Research Article

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Prolonged Nightly Fasting and Breast Cancer Risk: Findings from NHANES (2009–2010)

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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 $\geq$ 39 mmol/mol or 5.7%) and elevated 2-hour glucose (glucose $\geq$ 140 mg/dL). All models adjusted for age, education, race/ethnicity, body mass index, total kcal intake, evening kcal intake, and the number of eating episodes per day.

Results: Each 3-hour increase in nighttime fasting (roughly 1 SD) was associated with a 4% lower 2-hour glucose measurement [β, 0.96; 95% confidence interval (CI), 0.93–1.00; $P < 0.05$], and a nonstatistically 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 nonsignificantly reduced odds of elevated 2-hour glucose.

Conclusions: A longer nighttime 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. *Cancer Epidemiol Biomarkers Prev; 24(5); 783–9. ©2015 AACR.*

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 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 indicate 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 II diabetes mellitus have approximately a 23% higher risk of developing breast cancer [RR, 1.23; 95% confidence interval (CI), 1.12–1.34], and a 38% higher risk of breast cancer mortality (RR, 1.38; 95% CI, 1.20–1.58) compared with women without type II diabetes (3). Although the biologic 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 14 published studies using glycosylated hemoglobin (HbA1c) as a biomarker of hyperglycemia found a positive linear relationship between HbA1c and cancer incidence (7). Furthermore, a prospective case–control study of hormones and diet in the etiology of breast tumors (ORDET Study) found that the RR of breast cancer was more than 60% greater for women in the highest versus lowest quartile of fasting glucose (RR, 1.63; 95% CI, 1.14–2.32; ref. 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 (upward 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

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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 2-week intervention period, compared with the control condition ($P < 0.05$; ref. 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 article 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 NHANES; ref. 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 prediabetic and diabetic ranges, and better glucose tolerance during an oral glucose tolerance test.

Materials and Methods

Study sample

Data for this study were obtained from the 2009–2010 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 noninstitutionalized 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; ref. 24).

This 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 to 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. The USDA's Food Surveys Research Group 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-g 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, MN).

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 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, 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 (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. On the basis of the nonnormal 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 χ^2 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, body mass index (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 SD 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 $\geq$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. All analyses used sample weights to account for differential probabilities of selection into the sample, nonresponse, and noncoverage. SEs 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 five 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 SD) 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; 95% CI, 0.93–1.00; $P = 0.04$), and 6% decrease in the subsample of True Reporters (β, 0.94; 95% 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.

Table 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 h) $n = 770$	Fasting tertile 2 (11.5–13.49 h) $n = 746$	Fasting tertile 3 (≥13.5 h) $n = 770$	*P* 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 (h/night)	7.0 (0.0)	7.0 (0.1)	7.17 (0.1)	6.9 (0.1)	0.40
Daily energy intake (kcal/d)	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/d)	51.4 (5.6)	113.8 (12.2)	21.4 (3.5)	23.3 (5.4)	<0.001
Nighttime fasting duration (h)	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
Two-hour glucose[d] (mg/dL; median Q1 and 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 were missing for 4 study participants.
[c]Glycosylated hemoglobin.
[d]Concentration of glucose in plasma collected 2 hours after a 75-g oral glucose-equivalent challenge (oral glucose tolerance test). Two-hour glucose data were only available for the subsample of fasting participants ($n = 1,334$).

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, multiethnic, population-based sample of adult women, a 3-hour increase in nighttime fasting was associated with an approximate 20% lower likelihood of having HbA1c

Table 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*	β (95%CI)	*P*
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)	<0.001	0.16 (0.10–0.23)	<0.001
	2-hour glucose[c]			
	Full sample ($n = 1,066$)		True Reporters[b] ($n = 593$)	
	β (95%CI)	*P*	β (95%CI)	*P*
Nighttime fast duration	0.96 (0.93–1.00)	0.04	0.94 (0.90–0.98)	<0.01
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 10 pm	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)	<0.001	1.01 (1.01–1.02)	<0.001

NOTE: All models adjusted for age, education, and race/ethnicity.
[a]HbA1c presented as mmol/mol, per recommendations by the 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-g oral glucose-equivalent challenge (oral glucose tolerance test). Two-hour glucose data were only available for the subsample of fasting participants ($n = 1,334$). 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 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) OR (95% CI)	**True Reporters[b] (n = 1,334) 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) OR (95% CI)	**True Reporters[b] (n = 593) 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)

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

[a]Elevated HbA1c was defined as ≥39 mmol/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-g glucose-equivalent challenge (oral glucose tolerance test). Two-hour glucose data were available for fasting subsample of participants only (n = 1,334).

[d]Elevated 2-hour glucose was defined as ≥140 mg/dL (impaired glucose tolerance).

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 0.05 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, on the basis of 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 upward 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 II 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 that 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 proinflammatory 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 this 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/d).

The major limitation of this 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 this study indicated that the associations between nighttime fasting and glycemic control were stronger when we excluded women who over- or underreported total energy intake by 25% or more, as compared with their estimated energy needs.

Strengths of this study include use of a large, nationally representative sample of U.S. 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. Furthermore, 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.R. Marinac, L. Natarajan, S.J. Hartman, R.E. Patterson

Development of methodology: C.R. Marinac, R.E. Patterson

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.R. Marinac

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.R. Marinac, L. Natarajan, L.C. Gallo, S.J. Hartman, R.E. Patterson

Writing, review, and/or revision of the manuscript: C.R. Marinac, L. Natarajan, D.D. Sears, L.C. Gallo, S.J. Hartman, E. Arredondo, R.E. Patterson

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.R. Marinac

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