulin/insulin-like-growth-factor axis to breast cancer prognosis. Future, more highly powered studies should compare the associations between changes in carbohydrates intake, IGF-1R expression and prognosis while also considering the potential impacts of adjuvant therapies and estrogen receptor status of the primary cancer.

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Chapter 2 is currently being prepared for publication. I am the primary investigator and author of this material. Co-authors include Ruth E. Patterson, Loki Natarajan, Nissi M. Varki, Laarni R. Gapuz, John Nguyen, Susan Wancewicz, and John P. Pierce.

Table 2.1. Demographic and Baseline Lifestyle Characteristics by Case and Control Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis.[1,2]

		Case Status		
	Overall	**Control**	**Case**	**p-value[3]**
	N=265 (100%)	**N=174 (100%)**	**N=91 (100%)**	
Demographics				
Age at diagnosis*				
<45 years	4 (1.5%)	2 (1.2%)	2 (2.2%)	0.521
45-54 years	104 (39.3%)	68 (39.1%)	36 (39.6%)	
55-59 years	74 (27.9%)	53 (30.5%)	21 (23.1%)	
≥60 years	83 (31.3%)	51 (29.3%)	32 (35.2%)	
White, non-Latina	221 (83.4%)	144 (82.8%)	77 (84.6%)	0.832
College graduate	115 (43.4%)	76 (43.7%)	39 (42.9%)	>0.999
Baseline lifestyle				
BMI				
<25	78 (29.4%)	51 (29.3%)	27 (29.7%)	0.998
25-29.9	88 (33.2%)	58 (33.3%)	30 (33.0%)	
≥30	99 (37.4%)	65 (37.4%)	34 (37.4%)	
Physical activity level				
Inactive: <3.3 MHW	68 (26.3%)	46 (27.1%)	22 (24.7%)	0.946
Moderate: 3.3 - <10 MHW	73 (28.2%)	46 (27.1%)	27 (30.3%)	
Active: 10 - <20 MHW	60 (23.2%)	40 (23.5%)	20 (22.5%)	
High: ≥20 MHW	58 (22.4%)	38 (22.4%)	20 (22.5%)	

MHW: MET hours per week (44).
*Matching factor.
[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.
[2] Cases represent confirmed breast cancer recurrence. Percentages sum down columns.
[3] p-values are from Chi-squares tests for percentages.

Table 2.2. Clinical Features and Treatments by Case and Control Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis.[1,2]

		Case Status		
	Overall N=265 (100%)	**Control 174 (100%)**	**Case 91 (100%)**	**p-value[3]**
Primary cancer clinical characteristics				
Stage[*]				
I	66 (24.9%)	43 (24.7%)	23 (25.3%)	0.991
II	175 (66.0%)	115 (66.1%)	60 (65.9%)	
IIIA	24 (9.1%)	16 (9.2%)	8 (8.8%)	
Tumor differentiation				
Well-moderate	131 (49.4%)	86 (49.4%)	45 (49.5%)	0.459
Poor	99 (37.4%)	62 (35.6%)	37 (40.7%)	
Unspecified	35 (13.2%)	26 (14.9%)	9 (9.9%)	
Tumor size				
>2cm	140 (53.0%)	92 (53.2%)	48 (52.8%)	>0.999
Number of positive nodes				
0	122 (46.0%)	86 (49.4%)	36 (39.6%)	0.028
1-3	76 (28.7%)	53 (30.5%)	23 (25.3%)	
>3	67 (25.3%)	35 (20.1%)	32 (35.2%)	
ER Positive	190 (72.8%)	121 (70.8%)	69 (76.7%)	0.383
PR Positive	155 (60.1%)	97 (57.7%)	58 (64.4%)	0.360

Table continued next page

Table 2.2. Clinical Features and Treatments by Case and Control Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis,[1,2] Continued.

		Case Status		
	Overall	**Control**	**Case**	**p-value[3]**
	N=265 (100%)	**174 (100%)**	**91 (100%)**	
Treatments				
Surgery				
Lumpectomy	119 (44.9%)	82 (47.1%)	37 (40.7%)	0.445
Mastectomy	145 (54.7%)	91 (52.3%)	54 (59.3%)	
Neither	1 (0.4%)	1 (0.6%)	0 (0%)	
Chemotherapy	185 (70.1%)	117 (67.6%)	68 (74.7%)	0.291
Radiation therapy	168 (63.4%)	110 (63.2%)	58 (63.7%)	>0.999
Ever tamoxifen use[4]	199 (75.1%)	126 (72.4%)	73 (80.2%)	0.213

*Matching factor.
[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.
[2] Cases represent confirmed breast cancer recurrence. Percentages sum down columns.
[3] p-values are from Chi-squares tests for percentages.
[4] N=199 reported any anti-estrogen use, N=194 (97.5%) of which was tamoxifen.

Table 2.3. Demographic, Baseline Lifestyle, Clinical Features and Treatments by Case and Control Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis.[1,2]

	IGF-1R Status		
	Negative	**Positive**	**p-value[3]**
	N=132 (100%)	**N=133 (100%)**	
Demographics			
Age at diagnosis*			
<45 years	2 (1.5%)	2 (1.5%)	0.515
45-54 years	48 (36.4%)	56 (42.1%)	
55-59 years	35 (26.5%)	39 (29.3%)	
≥60 years	47 (35.6%)	36 (27.1%)	
White, non-Latina	116 (87.9%)	105 (79.0%)	0.074
College graduate	62 (47.0%)	53 (39.9%)	0.296
Baseline lifestyle			
BMI at baseline			
<25	41 (31.1%)	37 (27.8%)	0.312
25-29.9	53 (40.2%)	46 (34.6%)	
≥30	38 (28.8%)	50 (37.6%)	
Physical activity level			
Inactive: <3.3 MHW	32 (24.6%)	36 (27.9%)	0.443
Moderate: 3.3 - <10 MHW	41 (31.5%)	32 (24.8%)	
Active: 10 - <20 MHW	26 (20.0%)	34 (26.4%)	
High: ≥20 MHW	31 (23.9%)	27 (20.9%)	
Total CHO, grams/day, median (IQR)	244 (199-274)	246 (191-283)	0.784

Table continued next page

Table 2.3. Demographic, Baseline Lifestyle, Clinical Features and Treatments by Case and Control Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis,[1,2] Continued.

	IGF-1R Status		
	Negative	**Positive**	**p-value[3]**
	N=132 (100%)	**N=133 (100%)**	
Primary cancer clinical characteristics and treatments[4]			
PR Positive	67 (51.2%)	88 (69.3%)	0.004
Chemotherapy	103 (78.0%)	82 (62.1%)	0.007

MHW: MET hours per week (Hong et. al 2007); CHO: carbohydrates.
*Matching factor.
[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.
[2] IGF-1 receptor status scored analogous to HER-2 score and categorized as negative (0) versus positive (1,2,3). Percentages sum down columns.
[3] p-values are from Chi-squares tests for percentages or rank-sum test for medians.
[4] Only those measures that were significantly different by IGF-1R status ($p \leq 0.100$) presented.

Table 2.4. Adjusted Likelihood of Breast Cancer Recurrence by Change in Carbohydrate Intake over the First Year of Trial Enrollment and IGF-1R Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis.[1,2,3,4]

IGF-1R status of primary breast cancer[3]	Change in CHO intake[2]	Odds Ratios (95% CI)[5]		
		Overall	Within IGF-1R Status	
Negative	Stable/increased	1 (Referent)	1 (Referent)	--
Negative	Decreased	0.7 (0.2 – 1.7)	0.7 (0.2 – 1.7)	--
Positive	Stable/increased	5.5 (1.8 – 16.3)	--	1 (Referent)
Positive	Decreased	0.6 (0.2 – 1.3)	--	0.2 (0.03-0.3)

Likelihood Ratio Test, null hypothesis of no significant interaction: p=0.110[6]

CHO: Carbohydrates; CI: Confidence interval.

[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.

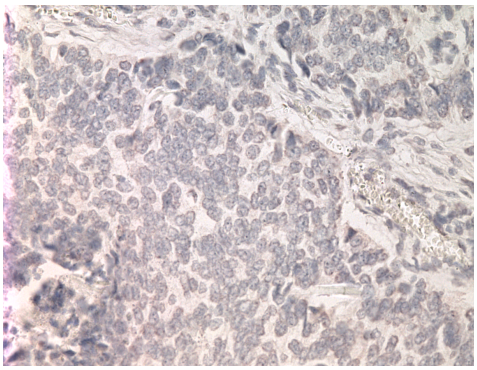
[2] Change in intake of carbohydrates based on tertiles of change in total carbohydrate intake over the first year of trial enrollment for the full cohort. Decreased intake reflects lowest tertile of change (< -26.7 grams/day) and stable/increased intake reflects upper two tertiles of change.

[3] IGF-1 receptor status scored analogous to HER-2 score and categorized as negative (0) versus positive (1,2,3).

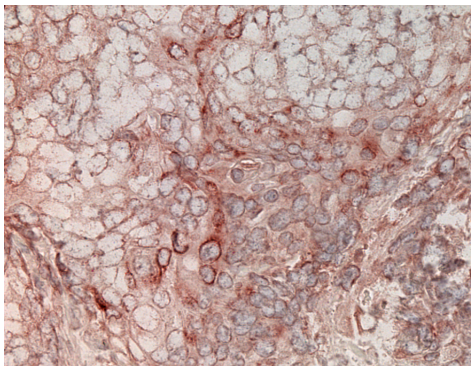
[4] Model also adjusted for total carbohydrate intake at baseline (grams/day), total caloric intake at baseline and change over one-year (kcals/day), fiber intake at baseline and change over one-year (grams/day), race/ethnicty, number of positive nodes, PR status and chemotherapy treatment.

[5] Odds ratios are based on exponentiation of the mean coefficient from 5,000 conditional logistic regression models; each model used a dataset with one control per each case; for each dataset, controls per each case were randomly sampled from the two available controls per each case. 95% Confidence intervals similarly reflect exponentiation of the 2.5th and 97.5th percentile of the distribution of model coefficients.

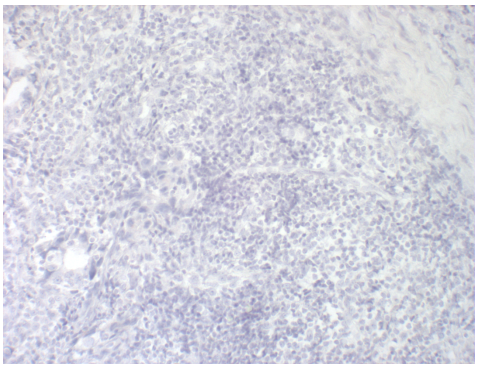
[6] p-value for Likelihood Ratio Test reflects median over 5,000 runs. Inter-quartile range: 0.038-0.260.



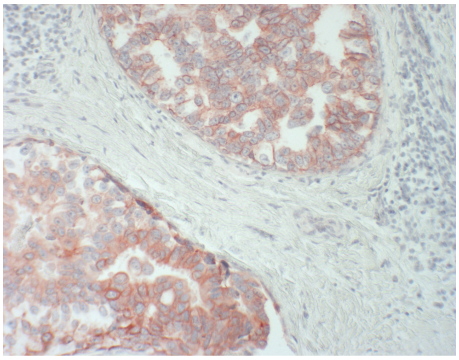
A. Negative control: TB333 breast cancer tissue plus BSA only



B. Positive control: TB333 breast cancer tissue plus IGF-1R antibody



C. Sample tissue scored negative for IGR-1R



D. Sample tissue scored positive for IGF-1R

Figure 2.1. Representative Images of Immunohistochemistry Results Staining Primary Breast Cancer Tissue for the IGF-1 Receptor: Postmenopausal Breast Cancer Survivors.[1,2]

TB333 in-house breast cancer sample; BSA: Bovine serum albumin; no active IGF-1R antibody included.
[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.
[2] IGF-1 receptor status scored analogous to HER-2 score and categorized as negative (0) versus positive (1,2,3).

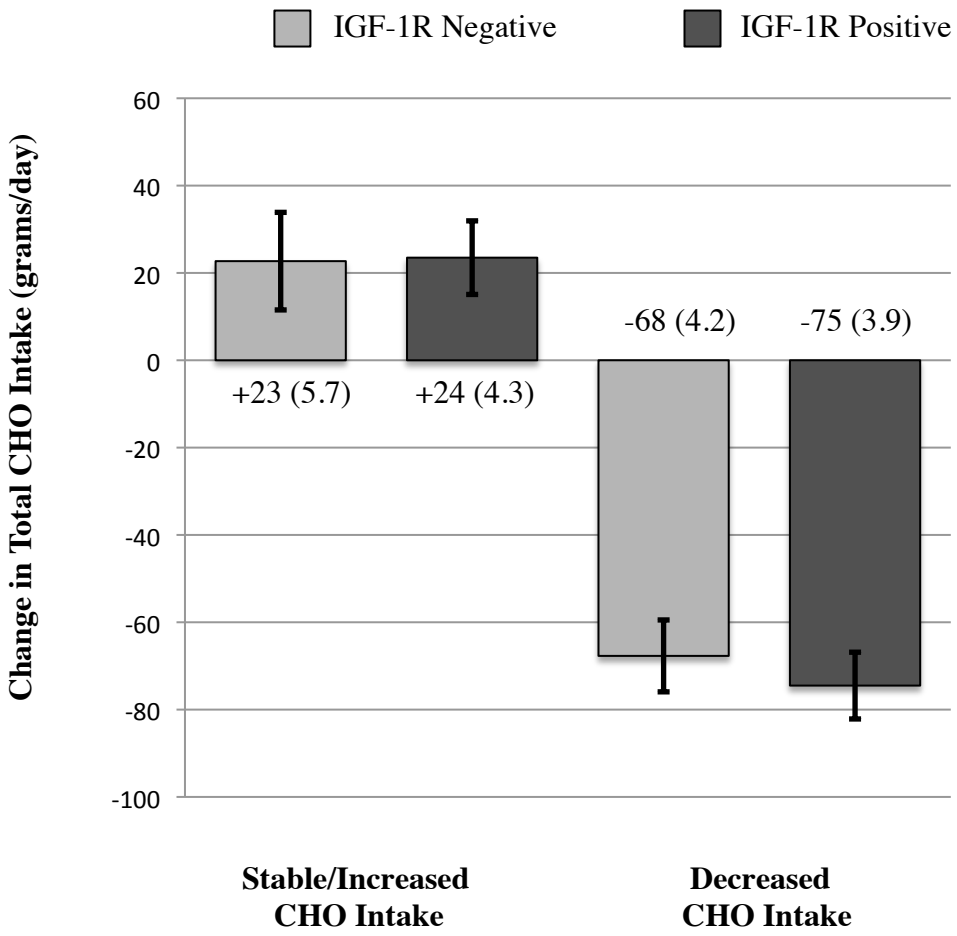


Figure 2.2. Change in Total Carbohydrate Intake (Grams/Day) over the First Year of Study Enrollment by a Net Decrease in Carbohydrate Intake and IGF-1R Status among N=265 Postmenopausal Breast Cancer Survivors Included in a Nested Case-Control Analysis.[1,2,3,4]

CHO: Carbohydrates.

[1] Participants sampled from cohort of N=2,111 postmenopausal breast cancer survivors enrolled in a dietary intervention trial.

[2] Change in intake of carbohydrates based on tertiles of change in total carbohydrate intake over the first year of trial enrollment for the full cohort. Decreased intake reflects lowest tertile of change (< -26.7 grams/day) and stable/increased intake reflects upper two tertiles of change.

[3] IGF-1 receptor status scored analogous to HER-2 score and categorized as negative (0) versus positive (1,2,3).

[4] Presented as means, standard errors of the mean, and 95% confidence intervals.

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CHAPTER 3

A Reduction in Low Quality Carbohydrate Intake Post-Diagnosis May Improve Prognosis among Postmenopausal Breast Cancer Survivors

Abstract

Background: Carbohydrate intake after a breast cancer diagnosis has the potential to impact prognosis among breast cancer survivors. Whether any modifications in carbohydrate intake differentially impact prognosis based on carbohydrate quality is unclear.

Methods: Secondary analysis of N=2,109 postmenopausal breast cancer survivors enrolled in a dietary intervention trial; total carbohydrate intake was not an intervention target. Women were a median 24 months post-diagnosis. Foods and beverages containing carbohydrates were classified by quality: postprandial blood glucose elevations were expected to be greater after consuming low quality carbohydrates (e.g, refined grains, sweets, starchy vegetables) as compared to high quality carbohydrates (e.g., fruits, non-starchy vegetables, dairy, whole grains). Time to recurrence and all-cause mortality were modeled on changes in intake of approximately one serving per day (15 grams of carbohydrates) of high and low quality carbohydrate-based foods over the first year of trial enrollment.

Results: Over a median 7.3 years of follow-up, there were 247 (11.7%) recurrences and 166 (7.9%) deaths. A decreased low quality carbohydrate intake (< -15 grams/day) was significantly associated with a decreased risk of recurrence (HR 0.62; 95%CI: 0.42-0.91; p=0.015) and all-cause mortality (HR 0.56; 95%CI: 0.35-0.90; p=0.016) in comparison to a minimal change in intake (within 15 grams/day). Associations were limited to participants who did not decrease their intake of high quality carbohydrates.

Conclusions: Carbohydrate quality may be more important than quantity when considering post-diagnosis dietary recommendations for postmenopausal breast cancer survivors.

Impact: Postmenopausal breast cancer survivors should be encouraged to decrease their intake of low quality carbohydrates.

Introduction

Dietary advice for breast cancer survivors has often emphasized a dietary pattern low in total fat (1-3) and many breast cancer survivors voluntarily decrease their total fat intake after treatment (4, 5). However, reducing total fat intake may result in an increased carbohydrate intake (FDA 2013), particularly when high-fat foods are replaced with low- or fat-free products containing added sugars (6). The metabolism of carbohydrates increases blood glucose concentrations, which ultimately increases circulating insulin concentrations (7). As circulating blood glucose and insulin concentrations appear to be positively associated with poor prognosis among breast cancer survivors (8-11), it is possible that a dietary pattern marked by high intakes of carbohydrates may worsen prognosis among breast cancer survivors (12, 13). Indeed, randomized trials are being conducted to assess the relative efficacy of low carbohydrate diets on breast cancer progression (14) and biomarkers of breast cancer prognosis among survivors (15).

However, dietary advice for breast cancer survivors based on carbohydrate content alone might be misleading considering that a variety of foods and beverages

provide dietary carbohydrates. For example, refined grains and products with added sugar are common sources of carbohydrates for Americans (6). Such foods are often energy dense and nutrient poor, and limiting the intake of such products is recommended for all Americans (6). Conversely, many fruits, vegetables, legumes and whole grains are nutrient-dense sources of carbohydrates, and their consumption is recommended to improve and maintain overall health (6, 16). In addition, many fruits, non-starchy vegetables, legumes and whole grains are also high in dietary fiber, which may have a beneficial impact on glucose regulation (17-19). It is important therefore to consider quality of carbohydrate intake in addition to quantity when considering the potential impact carbohydrate intake may have on breast cancer prognosis.

Dietary glycemic index (GI) and glycemic load (GL) are often used to estimate the glycemic response to carbohydrate metabolism. Specifically, GI reflects the relative impact that the metabolism of a carbohydrate-based food or beverage has on increasing blood glucose concentrations after ingestion; GL additionally considers the amount of carbohydrate (grams) consumed per food or beverage (20). GI values for commonly consumed foods and beverages are available in reference tables (20). A meta-analysis of 10 prospective studies found that diets marked by higher GI values were positively associated with an increased risk of incident breast cancer but no association was found between dietary GL and incident breast cancer (21). However, interpreting dietary recommendations for carbohydrate intake on GI values could lead to confusion for many breast cancer survivors. Foods with low GI values may or may not contain carbohydrates. Also, many foods may have high GI yet low GL values, and understanding the subtleties between GI and GL could be difficult. Findings from studies

that compare carbohydrate intake to prognosis using common servings sizes of carbohydrate-based foods groups while considering GI values may be more interpretable into dietary recommendations for breast cancer survivors.

In this study we compared changes in carbohydrate-based food and beverage intakes with prognosis among a cohort of postmenopausal breast cancer survivors who made considerable dietary changes within four years of their primary diagnosis. Carbohydrates were classified into food groups based on the expected impact their metabolism would have on glycemic response.

Methods

Study Sample

This current study is a secondary analysis of the multi-site, Women's Healthy Eating and Living (WHEL) dietary intervention trial (22). The WHEL study enrolled 3,088 breast cancer survivors between 1995-2000. The WHEL study was designed to test the effectiveness of a diet high in fruits, vegetables, and fiber and lower in total fat (target 20% total energy from fat) on breast cancer recurrence and overall survival. All women in the WHEL study provided written informed consent, and each study site's International Review Board approved of the study. Women with early stage breast cancer enrolled a median of 2 years post-diagnosis (range 6 months to 4 years). Dietary intervention targets were met by month 6 with change sustained over time. A subset analysis among N=2,922 women verified change in self-reported dietary intake with change in plasma carotenoid levels over the same one-year period (23). The dietary intervention was not associated with recurrence or mortality rates (22). As such, this

current analysis treats the WHEL sample as a cohort. The prevalence of insulin resistance is greater among postmenopausal women (24), and fluctuations in endogenous sex hormones among premenopausal women influence insulin sensitivity over the menstrual cycle (25). Thus, participants for this analysis were limited to postmenopausal breast cancer survivors. Participants were further limited to women who completed both the baseline and year one dietary assessments, who remained recurrence-free for 18 months post-baseline, and who had complete data for each of the scheduled 24-hour dietary recalls (N=2,109).

Dietary Intake

Baseline and year one dietary intake data were used in this analysis. Multiple telephone-based, 24-hour dietary recalls were used to assess dietary intake. Calls were completed over a three-week period at baseline and also at year one; three to four calls were completed at each time point and calls were stratified by weekday or weekend. Dietary intake data were collected and analyzed using Nutrition Data System (NDS) for Research software version 4.03 (1994-2006) developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

Food Group Classification

NDS entries for all food and beverages that provided carbohydrates consumed per each 24-hour dietary recall period were reviewed and categorized into food or beverage groups by the first author (JAE). Lists were also reviewed by another author (REP). Both brand name and item description were reviewed. Particular attention was paid to

classifying packaged and ready-to-eat grains such as crackers, chips, savory snacks and ready-to-eat cereals. Ingredient lists for products with the term "wheat" in the item name were verified by visiting the product manufacturer's website. If a whole grain (including whole wheat) was included as the first ingredient, the product was classified as a whole grain; otherwise the product was classified as a refined grain. For example, the item "crackers, brand name listing, Venus, Venus Stoned Wheat Wafers" was classified as a refined grain cracker since the first ingredient on the product label was "unbleached wheat flour" (26).

The foods and beverages identified by the above process were concatenated into one of nine carbohydrate-based food groups based on the American Diabetes Association's Dietary Exchange Lists (27). The Exchange Lists are primarily intended to help individuals with pre- or existing diabetes manage their blood glucose concentrations while considering overall nutrition (28). The Exchange Lists classify food and beverage items by the expected impact the metabolism of one serving (roughly 15 grams of carbohydrates) will have on blood glucose concentrations. Therefore the metabolism of foods or beverages within the same group is expected to have a similar impact on postprandial blood glucose concentrations. Finally, the final set of nine carbohydrate-based food groups was classified by quality to reflect the expected impact their metabolism would have on blood glucose concentrations or insulin sensitivity. High quality carbohydrate foods primarily reflected foods high in fiber and included whole grains, fruits and 100% fruit juice, beans/peas/lentils, non-starchy vegetables including 100% vegetable juice, and dairy. Dairy is an important dietary source of calcium, a critical nutrient for postmenopausal women (29). Furthermore, increasing dairy intake

over six months may improve insulin resistance among adults (29). Therefore, dairy products without added sugars were included with high quality carbohydrates. Low quality carbohydrate foods included refined grains, sweets and desserts, starchy vegetables, and spreads and condiments.

Primary Outcome

Breast cancer recurrence and all-cause mortality were the primary outcomes. WHEL study oncologists and study staff verified recurrence and death status, respectively. For each outcome, participants lost to follow-up were censored at date of last contact, and participants who remained event free after the WHEL trial end date (June 1, 2006) were censored at that date.

Additional Measures

Demographic, lifestyle, clinical and treatment characteristics were collected at baseline. Weight and height were measured at trial sites. Physical activity level was assessed using the Women's Health Initiative Personal Habits Questionnaire, and activity levels were categorized as inactive, mild to moderately active, active and very active based on MET hours per week (MHW) as previously reported (31).

Data Analyses

Intakes of high and low quality carbohydrates were computed as the sum of each individual food and beverage item within the respective food groups. Daily average intakes were the mean intakes per each food group based on the total number of 24-hour

dietary recalls at baseline or year one (i.e., 3 or 4). Baseline nutritional profiles for each carbohydrate-based food group are presented, and the contributions that each carbohydrate-based food group made to baseline total energy and total carbohydrate intakes of the entire sample are presented (32).

The primary goal of this study was to determine if a change in carbohydrate intake was associated with prognosis while considering both quality and quantity of carbohydrate. The main independent predictors for this analysis were therefore changes in intake of high and low quality carbohydrates. One serving of the carbohydrate-based foods included in the Exchange Lists is roughly equivalent to 15 grams of carbohydrates (27). Therefore dietary carbohydrate change was categorized as a decreased intake of at least 15 grams/day, minimal change (within 15 grams per day), or an increased intake of at least 15 grams/day.

The primary analysis modeled time to recurrence or all-cause mortality on change in intake of high and low quality carbohydrates. Delayed entry, Cox Proportional Hazard models were used. Model covariates included measures significantly related to recurrence status ($p<0.10$) or net change in total carbohydrate intake ($p<0.10$). The final set of covariates included clinic site, baseline measures (age, education, total energy intake, alcohol intake, BMI, physical activity level, hot flash status), clinical/treatment measures of primary cancer (stage, grade, number of positive nodes, tumor size, chemo- and radiation therapy, ever use of anti-estrogen therapy), and one-year changes in total energy and BMI. All analyses were run using the R Language and Environment for Statistical Computing, version 2.15.2 (http://www.R-project.org).

Results

The final sample consisted of N=2,109 postmenopausal breast cancer survivors who remained in remission up to 18 months after enrollment into the WHEL study and who had complete dietary recall data. Median age at enrollment was 56 years (SD 7.6), and women enrolled into the study a median 24 months after their primary diagnosis. The majority of the participants were White, non-Hispanics (86.2%) and college graduates (53.8%). Primary breast cancers were mainly earlier stage cancers (39.1% Stage I, 56.3% Stage II and 4.6% Stage IIIA per American Joint Committee on Cancer staging, version IV); 42.7% of primary cancers were node positive. Most primary cancers were positive for the estrogen or progesterone receptors (62.8% both ER+ and PR+, 12.6% ER+ only, 4.1% PR+ only, 18.3% were both ER and PR negative). The majority of participants received chemotherapy (68.5%) or radiation therapy (60.6%), 40.8% received both. Half (52.4%) of participants underwent a mastectomy, 47.6% underwent a lumpectomy, and most participants (72.8%) were taking tamoxifen at the time of enrollment into the WHEL study.

Baseline characteristics including demographic, lifestyle, clinical and treatment characteristics were compared to recurrence rates and results in this subset of postmenopausal survivors were consistent with the main WHEL trial (Pierce 2007). Specifically, the expected clinical (e.g., stage, grade, tumor size and number of positive nodes) and treatment (e.g., chemotherapy and radiation treatment) characteristics remained significantly related to recurrence rates (data not shown).

Table 3.1 presents baseline dietary intake overall and by high and low quality carbohydrate-based food groups. Participants consumed an average of 235 grams of

carbohydrates per day for roughly 55% of total energy intake. Participants consumed an average of 96 grams of high quality carbohydrates per day, and high quality carbohydrates provided an average 555 kcals and 14 grams of dietary fiber per day. Intakes of low quality carbohydrates were greater than intakes of high quality carbohydrates. Participants consumed an average of 135 grams of low quality carbohydrates per day, and low quality carbohydrates provided an average 754 kcals and 6 grams of dietary fiber per day.

Table 3.2 presents the contribution to total carbohydrate and total energy intake at baseline for high and low quality carbohydrates. High quality carbohydrates contributed 37% to total carbohydrate intake at baseline. Fruits and 100% fruit juice were the top sources of high quality carbohydrates, followed by whole grains and non-starchy vegetables. Low quality carbohydrates contributed 57% to total carbohydrate intake at baseline. Refined grains and sweets and desserts were the top sources of low quality carbohydrates, making a combined contribution of roughly one-half of total carbohydrate intake at baseline.

Over a median of 7.3 years follow-up, there were N=247 (11.7%) breast cancer recurrences and N=166 (7.9%) confirmed deaths during the study period; the majority of deaths (72.9%) were breast cancer related.

Change in the intakes of high and low quality carbohydrates was categorized as a change of at least 15 grams per day, where 15 grams roughly reflects one serving of a carbohydrate-based food or beverage. Most participants (53%) increased their intake of high quality carbohydrates by at least 15 grams per day. As shown in Table 3.3, there was no association between a change in intake of high quality carbohydrates and

recurrence (Chi-Square p=0.645) or all-cause mortality (Chi-Square p=0.623). Regarding a change in low quality carbohydrates, most participants (55%) *decreased* their intake of low quality carbohydrates by at least 15 grams per day. There was a significant main effect between a change in intake of low quality carbohydrates and recurrence (Table 3.3), with participants who decreased their intake of low quality carbohydrates having the lowest rate of breast cancer recurrence (Chi-Square p=0.038). Unadjusted rates of all-cause mortality also appeared lower among participants who decreased their intake of low quality carbohydrates, although results were not statistically significant at the p=0.050 level (Table 3.3).

A fully adjusted, proportional hazards model was created to fit time to recurrence on change in intake of high and low quality carbohydrates (data not shown). There were no main effects for a change in high quality carbohydrate intake on recurrence. However, there was a significant interaction between the main effect for a change in low quality carbohydrate intake by change in high quality carbohydrate intake on the risk of recurrence (Likelihood Ratio Test, $c^2(4)$ p=0.021). Specifically, a decreased intake of low quality carbohydrates was protective only among participants who did not concurrently decrease their intake of high quality carbohydrates (data not shown). Therefore, results for the fully adjusted model are presented for the subset of participants who did not decrease their intake of high quality carbohydrates (N=1,629).

Figure 3.1 presents the main effects for a change in low quality carbohydrate intake among participants who did not concurrently decrease their intake of high quality carbohydrates (N=1,629). Among this subset of participants, a decreased intake of low quality carbohydrates was significantly associated with a reduced risk of recurrence

(Panel A, HR: 0.62; 95% CI: 0.42-0.91; p=0.015) as well as a reduced risk of all-cause mortality (Panel B, HR: 0.56; 95% CI: 0.35-0.90; p=0.016). In comparison, among the N=480 participants who decreased their intake of high quality carbohydrates (data not shown), there were no significant associations between a decreased intake of low quality carbohydrates with recurrence (HR: 1.83; 95% CI: 0.83 – 4.03; p=0.133) or all-cause mortality (HR: 2.19; 95% CI: 0.79 – 6.03; p=0.130) compared to a minimal change in intake of low quality carbohydrates. Similarly, an increased intake of low quality carbohydrates among this same subset of participants was not related to recurrence (HR: 0.96; 95% CI: 0.44 – 2.09; p=0.912) or all-cause mortality (HR: 1.10; 95% CI: 0.40 – 3.02; p=0.847).

Discussion

These data indicate that a decreased intake of low quality carbohydrates, mainly refined grains, sweets and desserts, and starchy vegetables, improved prognosis among a subset of postmenopausal breast cancer survivors who do not concurrently decrease their intake of high quality carbohydrates. Specifically, a reduction of at least 15 grams per day of low quality carbohydrates, an amount roughly equal to one serving size of carbohydrates, reduced the risk of recurrence by 38% and the risk of early mortality by 44%. Furthermore, no beneficial effect was found between a net decrease in carbohydrate intake and prognosis once carbohydrate quality was considered.

Carbohydrate quality for this study was primarily based on the expected impact that the metabolism of each carbohydrate-based food or beverage would have on postprandial blood glucose concentrations. The metabolism of low quality carbohydrates

as defined in this study would therefore be expected to result in a rapid elevation of blood glucose and consequently, insulin. Glucose and insulin directly stimulate breast cancer growth (13), and elevated concentrations may also indicate activation of other factors along the insulin/insulin-like-growth-factor axis that could negatively influence prognosis (33, 34). Our findings that consumption of low quality carbohydrates may impact breast cancer prognosis are in agreement with a previous study that reported a statistically significant increased risk of breast cancer mortality related to an elevated intake of energy-dense, nutrient-low, sweets and grain-based foods among a cohort of N=603 breast cancer survivors (35).

Higher quality carbohydrates as defined in this study primarily reflected foods or beverages high in dietary fiber, and we did not find an association between prognosis and changes in high quality carbohydrate intake. Our results are in agreement with other studies that have not found significant associations between prognosis and usual intakes of high quality carbohydrates including fruits and vegetables (36, 37), overall dairy (36, 38), low fat-dairy (38), or dietary fiber (35, 36, 39) among breast cancer survivors. Dietary fiber can slow or even prevent the absorption of glucose into the blood stream (17, 18), and consumption of whole grains and legumes may improve insulin sensitivity (19) due to certain minerals present in these foods (40, 41). Adequate intakes of fruits, non-starchy vegetables and whole grains are important for overall health (6), and most free-living breast cancer survivors do not meet the recommended intakes of those foods (5). Thus, postmenopausal breast cancer survivors should continue to receive encouragement to meet the recommended intakes of fruits, non-starchy vegetables,

legumes and whole grains; and our results suggest that an increased intake of such high quality carbohydrates does not negatively impact prognosis.

Results of this study are confined to the impact of dietary changes made within approximately 6 months to 4 years post-diagnosis. There is evidence to suggest this may be a particularly critical time for lifestyle interventions that impact glucose regulation and insulin sensitivity. Specifically, in a study of N=535 breast cancer survivors without diabetes, Goodwin et al. (9) found that the positive associations between markers of insulin resistance with poor prognosis may be confined to the first five years after the primary diagnosis (9). If confirmed, these results suggest that there may be a particularly important time frame within which dietary changes must be made to influence prognosis.

A strength of this study is the classification of carbohydrates into common food groups. Results are immediately translatable to public health messages that most breast cancer survivors can interpret. The Exchange Lists are available on-line (28) and contain several practical examples of what one serving size of carbohydrates (roughly 15 grams of carbohydrates) would equal for the food groups used in this analysis. As a limitation, our study did not measure change in fasting glucose or insulin sensitivity. As such, we cannot prove that a decreased intake of low quality carbohydrates related to an improved prognosis by reducing circulating concentrations of glucose or insulin. Regardless, results demonstrate an association between the consumption readily absorbed carbohydrates and prognosis among postmenopausal breast cancer survivors.

In summary, these results suggest that among postmenopausal breast cancer survivors, total carbohydrate intake may not be a predictor of poor prognosis. However, postmenopausal breast cancer survivors may improve their prognosis by decreasing their

intakes of low quality carbohydrates such as refined grains, sweets and desserts, and starchy vegetables in the first few years post-diagnosis. Further, there does not appear to be a risk associated with increasing the intake of high quality carbohydrates such as fruits, non-starchy vegetables, legumes and whole grains. Overall, our findings suggest that carbohydrate quality may be more important than quantity in relationship to dietary recommendations targeted to postmenopausal breast cancer survivors.

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Chapter 3 is currently being prepared for publication. I am the primary investigator and author of this material. Co-authors include Ruth E. Patterson, Guadalupe X. Ayala, and John P. Pierce.

Table 3.1. Baseline Dietary Intake Overall and by High and Low Quality Carbohydrate-Based Food Groups among N=2,109 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1,2]

	Total CHO (g/day)	**Total Energy (kcals/day)**	**Protein (g/day)**	**Total Fat (g/day)**	**Sat. Fat (g/day)**	**Fiber (g/day)**
	< ---------- *Mean (SD)* ---------- >					
Overall	235 (62)	1703 (399)	68 (18)	56 (21)	18 (8)	21 (8)
By Food Group:						
High quality CHO	96 (46)	555 (222)	24 (11)	12 (7)	6 (4)	14 (7)
Fruits & fruit juice	44 (27)	176 (107)	2 (2)	1 (1)	--	5 (3)
Whole grains	24 (21)	124 (106)	5 (5)	2 (2)	--	4 (3)
Non-starchy vegetables	13 (9)	62 (39)	3 (2)	1 (1)	--	4 (3)
Dairy	9 (8)	159 (100)	11 (8)	9 (7)	5 (4)	--
Beans, peas, lentils	5 (8)	34 (48)	2 (3)	1 (1)	--	2 (2)
Low quality CHO	135 (54)	754 (290)	15 (6)	18 (11)	5 (4)	6 (3)
Refined grains	62 (29)	321 (150)	9 (4)	4 (3)	1 (1)	3 (2)
Sweets & desserts	55 (36)	271 (175)	4 (4)	5 (5)	2 (3)	1 (1)
Starchy vegetable	12 (11)	53 (46)	1 (1)	--	--	1 (1)
Spreads & condiments	6 (5)	109 (75)	1 (1)	9 (7)	2 (2)	--

CHO: Carbohydrates; kcals/day: kilocalories per day; g/day: grams per day. Sat. Fat: Saturated fat.
-- Denotes value <0.5
[1]Survivors were recurrence free 1.5 years after enrollment.
[2]Dietary intake assessed as the mean of three or four 24-hour, dietary recalls.

Table 3.2. Contribution to Total Carbohydrate and Total Energy Intake at Baseline by High and Low Quality Carbohydrate-Based Food Groups among N=2,109 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial.[1,2]

	% Contribution to baseline intake	
	Total CHO (g)	**Total Energy (kcal)**
High Quality CHO	**36.8%**	**23.2%**
Fruits and fruit juice	18.6%	10.3%
Fresh fruit	12.4%	6.8%
100% Fruit juice	4.2%	2.4%
Dried fruit	1.3%	0.7%
Whole grains	10.3%	7.3%
Ready-to-eat cereal	4.0%	2.7%
Bread	3.6%	2.6%
Cereals and grains (e.g., pasta, rice)	2.7%	1.9%
Non-starchy vegetable[3]	5.6%	3.6%
Carrots	1.2%	1.0%
Tomatoes and tomato products	1.3%	1.0%
Dairy	4.0%	9.3%
Beverages: Milk[4]	3.4%	4.0%
Beans, peas, lentils	2.3%	2.0%
Low Quality CHO	**57.2%**	**44.1%**
Refined grains	26.2%	18.8%
Cereals and grains (e.g., pasta, rice)	12.7%	8.5%
Bread	7.7%	5.8%
Crackers, chips, savory snacks	3.9%	3.3%
Ready-to-eat cereal	1.3%	0.8%

Table continued next page

Table 3.2. Contribution to Total Carbohydrate and Total Energy Intake at Baseline by High and Low Quality Carbohydrate-Based Food Groups among N=2,109 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial,[1,2] Continued.

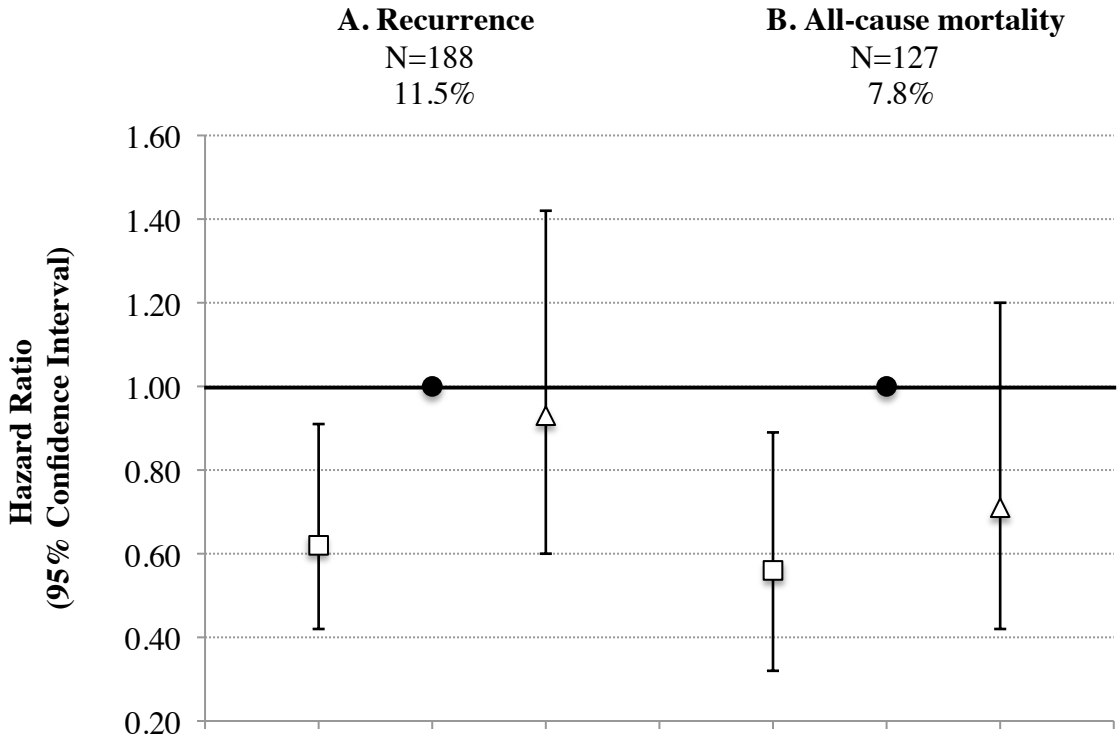
	% Contribution to baseline intake	
	Total CHO (g)	**Total Energy (kcal)**
Sweets and desserts	23.2%	15.8%
Beverages: Sugar-sweetened beverages	6.6%	3.7%
Added sugar	6.3%	3.3%
Cookies/cakes/muffins	3.1%	2.7%
Candy and chocolate	2.9%	2.3%
Dairy-based frozen treats	2.1%	2.1%
Yogurt with added sugar	1.0%	0.8%
Starchy vegetable	5.2%	3.1%
White potato	3.9%	2.3%
Corn	1.0%	<1%
Spreads and condiments[5]	2.6%	6.4%

CHO: Carbohydrates; kcals: kilocalories; g: grams.
[1]Survivors were recurrence free 1.5 years after enrollment.
[2]Subgroups contributing ≥1% of total carbohydrate intake presented per food group.
[3]Other major sources of non-starchy vegetables include alliums, bell peppers, broccoli, cabbage, leaf lettuce, mushrooms, spinach, string beans, and summer squashes. Tomato products include tomato sauce and paste.
[4]Milk with added sugar (e.g., chocolate milk) included as a sugar-sweetened beverage.
[5]Spreads and condiments include salad dressings, mayonnaise, imitation dairy, condiments, jams, jellies, and preserves.

Table 3.3. Breast Cancer Recurrence and All-Cause Mortality Rates among N=2,109 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial: Rates by Change in Intake of High and Low Quality Carbohydrates over the First Year of Study Enrollment.[1,2,3]

	Overall, N	Δ Total CHO Intake, g/day	Recurrence Rate		All-Cause Mortality Rate	
			N (%)	p-value[3]	N (%)	p-value[3]
		Median				
Overall	2,109 (100%)	-2.8	247 (11.7%)	--	166 (7.9%)	--
By change in high quality CHO						
Decreased intake (≤ 15 g/day)	480 (22.8%)	-46.8	59 (12.3%)	0.645	39 (8.1%)	0.623
Minimal change, (+/- 15 g/day)	510 (24.2%)	-13.9	54 (10.6%)		35 (6.9%)	
Increased intake (≥ 15 g/day)	1,119 (53.1%)	25.0	134 (12.0%)		92 (8.2%)	
By change in low quality CHO						
Decreased intake, (≤ 15 g/day)	1,165 (55.2%)	-26.7	118 (10.1%)	0.038	78 (6.7%)	0.056
Minimal change, (+/- 15 g/day)	507 (24.0%)	9.8	67 (13.2%)		51 (10.1%)	
Increased intake (≥15 g/day)	437 (20.7%)	43.4	62 (14.2%)		37 (8.5%)	

CHO: Carbohydrates; g/day: grams per day.
[1]Survivors were recurrence free 1.5 years after enrollment. Unadjusted recurrence and mortality rates presented.
[2]15 grams of carbohydrate equivalent to one serving (27, 28).
[3]p-value from Chi-square test.



	Δ Low Quality CHO:			Δ Low Quality CHO:		
	↓	Min Δ	↑	↓	Min Δ	↑
N:	954	384	291	954	384	291
Events, N:	91	53	44	60	40	27
Events, %:	9.5%	13.8%	15.1%	6.3%	10.4%	9.3%

Figure 3.1. Adjusted Relative Risk of Breast Cancer Recurrence (A) and Early All-Cause Mortality (B) among N=1,629 Postmenopausal Breast Cancer Survivors Enrolled in a Dietary Intervention Trial Who Did *Not* Decrease Their Intake of High Quality Carbohydrates: Risk by Change in Intake of Low Quality Carbohydrates.[1,2,3]

CHO: Carbohydrates.

[1]Survivors were recurrence free 1.5 years after enrollment. Model adjusted for site, baseline measures (age, education, total energy intake, alcohol intake, BMI, physical activity level, hot flash status), clinical/treatment measures of primary cancer (stage, grade, number of positive nodes, tumor size, chemo- and radiation therapy, ever use of anti-estrogen therapy) and one-year changes in total energy and BMI, as well as change in intake of high quality carbohydrates (increased intake versus minimal change). Change in intake of high quality carbohydrates non-significant for both recurrence (p=0.280) and all-cause mortality (p=0.295).

[2]Change in intake defined as a decrease of at least 15 grams/day (↓), within 15 grams/day (Min Δ), or an increase of at least 15 grams/day (↑). Fifteen grams of carbohydrates equivalent to one serving (27, 28).

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DISCUSSION

This dissertation examined the influence that a post-diagnosis change in carbohydrate intake had on the risk of breast cancer recurrence and early all-cause mortality among postmenopausal breast cancer survivors. Chapter 1 found a significant, linear association with a net change in carbohydrate intake and recurrence, and the point estimate for a decreased carbohydrate intake was borderline significant (p=0.056). Chapter 2 reported that the protective effect of a decreased carbohydrate intake was most pronounced for participants with primary cancers that expressed the IGF-1R, and Chapter 3 demonstrated that the protective effect was only related to a decreased intake of low quality carbohydrates.

The results from Chapter 1 highlight the complexity in measuring carbohydrate intake on the macronutrient level. In that analysis, a net decrease in carbohydrate intake was driven mainly by a decreased intake of refined grains and sweets and desserts. In comparison, a net increase in carbohydrate intake was driven mainly by an increased intake of fruits, whole grains, and non-starchy vegetables. Therefore, the significant linear trend over tertiles of change in total carbohydrate intake suggested that carbohydrate quantity impacted prognosis, regardless of the food source of carbohydrates. However, the results in Chapter 1 were not consistent across all carbohydrate subtypes, which would be expected if only carbohydrate quantity mattered in relationship to prognosis. For example, starch is a complex carbohydrate that is part of

refined grains, sweets and desserts, whole grains, and beans, peas, and lentils. Starch was the primary source of carbohydrates among participants in this cohort, yet the results for starch did not mirror the results for total carbohydrate intake. In fact, the rates of recurrence were lowest among participants who decreased their starch intake, yet no increased rate of recurrence was observed among participants who increased their starch intake compared to participants who made minimal change.

In comparison, only the results for fructose and maltose mirrored the findings for total carbohydrate intake in Chapter 1. Fructose does not impact blood glucose concentrations, yet increased fructose intakes have been associated with increased c-peptide concentrations, a marker of activated insulin (1). However, those associations between fructose and c-peptide concentrations may be limited to foods that included fructose as part of high-fructose corn syrup (1). Considering the results for changes in total carbohydrates, starch and fructose from Chapter 1, it is possible different biological mechanisms of action are responsible for the observed associations between a decreased carbohydrate intake and breast cancer outcomes compared to the observed associations between an increased carbohydrate intake and breast cancer outcomes. For example, a decreased intake of refined grains and sweets and desserts accounted for the majority of the decreased intake of carbohydrates observed in this cohort, items high in readily absorbed carbohydrates including starch and most likely high-fructose corn syrup. In comparison, the increased risk of recurrence associated with an increased carbohydrate intake may have been due to an increased intake of fructose only. Controlled feeding trials comparing the intakes of different carbohydrate-based foods on biomarkers of

breast cancer prognosis among postmenopausal breast cancer survivors could help answer some of those proposed hypotheses.

In Chapter 2, we hypothesized that a decreased carbohydrate intake would reduce the risk of a breast cancer recurrence due to limiting the proliferative signaling of breast cancer cells via the IGF-1R. Results from Chapter 2 supported that hypothesis: while expression of the IGF-1R was related to an increased risk of recurrence, a decreased carbohydrate intake markedly reduced that risk of recurrence. Results therefore suggested that dietary recommendations based on carbohydrate intake could be tailored for postmenopausal breast cancer survivors based on expression of the IGF-1R in the primary cancer tissue. Furthermore, dietary intervention trials comparing changes in carbohydrate intake could enroll breast cancer survivors who had IGF-1R positive cancers to better assess any associations between changes in the intakes of different carbohydrate-based foods on breast cancer recurrence. However, it is important to remember that participants who had IGF-1R negative cancers also benefited from a reduced carbohydrate intake. Given that expression of the IGF-1R increases the odds of a breast cancer recurrence as observed in this study and in several others, and given that IGF-1R expression had been associated with treatment resistance, results from Chapter 2 further support routine staining for the IGF-1R in primary breast cancer tissue in an effort to tailor adjuvant treatments.

In comparison to assessing carbohydrates at the macronutrient level, Chapter 3 presents the comparisons for a change in carbohydrate intake using carbohydrate-based food groups and common servings sizes. Results from Chapter 3 show that a net change in carbohydrate intake did not relate to prognosis: a reduced risk of recurrence was not

observed among those with the greatest net decrease in carbohydrate intake (i.e., a decrease in both high and low quality carbohydrates), and an increased risk of recurrence was not observed among participants with the greatest net increase in carbohydrate intake (i.e., an increased in both high and low quality carbohydrates). Instead, results from Chapter 3 showed that only a decreased intake in low quality carbohydrates was associated with reduced risk of recurrence, and increasing the intake of high quality carbohydrates did not impact prognosis. Those findings were limited to postmenopausal survivors who did not concurrently decrease their intakes of high quality carbohydrates, and support the importance of carbohydrate-based foods that are also high in dietary fiber when considering dietary recommendations for postmenopausal breast cancer survivors. Also, while not statistically significant, results from Chapter 3 suggested that a net decrease in carbohydrate intake might have been associated with an increased risk of breast cancer recurrence. Those findings are important as they demonstrate that a low carbohydrate diet may not be the best dietary pattern for postmenopausal breast cancer survivors to adopt. Instead, results from Chapter 3 suggest that postmenopausal breast cancer survivors should consume high quality carbohydrates such as fruits, non-starchy vegetables, whole grains, dairy, and beans, peas, and lentils, but need to reduce their intakes of low quality carbohydrates.

While the WHEL dietary trial did not find a significant association between the dietary intervention and breast cancer outcomes among breast cancer survivors, the WHEL intervention delivery methods (e.g., telephone counseling based on social cognitive theory), was effective in changing dietary intake among participants. Telephone counseling under the WHEL model helped participants make large dietary

changes through a series of smaller goals that could be evaluated and appraised (2). By accomplishing smaller goals, a participant's self-efficacy related to dietary behavior changes improved. Goals were tailored to each participant and participants worked with the same counselor throughout the trial. Dietary targets for the WHEL trial focused on increasing the intakes of plant-based foods, and participants were not specifically instructed to limit their intakes of low quality carbohydrates as defined in Chapter 3 (2). It is possible that an intervention model similar to that used in the WHEL study with more focus given to reducing the intakes of sweets and desserts and limiting the intake of refined grains and starchy vegetables may prove effective in influencing breast cancer outcomes. Finally, methods to easily identify low quality carbohydrates are needed to help postmenopausal breast cancer survivors make appropriate dietary changes. The use of food groups in Chapter 3 was useful to identify low quality carbohydrates; however, postmenopausal breast cancer survivors may further benefit from additional information to identify products with added sugars and to identify products made with whole grains. For example, in Chapter 3 we found that many packaged grain products that included 'wheat' in the name did not contain whole wheat. As part of the WHEL dietary intervention trial, participants in the intervention arm attended cooking classes that included educational objectives such as label reading. Future interventions could incorporate an online module to provide similar demonstrations of identifying quality carbohydrates for participants.

While our results assessing change in carbohydrate intake and all-cause mortality as reported in Chapters 1 and 3 were similar to the results for recurrence, the majority of

deaths among this cohort were breast cancer related. Therefore, results are limited with respect to causes of death other than breast cancer.

As demonstrated throughout this dissertation, there may be a critical time period for when changes in carbohydrate intake need to occur in order to impact breast cancer recurrence. Results from this dissertation stress the need for dietary intervention trials enrolling postmenopausal breast cancer survivors soon after completing treatment for their primary cancer. Dietary targets should reduce the intake of sweets and desserts and starchy vegetables, and encourage participants to replace refined grains with whole grains. Participants should also be encouraged to maintain an adequate intake of high quality carbohydrates as defined in Chapter 3. Importantly, blood glucose and insulin related measures should also be monitored to better understand biological mechanisms of action.

References

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