UNIVERSITY OF CALIFORNIA, SAN DIEGO
SAN DIEGO STATE UNIVERSITY

Prolonged Overnight Fasting as a Novel Intervention Strategy for Reducing Breast Cancer Risk

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

in

Public Health (Health Behavior)

by

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2016

The Dissertation of Catherine Rose Marinac is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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2016

DEDICATION

I dedicate this dissertation to my family:

To my parents, for their steady stream of love, unbiased support, and for instilling in me the value of hard work and education;

to Joseph, Liz, and Ben, whose belief in my abilities helped me to believe in myself;

and to Genevieve—may you also be motivated and encouraged to reach your dreams.

EPIGRAPH

Teach thy tongue to say, "I do not know," and thous shalt progress.

—Maimonides

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LIST OF ABBREVIATIONS

ACS, American Cancer Society

ADA, American Diabetes Association

BMI, body mass index

CI, confidence interval

CRP, C-reactive protein

HbA1c, hemoglobin A1c

HER2/neu, human epidermal growth factor receptor 2

HOMA-IR, homeostatic model of insulin resistance

HR, hazard ratio

MET, metabolic equivalent

NHANES, National Health and Nutrition Examination Survey

SD, standard deviation

WHEL, Women‘s Healthy Eating & Living

UC, University of California

LIST OF TABLES

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Chapter 3 is a reprint of the material as it appears in PLoS One: Marinac CR, Sears DD, Natarajan L, Gallo LC, Breen CI, Patterson RE. “Frequency and circadian timing of eating may influence metabolic risk of breast cancer.” *PLoS One.* 2015 10(8):e0136240. doi: 10.1371/journal.pone.0136240. Catherine Marinac was the primary investigator and author of this paper.

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INVITED LECTURES

1. **Prolonged Fasting, Metabolic Health, and Breast Cancer Risk.** Center for Circadian Biology Fall Workshop on Biological Timing, UC San Diego, November, 2015.

2. **Dietary Interventions for Chronic Disease Prevention.** Presented to public health undergraduate students (FPMU 110) at UC San Diego. April 2014.

3. **Female Specific Cancers.** Presented to public health master's level women's health course (PH 700) at San Diego State University, April 2012.

4. **Aging and Bone Health.** Presented to public health master's level women's health course (PH 700) at San Diego State University, April 2012.

ABSTRACT OF THE DISSERTATION

Prolonged Overnight Fasting as a Novel Intervention Strategy for Reducing Breast Cancer Risk

by

Catherine Rose Marinac

Doctor of Philosophy
in Public Health (Health Behavior)

University of California, San Diego, 2016
San Diego State University, 2016

Professor Ruth Patterson, Chair

Background: This dissertation examined whether the length of the nightly fasting interval was (1) associated with metabolic biomarkers putatively associated with breast cancer risk; and (2) predictive of breast cancer prognosis among women with a history of breast cancer. These analyses were conducted using data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) and the Women's Healthy Eating and Living Study (WHEL).

Methods: The NHANES sample included approximately 2000 non-diabetic women. Nightly fasting duration was calculated from one telephone-based dietary recall. Multivariable linear regression models were used to examine associations between the length of the nightly fasting interval and metabolic biomarkers putatively

associated with breast cancer risk, including hemoglobin A1c (HbA1c) and C-reactive protein (CRP). The WHEL sample included 2413 non-diabetic breast cancer survivors. Usual nightly fasting duration was assessed from 3-4 recalls that were collected at multiple time-points (i.e., baseline, year 1, year 4). Multivariable linear regression models were used to examine associations between the nightly fasting interval and factors hypothesized to influence breast cancer outcomes: HbA1c, CRP, sleep duration, and BMI. Delayed-entry Cox proportional hazard models were used to assess the association of usual nightly fasting duration, modeled as a time-varying covariate, with breast cancer recurrence, breast cancer-specific mortality, and all-cause mortality.

Results: In the NHANES sample of women, each 3-hour increase in nightly fasting duration was associated with significantly lower HbA1c and 2-hour post-prandial glucose measurements. In addition, nightly fasting duration was inversely associated with CRP concentrations among women who consumed <30% of their calories after 5 pm (p=0.01). Among WHEL breast cancer survivors, prolonged nightly fasting was associated with significantly lower HbA1c and longer sleep duration. Women who fasted <13 hours per night had a 36% increased risk of breast cancer recurrence compared to women who fasted ≥13 hours (HR: 1.36; 95% CI, 1.05 − 1.76). Nightly fasting duration was not associated with a statistically significant increased risk of breast cancer-specific or all-cause mortality.

Conclusions: Findings suggest that increasing the length of the nightly fasting interval could be a simple, feasible, and novel dietary strategy to regulate metabolic biomarkers putatively associated with breast cancer risk and reduce the risk of breast cancer recurrence.

CHAPTER 1

Introduction

INTRODUCTION

In 2015, an estimated 231,840 women in the United States were diagnosed with breast cancer and 40,290 women died of the disease.[1] Breast cancer is the most common form of cancer, and the second leading cause of all deaths among women in industrialized countries.[2] Therefore, the identification of feasible, population-level interventions to reduce breast cancer risk and mortality is an important goal.

It has long been hypothesized that diet is a major modifiable risk factor for breast cancer, and an extraordinary amount of time and money has been invested in the search for dietary determinants of breast cancer. However to date, randomized, controlled trials testing whether changes in dietary composition can reduce breast cancer risk have been disappointing.[3,4] For example, the Women's Health Initiative (n=48,835 postmenopausal women) found that a low-fat dietary pattern did not significantly reduce invasive breast cancer risk over an 8.1 year follow-up period.[3] Similarly, the Women's Healthy Eating and Living trial (n=3,088 breast cancer survivors) found that adoption of a diet high in fruit and vegetables had no effect on breast cancer recurrence or mortality over 7.3 years of follow-up.[4]

Landmark studies in rodents suggest that daily fasting schedules aligned with sleep-wake (circadian) cycles may be an important determinant of metabolic health and cancer risk. In mice, a high-fat diet induces obesity and metabolic diseases. However, an eating pattern consisting of a 16-hour fast during the sleep phase has been shown to normalize numerous markers associated with metabolic disease as well as cancer risk and progression.[5] Specifically, mice subjected to a 16-hour fasting regimen, without reducing calorie intake, were protected against hyperinsulinemia, hepatic steatosis, inflammation and obesity.[6] Notably, this circadian-aligned fasting schedule was found to have robust protective effects on cancer risk factors even

among mice fed a high-fat diet without reducing energy intake. These results suggest that the health impacts of time-restricted feeding may be independent of dietary composition and energy balance.

Data from animal models also suggest that cycles of fasting may reduce cancer incidence,[7] as well as impede tumor growth and enhance cancer-free survival among mice injected with a variety of cancer cell types including murine breast cancer cells.[8] In particular, a study examining the effects of various treatment regimens among mice injected with breast cancer 4T1 cells, found that a treatment regimen consisting of repeated fasting cycles reduced cancer metastases and extended survival.[8]

Whether fasting could be a strategy to reduce breast cancer risk and improve prognosis in humans is not known, although evidence is accumulating. A 2015 review of fasting and human metabolic health identified 13 human intervention trials testing the effects of various fasting regimens on outcomes relevant to metabolic health.[9] Of these 13 studies, 4 were trials that tested the effects of fasting schedules characterized by complete abstinence from food or drink for an extended period of time (i.e., prolonged fasting) on cancer-related biomarker endpoints. All 4 prolonged fasting studies observed significant protective effects of fasting on glucoregulatory parameters, with fasting glucose being the most consistent cancer-related biomarker that changed in response to fasting treatments.

Proposed Mechanisms Linking Nightly Fasting with Breast Cancer

Precisely how fasting regimens act to improve health and influence disease risk is not well understood. However, rodent and human studies of fasting have yielded some mechanistic insight. As shown in Figure 1, prolonged nightly fasting is postulated to positively influence a number of cancer-related metabolic factors through its effects on the circadian system, sleep, and weight control. Metabolic factors include an array of inflammatory factors,[10,11] sex hormones,[12] and glucoregulatory parameters[13,14] with etiologic ties to breast cancer. Favorable changes in these metabolic factors are hypothesized to prevent breast carcinogenesis.

Nightly Fasting and the Circadian System

Eating is a powerful stimulus (i.e. "zeitgeber"), influencing the alignment between circadian rhythms in the suprachiasmatic nucleus (SCN) and peripheral tissues.[15] Prolonged fasting intervals within a 24-hour day (particularly during the nighttime in humans) appear to impose a diurnal rhythm in food intake that improves oscillations in circadian clock gene expression; maintains alignment of circadian rhythms in the SCN and peripheral tissues; and optimizes molecular mechanisms of energy metabolism such as glucoregulation.[16] In contrast, eating continuously throughout the day and night (or at irregular time intervals) can offset circadian rhythms in peripheral tissues, resulting in misalignment between the circadian rhythms in the SCN and peripheral clocks. There is a wealth of evidence linking circadian misalignment to metabolic dysfunction,[17] and strong evidence that circadian misalignment is a predictor of higher cancer risk, more rapid tumor progression, and shorter survival.[18] Notably, the increased breast cancer risk among night shift workers has been attributed in part to circadian misalignment,[19] and in 2007 the World Health

Organization classified night shift work as a probable carcinogen due to circadian disruption.[20]

Nightly Fasting and Sleep

Considerable evidence suggests that inadequate sleep has negative effects on metabolic function and cancer risk.[21-25] Observational studies have reported that nighttime eating is associated with reduced sleep duration and poor sleep quality.[26-29] For example, a study by Baron and colleagues examining associations between sleep, meal-timing, and BMI, found a strong inverse correlation between calories consumed after 8 pm and sleep duration (P=-.57; p<.001) among 52 healthy men and women.[27] Experimental and epidemiologic evidence also suggest that sleep loss alters dietary behaviors,[30] and therefore, associations between sleep duration and feeding-fasting schedules may be bi-directional. There is also evidence that altered sleep influences the alignment between the sleep/wake cycle and the endogenous circadian timing system.[31]

Nightly Fasting and Body Weight Control

Most fasting regimens reduce the total number of hours available for eating and thereby reduce overall energy intake and opportunities for weight gain. However, evidence from both animal and human studies also suggest that prolonged nightly fasting may influence weight control independent of energy balance. In mouse models of diet-induced obesity, weight gain has been prevented without reducing calorie intake by subjecting mice to a 16-hour fasting regimen during the sleep phase.[6] In humans, experimental trials have shown that weight loss is more effective among adults who

consume a greater proportion of energy early vs. late in the day—even when overall energy intake is the same.[32]

There is also some evidence that disruptions in the circadian system can induce weight gain, which is a well-established risk factor for metabolic health and postmenopausal breast cancer prognosis.[33,34] Data from experimental trials in humans indicate that short-term circadian misalignment results in systematic and unfavorable changes in hormones regulating appetite and energy expenditure, such a leptin (a hormone that signals “fullness”). If maintained, these hormonal changes could contribute to weight gain and the development of obesity.[17]

Dissertation Chapters

This dissertation addresses a new and important question of whether a nightly fasting regimen could offer a nonpharmacological strategy to reduce the risk of breast cancer.

Chapter 2 examines associations of nightly fasting duration with biomarkers of breast cancer risk in a sample of 2,212 women in the 2009-2010 U.S. National Health and Nutrition Examination Survey (NHANES). Nightly fasting duration was calculated from 24-hour dietary recalls. Biomarkers of breast cancer risk include Hemoglobin A1c (HbA1c) and 2-hour postprandial glucose.

Chapter 3 describes associations between a variety of eating frequency and timing variables with metabolic biomarkers putatively associated with breast cancer risk in this same subset of NHANES women. The biomarkers examined in this chapter include C-reactive protein (a generalized biomarker of systemic inflammation) and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Chapter 4 investigates whether women's usual nightly fasting duration was associated with breast cancer recurrence and mortality, as well as the mechanisms by which prolonged nightly fasting have been postulated to improve breast cancer prognosis.

Together, these rich data sources substantially increase the generalizability of the study's results—offering findings that are relevant to the risk of incident breast cancer as well as breast cancer prognosis. To our knowledge, this is the first research conducted in humans to examine the relationships between nightly fasting duration and breast cancer risk and prognosis.

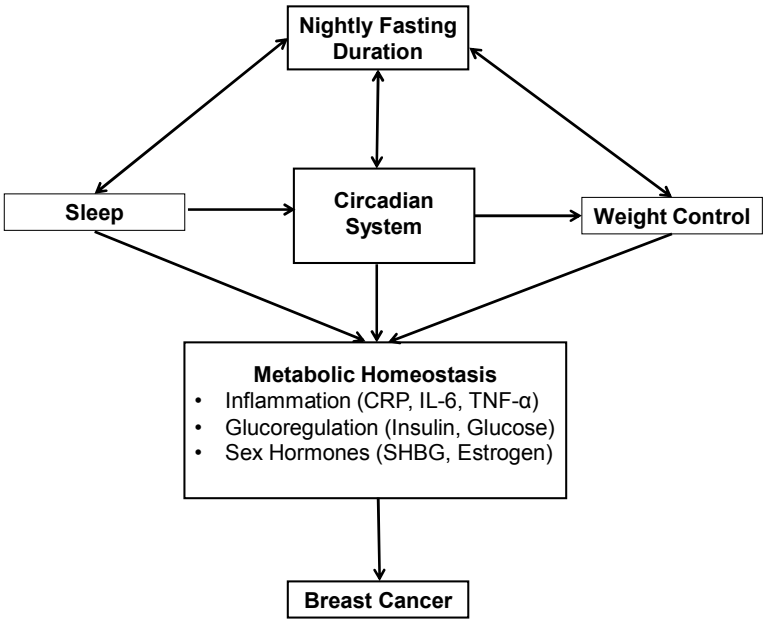

Figure 0.1. Simplified model illustrating the hypothesized relationships among nightly fasting, the circadian system, metabolic homeostasis, and breast cancer.

CRP=C reactive protein, IL-6=interleukin 6, TNF-α=Tumor necrosis factor alpha, SHBG=sex hormone binding globulin.

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CHAPTER 2

Prolonged Nightly Fasting and Breast Cancer Risk:

Findings from NHANES (2009-2010)

ABSTRACT

Background: A novel line of research has emerged suggesting that daily feeding-fasting schedules that are synchronized with sleep-wake cycles have metabolic implications that are highly relevant to breast cancer. We examined associations of nighttime fasting duration with biomarkers of breast cancer risk among women in the 2009-2010 U.S. National Health and Nutrition Examination Survey.

Methods: Dietary, anthropometric and HbA1c data were available for 2,212 women, and 2-hour postprandial glucose concentrations were available for 1,066 women. Nighttime fasting duration was calculated using 24-hour food records. Separate linear regression models examined associations of nighttime fasting with HbA1c and 2-hour glucose concentrations. Logistic regression modeled associations of nighttime fasting with elevated HbA1c (HbA1c ≥ 39 mmol/mol or 5.7%) and elevated 2-hour glucose (glucose ≥ 140 mg/dL). All models adjusted for age, education, race/ethnicity, BMI, total kcal intake, evening kcal intake, and the number of eating episodes per day.

Results: Each 3-hour increase in nighttime fasting (roughly one standard deviation) was associated with a 4% lower 2-hour glucose measurement (β 0.96, 95% CI 0.93-1.00; $p<0.05$), and a non-statistically significant decrease in HbA1c. Logistic regression models indicate that each 3-hour increase in nighttime fasting duration was associated with roughly a 20% reduced odds of elevated HbA1c (OR 0.81, 95% CI 0.68, 0.97; $p<0.05$) and non-significantly reduced odds of elevated 2-hour glucose.

Conclusions: A longer nighttime fasting duration was significantly associated with improved glycemic regulation.

Impact: Randomized trials are needed to confirm whether prolonged nighttime fasting could improve biomarkers of glucose control, thereby reducing breast cancer risk.

INTRODUCTION

Breast cancer is the most common form of cancer and the leading cause of death among women in industrialized countries. According to data from the Surveillance, Epidemiology, and End Results (SEER) Program, an estimated 232,670 women in the United States will be diagnosed breast cancer in 2014, and 44,000 will die from the disease.[1] Breast cancer incidence rates are projected to remain stable in years to come; therefore, the identification of population-level strategies to reduce the breast cancer risk among women is an important goal.

Converging lines of epidemiologic evidence indicates that diabetes is a risk factor for a several cancer types including breast cancer.[2-4] A meta-analysis of studies published after 2007 indicates that women with clinically diagnosed type 2 diabetes mellitus have approximately a 23% higher risk of developing breast cancer (RR 1.23; 95%CI: 1.12-1.34) and a 38% higher risk of breast cancer mortality (RR 1.38; 95%CI 1.20-1.58) compared to women without type 2 diabetes.[3] Although the biological mechanisms linking diabetes with cancer development may involve multiple signaling pathways, hyperglycemia (a hallmark of diabetes) is thought to be a key pathogenic component of the link [5] and is associated with increased breast cancer risk in numerous studies.[6-11] For example, a 2014 meta-analysis of fourteen published studies using glycosylated hemoglobin (HbA1c) as a biomarker of hyperglycemia found a positive linear relationship between HbA1c and cancer incidence.[7] Further, a prospective case control study of hormones and diet in the etiology of breast tumors (ORDET Study) found that the relative risk of breast cancer was more than 60% greater for women in the highest vs. lowest quartile of fasting glucose (RR 1.63; 95% CI 1.14-2.32).[10]

There is mounting evidence suggesting that time-restricted feeding, the practice of consuming ad-libitum energy within a restricted window of time and fasting thereafter (upwards of 12-16 hours), has favorable effects on glucose metabolism and may reduce the risk of chronic diseases such as diabetes and cancer. In particular, synchronizing feeding/fasting regimens with daily circadian rhythms (e.g., eating at night for nocturnal mice) appears to reset the body's peripheral clocks, resulting in improved oscillations in circadian clock gene expression and enhanced energy metabolism.[12] A 2014 review by Rothschild and colleagues [13] identified four published studies that have examined the impact of time-restricted feeding on glycemic control in mice.[14-17] Regimens of time-restricted feeding favorably influenced fasting glucose in all but one study.[14] There is also evidence that fasting regimens have direct effects on cell proliferation.[18-20] For example, a study examining the effects of different treatment regimens in mice injected with a variety of cancer cell types (including breast cancer 4T1 cells), found that a treatment regimen consisting of repeated fasting cycles resulted in reduced cancer metastases.[20] The authors of this study reported that the therapeutic effects of repeated fasting cycles were as effective as (if not superior to) chemotherapeutic treatments in mice.

Conversely, eating out of synchronization with daily circadian rhythms has been shown to result in a phase shift and misalignment of normal daily circadian oscillations in rodents—subsequently altering metabolic hormone concentrations, inducing obesity-related diseases, and accelerating the development of certain cancers.[12,21] Consistent with rodent models, data from numerous epidemiologic investigations in humans indicate that circadian misalignment due to lifestyle is associated with various diseases and metabolic disorders. Increased incidence of breast cancer observed in night-shift workers is a notable example.[22] Although a small number of experimental studies have

explored the effects of time-restricted feeding on human health, we are only aware of one published study to have explored the impact of a fasting regimen that is synchronized to daily circadian rhythms in humans. In a randomized cross-over study among 29 healthy college men, restricting energy intake between 7 pm and 6 am (a prolonged nighttime fast) resulted in significantly reduced overall daily energy intake and body weight during the two-week intervention period, compared to the control condition ($p<0.05$).[23] No large-scale study, to our knowledge, has explored this type of circadian synchronized eating pattern on metabolic health or chronic disease risk among women.

The objective of this paper was to investigate whether prolonged nightly fasting is associated with reduced HbA1c and postprandial glucose concentrations and thereby reduced risk of breast cancer. We used data from a nationally representative and diverse sample of women who participated in the 2009-2010 National Health and Nutrition Examination Survey.[24] We hypothesized that longer nighttime fasting periods would be associated with more favorable breast cancer risk profiles, as evidenced by lower HbA1c, reduced likelihood of having HbA1c values in the pre-diabetic and diabetic ranges, and better glucose tolerance during an oral glucose tolerance test.

METHODS

Study Sample

Data for this study were obtained from the 2009-2010 National Health and Nutrition Examination Survey (NHANES), which is a continuous annual survey conducted by the National Center for Health Statistics. NHANES is comprised of a nationally representative sample of the U.S. civilian non-institutionalized population,

selected by a complex, multistage, stratified, clustered probability design. The survey consists of two components: (1) an in-home interview; and (2) an in-person comprehensive medical examination at the mobile exam center, which includes an array of laboratory tests. The National Center for Health Statistics Research Ethics Review Board approval was granted and documented consent was obtained from all study participants. Details of the study procedures have been published elsewhere (http://www.cdc.gov/nchs/nhanes.htm).[24]

The current study sample consisted of 2,212 adult women from the NHANES 2009-2010 survey year who completed the in-person comprehensive medical exam at the mobile exam center. We excluded women who did not have the telephone-based dietary recalls in the online database, women who had diabetes (self-report), were taking medication for diabetes, and women who were pregnant. Outcomes requiring a fasting blood draw, such as 2-hour postprandial glucose concentrations, were only available for a subsample of adult women who were scheduled for morning blood draws (OGTT subsample; n=1,334).

Dietary Assessment

One 24-hour dietary recall was conducted by telephone 3-10 days after the in-person medical exam. The dietary recall was conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). USDA's Food Surveys Research Group (FSRG) was responsible for the dietary data collection methodology, maintenance of the databases used to code, review, and processing of the data.

Outcome and Covariate Assessment

Biomarkers of Glucose Control. Assays for HbA1c concentrations, which reflect average plasma glucose across the past 120 days, were performed on a Tosoh A1C G7. HbA1c values were converted to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standardized units.[25]

In the subsample of individuals assigned a morning examination, 2-hour postprandial glucose concentrations were assessed after a 75-gram oral glucose-equivalent challenge (Oral Glucose Tolerance Test). Plasma glucose concentrations were approximated by a hexokinase method using plasma blood specimens. All blood specimens were obtained by trained medical professionals in mobile examination centers and were analyzed at the Fairview Medical Center Laboratory at the University of Minnesota, Minneapolis Minnesota.

Nighttime Fasting Duration. We estimated nighttime fasting duration by calculating the time between the first and last calorie-containing (>5 kcal) food or beverage consumed for each 24-hour dietary recall day and subtracting this number from 24.

Dietary factors. We identified other dietary covariates that could confound the association of nighttime fasting with glucose regulation, such as total energy intake, and the number of eating episodes per day. The number of eating episodes per day variable was defined as the number of time-stamps associated with calorie-containing food or beverage consumption. We also calculated kcals consumed after 10 pm as a means of controlling for fasting initiation times (e.g., starting nighttime fast at 6 pm vs.

11 pm), given the evidence that nighttime eating may have deleterious effects on metabolic health.[26,27]

Other covariates. Height and weight measurements were obtained using standardized techniques and equipment. Physical activity was assessed using the physical activity questionnaire (PAQ), which includes questions related to daily activities, leisure time activities, and sedentary activities.[28] Responses were used to calculate an estimate of weekly metabolic equivalents (METs) using the analytic notes and suggested MET scores outlined in the NHANES online documentation (http://www.cdc.gov/nchs/nhanes.htm). Briefly, work-related activities and vigorous leisure-time physical activities were assigned MET values of 8.0; moderate work-related activities, walking or bicycling for transportation, and moderate leisure-time physical activities were assigned MET values of 4.0. Based on the non-normal distribution of weekly MET values, we present the data by tertiles of weekly MET scores. Sleep duration was assessed using the single item question, “How much sleep do you usually get per night on weekdays or workdays?”

The Family and Sample Person Demographics questionnaire ascertained demographic data on survey participants. This questionnaire was administered in the home, by trained interviewers using the Computer-Assisted Personal Interviewing system. Demographic covariates used in regression analyses include age (continuous variable), ethnicity (categorical variable: non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and others), and education (did not complete high school, completed high school, and attended/completed college or advanced degree).

Statistical Analysis

Descriptive statistics characterized the study population, and chi-square tests and univariate regression analyses were conducted to examine differences in participant characteristics by tertiles of nighttime fasting duration. Separate linear regression models examined associations of nighttime fasting (independent variable) with biomarkers of interest (HbA1c and 2-hour postprandial glucose). We controlled for the primary covariates of age, education, and race/ethnicity. We also controlled for lifestyle confounders associated with dietary behaviors and biomarkers of glucose control, such as total kcal, BMI, number of eating episodes per day, and "calories consumed after 10 pm". To simplify interpretation of the parameter estimates, we used a 3-hours unit of analysis for the nighttime fasting duration variable, which is approximately 1-standard deviation of the nighttime fasting variable. We also used a 100 kcal unit of analysis in regression models for the total kcal variable. The calories consumed after 10 pm variable was highly skewed and so was dichotomized for use in regression models (any vs. no calories after 10 pm). HbA1c concentrations were normally distributed and treated as a continuous variable in linear regression models. Two-hour glucose concentrations were log transformed to better approximate Gaussian distribution, and treated as a continuous variable linear regression models. Physical activity and sleep were not included in the final models, as they did not meaningfully change the relationship between nighttime fasting and either HbA1c or 2-hour postprandial glucose concentrations. Logistic regression analysis was used to model associations of nighttime fasting with elevated HbA1c and 2-hour glucose. We characterized elevated HbA1c as any value at or above 39 mmol/mol (5.7%), which is the American Diabetes Association's recommended threshold for identifying individuals at high risk for hyperglycemia.[29] Elevated 2-hour glucose (impaired glucose tolerance)

was scored as glucose concentrations at or above the American Diabetes Association cutoff for prediabetes of ≥ 140 mg/dL.[29] The logistic regression models adjusted for the same covariates as linear regression models described previously. As a sensitivity analysis, we analyzed these same linear and logistic regression models using only participants whose reported dietary intake was within 25% of predicted energy expenditure (caloric level appropriate for maintaining body weight) using the Mifflin-St Jeor formula, which has been validated in broad populations of adults.[30] We hereafter refer to this subsample as the "True Reporters" subsample. The True Reporters subsample excludes individuals who report unusually low or high kcal based on their estimated caloric needs or who are practicing extreme dietary or exercise regimens likely to result in weight change, which may obscure the true relationships between nighttime fasting and the biomarkers of interest. Data were analyzed using SAS version 9.3 (Carey, NC). All analyses used sample weights to account for differential probabilities of selection into the sample, nonresponse, and noncoverage. Standard errors were estimated using Taylor Series Linearization. All statistical tests were set at an overall significance level cutoff of $P<0.05$.

RESULTS

There were minimal differences between demographic and behavioral characteristics in the full and the subsample of women with 2-hour glucose data; thus, we present population-weighted demographic and behavioral characteristics in the full sample only (Table 1). The sample population had an average age of approximately 47 years and a mean BMI of 28 kg/m^2. This was a diverse sample: approximately half of the women were non-Hispanic white, 16% were non-Hispanic black and 17% were

Mexican American. Women in our sample reported an average of 5 eating occasions per day and their mean nighttime fasting duration was 12.4 hours (SEM=0.08).

We categorized nighttime fasting duration into tertiles (Table 1). Mean fasting duration was 9.5 hours in tertile 1, 12.3 hours in tertile 2, and 15.1 hours in tertile 3 ($p<0.001$). Nighttime fasting duration was significantly associated with several other dietary behaviors. In particular, women who reported longer nighttime fasts also reported consuming fewer calories per day, ate fewer calories after 10 pm, and had considerably fewer eating episodes per day ($p<0.001$ for all). However, women who reported a longer nighttime fast had a significantly higher BMI than those who reported a shorter fast. In these univariate analyses, there were no significant associations between tertiles of fasting duration and HbA1C or 2-hour glucose.

Table 2 presents multivariable linear regression models of the associations of nighttime fasting with biomarkers of glucose control in the full sample and among True Reporters. The parameter estimates of the linear regression models should be interpreted as a 1-point increase in the exposure per 3-hour increase (roughly 1 standard deviation) in nighttime fasting duration. Each 3-hour increase in nighttime fasting duration was associated with roughly a 0.40 mmol/mol lower HbA1c measurement (β -0.39, 95% CI -0.84-0.05; $p=0.08$) among the full sample of women, and a 0.50 mmol/mol lower measurement among True Reporters (β -0.48, 95% CI -0.85--0.10; $p=0.02$). Significant trends were observed for the associations of nighttime fasting and 2-hour postprandial glucose in fully adjusted models. According to the back-transformed parameter estimates presented in Table 2, each 3-hour increase in nighttime fasting duration was associated with a 4% decrease in postprandial glucose in the full sample (β 0.96, CI 0.93-1.00; $p=0.04$), and 6% decrease in the subsample of True Reporters (β 0.94, CI 0.90-0.98; $p<0.01$). BMI was the only other significant

predictor of biomarkers of glucose control in the full sample and among True Reporters.

Multivariable logistic regression models of the associations of nighttime fasting with elevated HbA1c and 2-hour postprandial glucose are presented in Table 3. Each 3-hour increase in nighttime fasting was associated with 19% lower odds of elevated HbA1C in the full sample (OR 0.81, 95% CI 0.68-0.97) and a 23% lower odds of elevated HbA1c among True Reporters (OR 0.77, 95% CI 0.61-0.98). Nighttime fasting duration was not significantly associated with elevated 2-hour postprandial glucose in the full sample. However, in the True Reporters subsample, each 3-hour increase in nighttime fasting was associated with a greater than 50% reduction in odds of having elevated 2-hour glucose ($p<0.05$).

DISCUSSION

In this large, multi-ethnic, population-based sample of adult women, a 3-hour increase in nighttime fasting was associated with an approximate 20% lower likelihood of having HbA1c concentrations at or above the prediabetic threshold. The association of nighttime fasting with elevated HbA1c was independent of caloric intake, BMI, and other potential confounders. The linear trends in this study also indicated that each 3-hour increase in nighttime fasting was associated with roughly a 0.4 mmol/mol decrease in HbA1c concentrations. This translates to a reduction of 0.05% when HbA1c is expressed as a percentage. Although this association appears modest, previous epidemiologic investigations of HbA1c and health outcomes suggest that even small changes in HbA1c concentrations could have a considerable population-level impact. For example, based on projections using data from 4,462 older adult men

in the EPIC-Norfolk cohort, a reduction in the HbA1c of 0.1% throughout the whole population is estimated to result in a reduction in excess mortality by upwards of 5%.[31]

Nighttime fasting was also associated with postprandial 2-hour glucose concentrations. We found that a 3-hour unit increase in nighttime fasting was associated with 2-hour glucose concentrations after an oral glucose challenge. Our finding that nighttime fasting was associated with both postprandial glucose and HbA1c concentrations is interesting, given the evidence that postprandial hyperglycemia may occur even when overall glycemic control appears to be adequate as assessed by HbA1c.[32] Specifically, postprandial hyperglycemia is part of a progressive decline in peripheral insulin sensitivity and beta cell function that precedes abnormal HbA1c and development of type 2 diabetes.[33]

The finding that longer nighttime fasting was associated with better glucose control is biologically plausible. In rodents, various fasting regimens have been shown to increases fatty acid oxidation in the liver and muscle,[34] which may have downstream effects in insulin sensitivity and glucose control. There is also evidence that fasting activates the Forkhead Box A (FOXA) genes which encode transcription factors involved in gluconeogenesis [35] and such activation could influence homeostatic regulation of glucose in the liver.[36] Furthermore, circadian light cycle disruption in mice (equating to nighttime feeding in humans) has been shown to increase intestinal permeability (i.e., gut leakiness) and circulating pro-inflammatory endotoxins, which are known to induce pathologic inflammation and metabolic states associated with impaired hepatic glucose and lipid metabolism.[37] Thus, nighttime fasting would support maintenance of intestinal barrier integrity and minimize detrimental effects of gut microbe-produced endotoxins on hepatic glucose regulation.

Many types of fasting regimens have been associated with reductions in total daily energy intake. For example, studies of alternate day fasting (i.e., rotating “fasting days” with “eating days”) generally result in weight loss via reductions in overall energy intake.[38,39] Similarly, findings from the current study indicate that women who engaged in longer nighttime fasts reported consuming fewer calories throughout the day. Despite these associations of longer fasting with reduced caloric intake, the favorable effects of fasting on glycemic control parameters were observed even after adjustment for total daily energy intake (kcals/day).

The major limitation of the current study was the use of a single day of self-reported dietary intake to assess a woman’s usual nighttime fasting interval. It is unclear whether self-reported timing of energy intake is susceptible to the same biases as self-reported diet, as we are not aware of any published studies that have explored this concept. Nonetheless, numerous studies have shown that self-reported dietary consumption is underreported—particularly among overweight or obese adults [40]. Therefore, we hypothesize that the magnitude of the associations between nighttime fasting and glycemic parameters observed in this study are attenuated. In support of this conjecture, sensitivity analyses conducted in the current study indicated that the associations between nighttime fasting and glycemic control were stronger when we excluded women who over-or under-reported total energy intake by 25% or more, as compared to their estimated energy needs.

Strengths of this study include use of a large, nationally representative sample of US women with a wide array of demographic, anthropometric, and dietary variables available. Our results are also strengthened by the use of HbA1c and 2-hour postprandial glucose data to assess glycemic regulation. HbA1c reflects average plasma glucose over the past 12 weeks and is a considered a reliable measure of

chronic glycemic control. Further, an OGTT is the recommended “gold standard” for diabetes diagnostic purposes by large public health organizations such as the WHO.[41,42]

To our knowledge, this is the first study to document that a longer nighttime fasting duration in women was significantly associated with improved glycemic regulation and putatively with reduced breast cancer risk. Large-scale randomized trials are needed to confirm whether a habitual prolonged nighttime fasting regimen results in favorable changes in biomarkers of glycemic control and breast cancer risk. If these findings are confirmed, recommendations for prolonged nightly fasting could be provided as a simple and understandable dietary guideline.

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Table 2.1. Demographic, Lifestyle, and Dietary Characteristics of Women from the NHANES 2009-2010 Survey. Data are Presented for the Full Sample and by Tertiles of Nighttime Fasting Duration.

Characteristics mean (SEM) unless specified	**Full Eligible Sample** n=2,212	**Fasting Tertile 1** (<11.5 hrs) n=770	**Fasting Tertile 2** (11.5-13.49 hrs) n=746	**Fasting Tertile 3** (≥13.5 hrs) n=770	*P-value for difference*[a]
Age	46.8 (0.7)	48.4 (1.0)	46.6 (0.9)	45.5 (0.7)	0.03
Ethnicity n(%)					
Non-Hispanic White	1,138 (51.4)	386 (55.5)	399 (53.5)	353 (45.8)	<0.001
Non-Hispanic Black	355 (16.0)	110 (15.8)	100 (13.4)	145 (18.8)	
Mexican American	379 (17.1)	80 (11.5)	140 (18.8)	159 (20.6)	
Other race(s)	340 (15.4)	120 (17.2)	107 (14.3)	113 (14.7)	
Educational Attainment[b] n(%)					
No High School Diploma	549 (24.8)	138 (19.8)	193 (25.9)	218 (28.8)	0.47
High School Diploma	494 (22.3)	154 (22.1)	151 (20.2)	189 (24.5)	
Some College	682 (30.8)	231 (33.2)	229 (30.7)	222 (28.7)	
College Degree	483 (21.8)	171 (24.6)	172 (23.0)	140 (18.2)	
BMI kg/m^3	28.2 (0.2)	27.5 (0.4)	27.9 (0.4)	29.1 (0.3)	0.01
Physical Activity n(%)					
Low	718 (32.5)	216 (31.0)	235 (25.9)	267 (28.3)	0.99
Med	742 (33.5)	236 (33.9)	256 (34.3)	250 (32.5)	
High	752 (34.0)	244 (35.1)	255 (34.2)	253 (32.8)	
Sleep (hours/night)	7.0 (0.0)	7.0 (0.1)	7.17 (0.1)	6.9 (0.1)	0.40
Daily Energy Intake (kcal/day)	1,773.1 (22.5)	1,914.1 (51.8)	1,797.5 (39.2)	1,623.3 (32.4)	<0.001
Daily Energy Intake After 10 pm (kcal/day)	51.4 (5.6)	113.8 (12.2)	21.4 (3.5)	23.3 (5.4)	<0.001
Nighttime Fasting Duration (hours)	12.4 (0.1)	9.5 (0.1)	12.3 (0.0)	15.1 (0.1)	<0.001
Number of Eating Episodes Per Day	4.7 (0.1)	5.5 (0.1)	4.8 (0.1)	3.8 (0.1)	<0.001
HbA1c (mmol/mol)[c]	36.1 (0.2)	36.5 (0.2)	36.0 (0.2)	35.9 (0.3)	0.18
2-hour glucose[d] **(mg/dL)(median Q1,Q3)**	104.1 (85.6, 131.3)	104.7 (87.8,135.1)	103.9 (84.8,128.5)	104.0 (84.8,131.2)	0.38

[a]P-values reflect statistical comparisons of participant characteristics by tertiles of nighttime fasting duration.

[b]Data on educational attainment was missing for 4 study participants.

[c]Glycosylated Hemoglobin.

[d]Concentration of glucose in plasma collected 2-hours after a 75-gram oral glucose-equivalent challenge (Oral Glucose Tolerance Test). 2-hour glucose data were only available for the subsample of fasting participants (n=1,066).

Table 2.2 Associations of Eating Patterns and Lifestyle Factors with Glycemic Control in Women from the NHANES 2009-2010 Survey.

	HbA1c (mmol/mol)[a]			
	Full Sample (n=2,212)		True Reporters[b] (n=1,334)	
	β (95%CI)	p-value	β (95%CI)	p-value
Nighttime Fast Duration	**-0.39 (-0.84-0.05)**	**0.08**	**-0.48 (-0.85--0.10)**	**0.02**
Eating Episodes	-0.12 (-0.34-0.10)	0.26	-0.16 (-0.42-0.10)	0.21
Total Kcal	0.02 (-0.02-0.51)	0.26	0.01 (-0.03-0.04)	0.54
Kcal after 10 pm	-0.03 (-0.73-0.66)	0.92	-0.02 (-0.98-0.93)	0.96
BMI (kg/m^2)	0.16 (0.12-0.20)	<.001	0.16 (0.10-0.23)	<.001
	2-Hour Glucose[c]			
	Full Sample (n=1,066)		True Reporters[b] (n=593)	
	β (95%CI)	p-value	β (95%CI)	p-value
Nighttime Fast Duration	**0.96 (0.93-1.00)**	**0.04**	**0.94 (0.90-0.98)**	**<.01**
Eating Episodes	0.99 (0.96-1.01)	0.32	0.98 (0.94-1.01)	0.13
Total Kcal	1.00 (0.99-1.01)	0.86	1.00 (0.99-1.01)	0.87
Kcal after 10pm	1.01 (0.94-1.10)	0.73	0.99 (0.90-1.09)	0.79
BMI (kg/m^2)	1.01 (1.01-1.02)	<.001	1.01 (1.01-1.02)	<.001

*All models adjusted for age, education, and race/ethnicity.

[a]HbA1c presented as mmol/mol, per recommendations by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

[b]True Reporters subsample includes participants who reported calorie intake within 25% of their predicted energy expenditure.

[c]Concentration of glucose in plasma collected 2-hours after a 75-gram oral glucose-equivalent challenge (Oral Glucose Tolerance Test). 2-hour glucose data were only available for the subsample of fasting participants (n=1,066). Parameter estimates were back-transformed for ease of interpretation, and should be interpreted as a percent change in 2-hour glucose per unit increase in nighttime fasting.

Table 2.3. Separate Multivariable Logistic Regression Modeling Associations of Nighttime Fasting Duration with Odds of Elevated HbA1c and 2-hour Glucose Levels in a Sample of Women from the NHANES 2009-2010 Survey.

	Elevated HbA1c[a]	
	Full Sample (n=2,212)	True Reporters[b] (n=1,334)
	OR (95%CI)	OR (95%CI)
Nighttime Fast Duration	**0.81 (0.68-0.97)**	**0.77 (0.61-0.98)**
Eating Episodes	0.94 (0.80-1.11)	0.92 (0.73-1.61)
Total Kcal	1.02 (0.99-1.04)	1.01 (0.98-1.04)
Kcal after 10 pm	0.80 (0.51-1.24)	0.70 (0.63-1.38)
BMI (kg/m^2)	1.07 (1.05-1.08)	1.07 (1.05-1.10)
	Elevated 2-hour Glucose[c,d]	
	Full Sample (n=1,066)	True Reporters[b] (n=593)
	OR (95%CI)	OR (95%CI)
Nighttime Fast Duration	**0.78 (0.53-1.15)**	**0.54 (0.36-0.80)**
Eating Episodes	0.88 (0.74-1.05)	0.73 (0.58-0.91)
Total Kcal	0.99 (0.96-1.03)	1.00 (0.95-1.04)
Kcal after 10 pm	1.50 (0.79-2.82)	1.01 (0.54-2.05)
BMI (kg/m^2)	1.07 (1.05-1.08)	1.07 (1.04-1.10)

*All models adjusted for age, education, and race/ethnicity.
[a]Elevated HbA1c was defined as ≥39mmol/l (5.7%).
[b]True Reporters subsample includes participants who reported to have eaten within 25% of predicted energy expenditure.
[c]Concentration of glucose plasma collected 2-hours after a 75-gram glucose-equivalent challenge (Oral Glucose Tolerance Test). 2-hour glucose data were available for fasting subsample of participants only (n=1,066).
[d]Elevated 2-hour glucose was defined as ≥140 mg/dL(impaired glucose tolerance).

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CHAPTER 3

Frequency and circadian timing of eating may influence biomarkers of inflammation and insulin resistance associated with breast cancer risk

ABSTRACT

Emerging evidence suggests that there is interplay between the frequency and circadian timing of eating and metabolic health. We examined the associations of eating frequency and timing with metabolic and inflammatory biomarkers putatively associated with breast cancer risk in women participating in the National Health and Nutrition Examination 2009-2010 Survey. Eating frequency and timing variables were calculated from 24-hour food records and included (1) proportion of calories consumed in the evening (5pm-midnight), (2) number of eating episodes per day, and (3) nighttime fasting duration. Linear regression models examined each eating frequency and timing exposure variable with C-reactive protein (CRP) concentrations and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Each 10 percent increase in the proportion of calories consumed in the evening was associated with a 3 percent increase in CRP. Conversely, eating one additional meal or snack per day was associated with an 8 percent reduction in CRP. There was a significant interaction between proportion of calories consumed in the evening and fasting duration with CRP ($p=0.02$). A longer nighttime fasting duration was associated with an 8 percent lower CRP only among women who ate less than 30% of their total daily calories in the evening ($p=0.01$). None of the eating frequency and timing variables were significantly associated with HOMA-IR. These findings suggest that eating more frequently, reducing evening energy intake, and fasting for longer nightly intervals may lower systemic inflammation and subsequently reduce breast cancer risk. Randomized trials are needed to validate these associations.

INTRODUCTION

There is compelling evidence that persistent low-grade inflammation is a significant underlying contributor to numerous cancers[1-4] (including breast) and is associated with several chronic metabolic conditions linked to breast cancer (e.g., obesity, diabetes).[5,6] Laboratory studies have demonstrated that chronic inflammation predisposes tissue to cancer development, whereby tumors generally arise within inflamed tissues.[7] Consistent with this evidence, data from epidemiologic cohorts of women have shown that elevated C-reactive protein (CRP), a biomarker of systemic inflammation, is associated with breast cancer risk.[3,4] For example, a case-control study among postmenopausal women enrolled in the Multiethnic Cohort Study (706 cases; 706 controls) found that women in the highest quartile of CRP were significantly more likely to develop breast cancer than women in the lowest quartile (OR: 1.41; 95% CI 1.01-1.96; P_{trend}=.01).[4]

Insulin resistance is another metabolic condition that appears to play a significant role in the etiology of breast cancer and related metabolic diseases. Although the exact mechanisms linking insulin resistance to these conditions are unknown, experimental data indicate that elevated circulating insulin within the hyperinsulinemic range is a fundamental component of these links.[8,9] For example, in a sample of women enrolled in the Women's Health Initiative Observational Study, investigators found that women in the highest tertile of baseline insulin had nearly double the risk of breast cancer relative to women in the lowest tertile (HR: 2.22; 95% CI 1.39-3.53).[9] The authors of this study also reported that the estimate of breast cancer risk based on insulin was even higher in the subsample of women who did not use hormone replacement therapy (HR: 3.15; 95% CI 1.61-6.17). Insulin resistance is also a hallmark of type 2 diabetes and type 2 diabetes risk,[10] and has been associated

with numerous other cancers such as liver, kidney, and pancreatic cancer.[11]

Recent evidence suggests that dietary behaviors such as eating frequency and timing influence insulin secretion and systemic inflammation, and may have downstream effects inflammation and insulin resistance. The majority of this initial evidence has come from mechanistic studies in animals. Landmark studies in rodents have demonstrated that eating regimens that restrict food intake to within a 4- to 8-hour window of time (i.e., 'time-restricted feeding'), thereby increasing the number of hours spent fasting, can improve metabolic processes related to insulin resistance and inflammation. Specifically, mice subjected to these feeding-fasting regimens appear to be protected from hyperinsulinemia, hepatic steatosis, and inflammation.[12] Notably, the most profound effects of these feeding-fasting regimens on metabolism seem to occur when synchronized with daily sleep-wake cycles (i.e., eating only during the night and fasting during the day for nocturnal mice).[13] To date, only small-scale human studies have examined the impact of fasting regimens on human metabolic health. Results of these studies generally indicate that fasting has favorable effects on insulin resistance parameters but provide little data on inflammation.[14] It is difficult to extrapolate from these small-scale studies of fasting to our research question regarding the health impacts of prolonged nightly fasting. However, our recently published analysis of NHANES data did find that prolonged nightly fasting was associated with significantly lower levels of HbA1c.[15]

Although data from human studies are sparse, there is evidence that other eating frequency and timing behaviors can influence biomarkers of breast cancer and metabolic disease risk. In particular, eating large meals in the evening appears to have deleterious effects on insulin metabolism.[16,17] A cross-over study among 6 healthy men found insulin sensitivity to be significantly impaired among men consuming a high-

energy (60 percent of daily calories) meal at dinner vs. in the morning.[17] A 12-week randomized parallel-arm trial among 93 overweight and obese women found that insulin (fasting and postprandial) and HOMA-IR were significantly improved when the women ate a high-calorie breakfast vs. high-calorie dinner (total daily caloric intake was the same).[16] Evidence from controlled feeding studies generally suggests that grazing (small, frequent meals) as opposed gorging (fewer, large meals) may improve regulation of metabolic processes.[18,19] For example, a recent trial among 54 patients with type 2 diabetes randomized to isocaloric diets consumed as either 2 meals or 6 meals per day found that more frequent meals resulted in significantly reduced body weight and fasting glucose concentrations.[20] Less is known about optimal meal frequency in nondiabetics, and recommendations on meal frequency for reducing chronic disease risk remain highly controversial.

The purpose of this study was to further examine the associations between eating frequency and timing behaviors with inflammation and insulin resistance biomarkers putatively associated with increased breast cancer risk. Eating frequency and timing variables were (1) proportion of calories consumed in the evening (between 5pm and midnight); (2) number of eating episodes per day; and (3) nighttime fasting duration (a time-restricted feeding schedule that is aligned with sleep-wake cycles). We used data from a population-based sample of women participating in the 2009-2010 National Health and Nutrition Examination Survey.

MATERIALS AND METHODS

Study Sample

Data for this study were obtained from the 2009-2010 National Health and Nutrition Examination Survey (NHANES), which is a continuous annual survey

conducted by the National Center for Health Statistics. NHANES comprises a nationally representative sample of the U.S. civilian non-institutionalized population, selected by a complex, multistage, stratified, clustered probability design. The survey consists of two components: (1) an in-home interview; and (2) an in-person comprehensive medical examination at the mobile exam center, which includes an array of laboratory tests. The National Center for Health Statistics Research Ethics Review Board approval was granted and documented consent was obtained from all study participants. Details of the study procedures have been published elsewhere (http://www.cdc.gov/nchs/nhanes.htm) [21].

The current study sample consisted of 2,650 adult women from the NHANES 2009-2010 survey year who completed the in-person comprehensive medical exam at the mobile exam center. We excluded women who did not have the telephone-based dietary recalls in the online database, women who had diabetes or borderline-diabetes (self-report), were taking medication for diabetes, and women who were pregnant (n=438 excluded). Outcomes requiring a fasting blood draw, such as fasting insulin and glucose (used to calculate HOMA-IR), were only available for a subsample of adult women who were scheduled for morning blood draws (HOMA-IR subsample; n=1,034).

Dietary Assessment

Eating behavior data used in our analyses were collected from each participant via a 24-hour dietary recall that was conducted by telephone 3-10 days after the in-person medical exam. The dietary recall was conducted in partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). USDA's Food Surveys Research Group (FSRG) was responsible for the dietary data collection methodology and database maintenance.

Outcome and Covariate Assessment

C-reactive protein (CRP). All blood specimens were obtained by trained medical professionals in mobile examination centers and were analyzed at the Fairview Medical Center Laboratory at the University of Washington. Participants were not required to fast prior to the collection of blood specimens used in the determination of CRP, given that this biomarker can be measured accurately in non-fasting conditions.[22] There were also no restrictions as to the time of day in which these blood specimens were collected, as CRP does not exhibit diurnal variation.[23]. High-sensitivity assays performed on a latex-enhanced Behring Nephelometer were used to quantify CRP concentrations. CRP concentrations were calculated using a calibration curve. Data reduction of the signals was performed by using a storable logit-log function for the calibration curve.

HOMA-IR. Individuals who were assigned a morning examination provided fasting blood specimens for insulin and glucose measures. Blood specimens were collected after a 9-hour fast using standard procedures and were sent to the University of Minnesota for analysis. Insulin concentration was quantified using a Merocodia Insulin ELISA, which is a two-site enzyme immunoassay. Plasma Glucose concentrations measured by a hexokinase-mediated reaction Roche/Hirachi Modular P Chemistry Analyzer using plasma blood specimens. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used to estimate the degree of insulin resistance in each participant using the following equation[24]:

HOMA-IR=fasting glucose (mml/L) x fasting insulin (uU/ml)/22.5

Eating frequency and timing. The number of eating episodes per day variable

was defined as the number of time-stamps associated with calorie-containing food or beverage consumption. It is unclear how many ingested calories are needed for initiation of metabolic processes; therefore, we used a conservative 5 kcal cutoff to define an eating episode. We re-processed the dietary data using more liberal calorie cutoffs to define an eating episode (e.g., 10 kcal, 25 kcal); however these liberal cutoffs did not meaningfully change the study findings or conclusions rendered. Evening caloric intake was calculated by summing the total calories consumed between 5 pm and midnight. Nighttime fasting duration was estimated by (1) calculating the elapsed time between the first and last calorie-containing food or beverage consumed for each 24-hour dietary recall day; and (2) subtracting this elapsed time from 24.

Other covariates. The Family and Sample Person Demographics questionnaire ascertained demographic data on survey participants. This questionnaire was administered in the home, by trained interviewers using the Computer-Assisted Personal Interviewing system. Demographic covariates used in regression analyses include age (continuous variable), ethnicity (categorical variable: non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and others), and education (did not complete high school, completed high school, and attended/completed college or advanced degree. Physical activity was assessed using the physical activity questionnaire, which includes questions related to daily activities, leisure time activities, and sedentary activities [25]. Responses were used to calculate an estimate of weekly metabolic equivalents (MET) using the analytic notes and suggested MET scores outlined in the NHANES online documentation (http://www.cdc.gov/nchs/nhanes.htm). Briefly, work-related activities and vigorous leisure-time physical activities were assigned MET values of 8.0; moderate work-related activities, walking or bicycling for

transportation, and moderate leisure-time physical activities were assigned MET values of 4.0. Sleep duration was assessed using the single item question, “How much sleep do you usually get per night on weekdays or workdays?”). Height and weight measurements were obtained using standardized techniques and equipment during the medical exam.

Statistical Analysis

Descriptive statistics were used to characterize the study population. Outcome variables were assessed for linearity and normality. Both HOMA-IR and CRP were non-normally distributed and were log-transformed to better approximate Gaussian distribution. Multivariable linear regression models were used to examine the joint-effects of the eating frequency and timing exposure variables (all three exposures modeled together) with the inflammation and insulin resistance biomarkers of interest (CRP and HOMA-IR). Models controlled for the primary confounders of age, education, and race/ethnicity.

We hypothesized that fasting is more beneficial when nighttime eating is restricted (i.e., fasting during the night and eating during the day). Therefore, we tested for effect modification between hours of nighttime fasting and proportion of calories consumed in the evening, using tests of statistical interaction, and in models stratified by the proportion of evening caloric intake (<30% vs. ≥30%) based on quartile distributions (bottom quartile vs. upper three). Interaction and stratified models controlled for the same covariates described above, as well as the other eating frequency and timing variables.

When modeling CRP, we excluded individuals with CRP concentrations greater than or equal to 15 mg/L, given that our goal was to model chronic inflammation (very

high CRP may indicate acute inflammation); however sensitivity analyses revealed that excluding individuals with high CRP did not influence the associations we report. We explored adjustment for physical activity, total caloric intake, as well as other dietary exposures (e.g., total fat, carbs), however, these variables were not statistical confounders and, thus, were not included final models. To ease interpretation and clinical relevance of the parameter estimates for the Evening Calories variable, we used a 10 percent unit of analysis. Using this approach, a 10 percent increase in the proportion of total calories consumed in the evening (after 5 pm) corresponds to a 1-unit change in the biomarker outcome of interest. Data were analyzed using SAS Studio and SAS version 9.4 and (Carey, NC). Sample weights were used in all analyses to account for differential probabilities of selection into the sample, nonresponse, and noncoverage. Standard errors were estimated using Taylor Series Linearization. All statistical tests were set at an overall significance level cutoff of $p<0.05$.

RESULTS

Sociodemographic, behavioral, and biomarker characteristics of the eligible (n=2,212), excluded (n=438), and HOMA-IR subsample (n=1,034) are presented in Table 1. The primary reason women were excluded from the analytic sample was due to having diabetes (self-report) and/or taking antidiabetic medication. The mean age of women in the eligible sample was 46.8 (SEM=0.7) years. They consumed an average of 774 (SEM=17.1) kcals between 5 pm and midnight, fasted 12.4 hours per night (SEM=0.1), and ate 4.7 (SEM=0.1) times per day. These characteristics for the HOMA-IR subsample were similar to those of all eligible women.

Eating frequency and timing variables were generally modestly but statistically significantly correlated (data not shown). According to weighted Pearson correlation coefficients, eating frequency was positively correlated with the Evening Calories variable (r=0.2, $p< 0.001$) and inversely associated with nighttime fasting duration (r=-0.51, $p<0.001$). Nighttime fasting duration was also inversely correlated with Evening Calories (r=-0.15, $p<0.001$). However, statistical tests revealed no evidence of collinearity (low variance inflation factor [<3]). There were also statistically significant positive associations between the CRP and HOMA-IR (r=0.45, $p<0.001$), suggesting that the two biomarker outcomes are distinct yet moderately related.

Associations of eating frequency and timing exposure variables with CRP and HOMA-IR are presented in Table 2. As described previously, the parameter estimates for the Evening Calories variable should be interpreted as 1 point increase/decrease in the outcome of interest (CRP or HOMA-IR), per 10 percent increase in the proportion of total daily calories that were consumed in the evening (after 5 pm). The back-transformed parameter estimates indicate that each 10 percent increase in proportion of calories consumed in the evening was associated with a 3 percent increase in CRP (β 1.03; 95% CI 1.01-1.06; p=0.02). Eating frequency was inversely related to CRP. Specifically, eating one additional meal per day was associated with an 8 percent reduction in CRP (β 0.92; 95% CI 0.86-0.99; p=0.03). Nighttime fasting duration was not significantly associated with CRP; and none of the eating frequency or timing behaviors were associated with HOMA-IR.

There was a statistically significant interaction between nighttime fasting and the Evening Calories variable (p=0.02). Accordingly, exploratory models examined the association of nighttime fasting stratified by portion of calories consumed in the evening (<30% calories after 5pm vs. ≥30% calories)(Table 3). Among women who ate

fewer than 30% of their calories in the evening, nighttime fasting duration was inversely associated with CRP concentrations. Specifically, according to the back-transformed parameter estimates, each additional hour of fasting was associated with an 8 percent lower concentration of CRP (β 0.92; 95% CI 0.87-0.98; p=0.01); however this association was not statistically significant when evening calories were greater than or equal to 30 percent. Stratified models revealed no significant associations between nighttime fasting duration and HOMA-IR.

DISCUSSION

The degree to which timing and frequency of food intake influences metabolic health and chronic disease risk is an exciting new area of dietary research.[14] Data from longitudinal studies suggests that over time, adults are eating a greater proportion of their daily calories in the evening,[26] although this shift in the distribution energy intake towards the evening hours may have deleterious effects on human health.[27] The current study found significant associations between evening caloric intake and circulating concentrations of biomarkers of generalized inflammation. Specifically, each 10 percent increase in the proportion of daily calories consumed after 5 pm was associated with a significantly higher concentration of CRP – a biomarker that has been associated with increased risk of breast cancer, as well as a variety of chronic conditions.[28] This association between evening caloric intake and CRP was independent of potential lifestyle confounders, as well as the other dietary variables hypothesized to influence metabolic heath (e.g., nighttime fasting duration and eating frequency).

Animal and human studies have examined the impact of meal frequency on metabolism with conflicting results. In particular, although there is a wealth of evidence

from rodent models that meal skipping (a form of intermittent fasting) has favorable effects on insulin metabolism and inflammation,[13] meal frequency studies in humans have not supported these associations.[14] It is notable that in most instances, human studies were done over a short period with small sample sizes, limiting the conclusions that can be drawn. For example, a study by Rashidi and colleagues compared the effect of a diet composed of 3 meals vs. 9 snacks per day in 15 healthy men. After three weeks on these diets, men on the snacking diet had reduced fasting insulin concentration ($p<0.01$).[29] In contrast, an 8-week controlled feeding study compared the effect of repeatedly consuming 1 vs. 3 meals per day in a sample of 10 healthy adult men and women and found no difference in fasting plasma insulin concentration or proinflammatory cytokine expression with respect to meal frequency.[30] The results of the current study were similarly mixed: we found no associations between eating frequency or fasting variables and insulin resistance (HOMA-IR); however we did observe a significant protective effect of increased eating frequency on plasma CRP concentration, a measure of systemic inflammation. It is possible that the lack of association observed between eating frequency and insulin resistance may be due to measurement error in the biomarker concentrations used to construct the HOMA-IR variable (i.e., fasting insulin and glucose). Specifically, although HOMA-IR is one of the most frequently used methods of determining insulin resistance in large population-based studies, it is approximated from single fasting glucose and insulin measurements, which may have a large amount of day-to-day variability compared to the euglycemic-hyperinsulinemic clamp method (gold standard approach for measuring insulin resistance). This variability could have obscured significant relationships between eating behaviors and insulin resistance parameters.

Accumulating evidence from studies in rodents suggest that eating patterns that restrict the number of hours that food can be consumed on a given day and increase the number of hours spent fasting have favorable effects on a number of parameters related to metabolic health.[12] These rodent studies also highlight the importance of aligning fasting regimens with sleep-wake cycles.[12,31,32] In particular, fasting patterns that reduce or eliminate nighttime eating have been shown to protect against a number of disease risk factors, including hyperinsullinemia, hepatic steatosis, and inflammation.[12] Similar to the evidence from rodent studies, we found that longer nighttime fasting intervals were associated with significantly lower concentrations of CRP and non-significantly lower concentrations of insulin resistance biomarkers. However, these favorable effects of nighttime fasting on inflammation and insulin resistance biomarkers were only present among a subsample of women who ate <30% daily energy intake after 5 pm. Although the size of this subsample was relatively small and models were exploratory, these findings suggest that lengthening the nighttime fasting intervals may only be an effective disease prevention strategy when the nighttime fast is initiated early in the evening. In other words, eating late at night but skipping breakfast (which could also result in a long fasting interval) may be a biologically different behavior.

The underlying associations between meal frequency and timing variables with inflammation may involve circadian rhythm parameters. Feeding is a powerful signal (or 'zeitgeber'), influencing alignment of circadian rhythms in peripheral tissues. Data from animal models have shown the eating at the 'wrong' circadian time (e.g., late at night) can reset some peripheral clocks almost entirely, resulting in a phase shift and misalignment of daily rhythms[31-33] and increased expression of proinflammatory genes such as *TNF-α*, *IL6*, and *CXCL2.*[12] Gene knockout studies have confirmed links

between circadian disruption and inflammation: removal of the core clock component protein cryptochrome has been shown to result in constitutive elevation of proinflammatory cytokines.[34] In humans, controlled simulations of forced circadian misalignment have also demonstrated evidence of a causal link between circadian misalignment and inflammation. For example, a study by Wright and colleagues investigated the effect of forced circadian disruption in healthy men and women using a 25-day laboratory entrainment protocol. The authors of this study reported that plasma concentrations of proinflammatory proteins TNF-α and CRP were significantly higher during the period of forced circadian misalignment.[35]

Although there are numerous advantages to using 24-hour dietary recalls for measuring dietary patterns, the limitations are noteworthy. For example, 24-hour dietary recalls are prone to response biases.[36] We are not aware of any published studies, however, that have documented random error or bias in self-report of meal timing. Diets may also vary considerably from day to day, and thus our measure of nighttime fasting duration derived from a single 24-hour recall likely has considerable measurement error. Research has also demonstrated that CRP measurements are susceptible to this same type of measurement error (day-to-day within-subject variability,[37] and CRP concentrations can be elevated acutely by other factors, such as the common cold. Nevertheless, these random measurement errors would attenuate associations between dietary exposures and outcomes;[36] thus, it is likely that the parameter estimates and odd ratios presented in this study are conservative.

Strengths of this study include the diverse, population-based sample of women with extensive information on potential confounding demographic, clinical, and behavioral variables. Findings are also strengthened by the large sample size with

sufficient power to detect associations between dietary exposures and biomarker outcomes of interest.

In conclusion, results of this study suggest that reducing evening energy intake, eating more frequently, and fasting for longer nightly intervals (when fasting is initiated early in the evening) may reduce systemic inflammation in the body which could subsequently reduce breast cancer and chronic disease risk. To our knowledge, this is the first population-based study to document these associations. Adequately powered randomized trials are needed to validate whether changing these meal frequency and timing behaviors significantly influence inflammatory processes. If the current findings are confirmed, reducing evening energy intake, eating more frequently, and fasting for longer nightly intervals (when initiated early in the evening), could be recommended as simple and effective behavioral strategies for breast cancer and disease prevention.

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Table 3.1. Demographic, Lifestyle, and Dietary Characteristics of a Nationally-Representative Sample of Adult Women From the NHANES 2009-2010 Survey.

Characteristics mean (SEM) unless otherwise noted	**Eligible** n=2,212	**Excluded** n=438	**HOMA-IR**[a] n=1,034
Age	46.8 (0.7)	52.5 (0.9)	47.2 (0.7)
Ethnicity n(%)			
Non-Hispanic White	1,138 (51.4)	164 (38.1)	514 (49.7)
Non-Hispanic Black	355 (16.0)	106 (24.7)	151 (14.6)
Mexican American	379 (17.1)	93 (21.6)	195 (18.9)
Other race(s)	340 (15.4)	75 (17.4)	174 (16.8)
Education[b] n(%)			
No High School Diploma	549 (24.8)	161 (37.4)	252 (24.4)
High School Diploma	494 (22.3)	93 (21.6)	225 (21.8)
Some College	682 (30.8)	127 (29.5)	322 (31.1)
College Degree	483 (21.8)	54 (12.6)	233 (22.5)
BMI	28.2 (0.2)	33.4 (0.6)	28.4 (0.2)
Sleep (hours)	7.0 (0.0)	7.0 (0.1)	7.0 (0.1)
Daily Energy Intake (kcal/day)	1,773.1 (22.5)	1,636.6 (52.9)	1,772.5 (20.7)
Daily Energy Intake After 5pm (kcal/day)	773.7 (17.1)	677.9 (27.5)	772.0 (18.4)
Nighttime Fasting Duration (hours)	12.4 (0.1)	13.0 (0.2)	12.2 (0.1)
Number of Eating Episodes per day	4.7 (0.1)	4.4 (0.1)	4.7 (0.1)
CRP[c] **(median Q1,Q3)**	0.4 (0.6, 0.4)	0.7 (0.2, 0.8)	0.4 (0.1, 0.5)
Insulin (median Q1,Q3)	--	16.8 (9.3, 20.8)	12.1 (6.2, 15.1)
Glucose (median Q1,Q3)	--	6.8 (5.3, 7.7)	5.4 (4.9, 5.6)
HOMA-IR[a] **(median Q1,Q3)**	--	5.2 (2.4, 6.1)	3.0 (1.4, 3.7)

[a]Homeostatic Model Assessment of Insulin Resistance.
[b]Data on educational attainment was missing for 4 study participants.
[c]C-reactive protein.

Table 3.2. Associations of Eating Patterns and Lifestyle Factors with C-Reactive Protein (CRP) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) in Adult Women Participants From the NHANES 2009-2010 Survey Year.

	CRP[a] (n=2,019)	
	β (95%CI)	*p*-value
Evening Calories[b]	1.03 (1.01-1.06)	0.02
Eating Frequency	0.92 (0.86-0.99)	0.03
Nighttime Fasting Duration	1.01 (0.97-1.05)	0.70
	HOMA-IR[a] (n=1,034)	
	β (95%CI)	*p*-value
Evening Calories[b]	1.00 (0.98-1.02)	0.96
Eating Frequency	0.97 (0.92-1.03)	0.26
Nighttime Fasting Duration	1.00 (0.98-1.02)	0.98

*Models controlled for age, race/ethnicity, and education.
[a]Parameter estimates have been back transformed to reflect the percent change in each outcome (CRP and HOMA-IR) associated with a 1-unit increase in each dietary exposure variable.
[b]Calories consumed in the evening (between 5pm and midnight), divided by total energy intake.

Table 3.3. Stratified Analyses of Associations of Nighttime Fasting Duration with CRP and HOMA-IR, by Percent of Calories Consumed in the Evening[a].

	CRP[b]			
	Evening Calories < 30% (n=543)		**Evening Calories ≥ 30% (n=1476)**	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Nighttime Fasting Duration	0.92 (0.87-0.98)	.01	1.04 (1.00-1.08)	0.07
	HOMA-IR[b]			
	Evening Calories < 30% (n=295)		**Evening Calories ≥ 30% (n=739)**	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Nighttime Fasting Duration	0.98 (0.91-1.05)	0.48	1.01 (0.99-1.04)	0.91

*Models controlled for age, race/ethnicity, and education; as well as evening calories and eating frequency.

[a]Calories consumed in the evening (between 5pm and midnight), divided by total energy intake.

[b]Parameter estimates have been back transformed to reflect the percent change in each outcome (CRP and HOMA-IR) associated with a 1-unit increase in each dietary exposure variable.

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CHAPTER 4

Prolonged nightly fasting and breast cancer prognosis

CHAPTER 5

Discussion

DISCUSSION

Breast cancer morbidity and mortality have a significant impact on public health in the United States. In 2015, an estimated 231,840 women in the United States were diagnosed with, and 40,290 women died of the disease.[1] Weight loss is a generally recommended strategy for cancer prevention at the population-level, given the (1) well-established links between obesity and breast cancer (particularly among postmenopausal women),[2,3] and (2) high prevalence of obesity among US adults.[4] While data from randomized trials have demonstrated that obesity can be ameliorated in high-risk individuals by intensive lifestyle interventions,[5] obesity prevention strategies have demonstrated limited effectiveness for long-term weight reduction in "real world" settings. Therefore, the identification of novel breast cancer prevention strategies that are both feasible and effective is needed.

Prolonged Nightly Fasting and Breast Cancer

Humans evolved to be active during daylight hours and rest during darkness. But the advent of artificial lighting around the turn of the 20th century changed human rest-activity patterns immensely. Work and social schedules became extended, average sleep duration shortened, and meal consumption began to stretch into the nighttime.[6] A growing body of evidence suggests that departures from eating and sleeping in accordance with the natural light/dark cycle have contributed to a host of health problems in humans, including obesity, diabetes, cardiovascular disease, and many cancers.[7,8]

This dissertation used data from two complementary populations of women to explore the premise that eating in accordance with the 24-hour light/dark cycle, as characterized by a prolonged nightly fasting schedule, may be a novel breast cancer

prevention strategy in women. Specifically, this dissertation investigated the hypothesis that woman's usual nightly fasting duration influences multiple metabolic pathways associated with breast cancer risk and progression, and has direct effects on prognosis among women with a history of breast cancer.

To briefly summarize our findings, Chapter 2 demonstrated that nightly fasting duration was significantly and inversely associated with glycemic control biomarkers putatively associated with breast cancer in a representative sample of adult women.[9] Chapter 3 found that a longer nightly fasting duration was significantly associated with lower concentrations of systemic inflammation among women who consume less than 30% of their total daily energy intake after 5 pm.[10] Results of Chapter 4 indicate nightly fasting duration predicts breast cancer recurrence risk among survivors of early stage breast cancer.[11] Specifically, fasting less than 13 hours per night was associated with a significantly increased risk of breast cancer recurrence but was not statistically significantly associated with increased risk of breast cancer-specific or all-cause mortality. Findings from Chapter 4 also indicate that improvements in sleep duration and glycemic control may be mechanisms linking nightly fasting with improved breast cancer prognosis.

Taken together, these first-in-human data on the association of nightly fasting intervals with disease risk are supportive of the robust literature on this dietary exposure provided by rodent studies.[12-16]

Future Directions

A recommendation to increase the length of the nightly fasting interval has promise to be a simple and effective public health strategy for cancer prevention, and evidence suggests that the incorporation of a prolonged nightly fasting period into daily

eating patterns may be an achievable lifestyle change that many women can adopt. Pilot studies led by Ruth Patterson and Linda Gallo have demonstrated that a prolonged nightly fasting regimen is feasible among ethnically-diverse samples of women (Patterson RE, personal communication). Specifically, obese, postmenopausal women were enrolled in pilot studies and asked to adopt a prolonged nightly fasting dietary pattern for a 1-month period (nightly fasting goal was 12-14 hours fasting per night). A total of 10 predominantly white women were recruited in the first pilot study from a clinic in La Jolla. The second pilot study recruited 10 Latina women from a clinic in the South Bay, San Diego area. Overall, women in these pilot studies increased their mean nightly fasting duration by 22% over the 1-month intervention period. According to the combined sample, participants fasted 12 or more hours on 96% of all nights, 13 or more hours on 46% of nights, and 14 or more hours on 18% of nights. Importantly, 90% of participants in the studies reported feeling that the intervention was simple and acceptable. Collectively, results of these pilot studies indicate that a prolonged nightly fasting intervention is feasible and has the potential to reach broad populations of women.

Although our data suggest numerous positive effects of prolonged nightly fasting on health and disease outcomes, a number of questions remain. For example, there are insufficient data to determine the minimal and optimal frequency and duration of fasting for improving metabolic health and reducing disease risk; or whether there is an ideal window of time that an individual must initiate the fast (e.g., between 5-7 pm) to maximize the health benefits of nightly fasting. Randomized trials comparing different nightly fasting and nighttime dietary restriction regimens are needed to determine the optimal nightly fasting regimen for improving metabolic health and modulating breast cancer risk. These randomized trials should include objective

assessments of hypothesized intermediate outcomes, such as objectively-measured sleep, physical activity, and circadian rhythm parameters. These trials should also collect blood specimens at multiple study time points to quantify circulating concentrations of biomarkers with known relationships to breast cancer. Data from these intervention studies will permit a better understanding of role that nightly fasting may have in breast cancer risk and prognosis.

Conclusions

Given the individual and societal cost of breast cancer, it is an important goal to identify feasible lifestyle interventions that can reduce breast cancer risk and improve breast cancer prognosis. Findings from this dissertation support the hypothesis that a prolonged nightly fasting regimen could be a public health strategy to reduce breast cancer incidence and reduce the risk of breast cancer recurrence among women with a history of early stage breast cancer. A prolonged nightly fasting intervention could also potentially aid in the prevention of other cancers, as well as metabolic conditions that have etiologic ties to cancers, such as type 2 diabetes mellitus. If supported by randomized trials, prolonged nightly fasting could be an innovative strategy for improving health and preventing disease at the population level.

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