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Original Article

Obesity-related markers and breast cancer in CPS-II Nutrition Cohort

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Abstract: Low circulating levels of adiponectin and high levels of insulin-like growth factor-1 (IGF-1), C-reactive protein (CRP), and C-peptide have been shown to be related to postmenopausal breast cancer risk, and to partially mediate the obesity-postmenopausal breast cancer association; however, data from prospective studies, especially those limited to non-users of postmenopausal hormones, are sparse. To further evaluate these associations, we measured these markers in a case-control study nested in the Cancer Prevention Study-II (CPS-II) Nutrition Cohort. Plasma samples from 302 postmenopausal breast cancer cases and matched controls were analyzed. None of the women were taking postmenopausal hormones at blood draw. Multivariable-adjusted odds ratios (OR) and 95% confidence intervals (CI) were estimated using conditional logistic regression models. Low levels of total adiponectin and high levels of total IGF-1 and CRP were associated with increased breast cancer risk, but associations were not statistically significant. The association with C-peptide was statistically significant (T3 vs. T1: OR=1.63, 95% CI 1.08-2.45; *p*-value for linear trend=0.001), but was slightly attenuated after further adjustment for BMI (T3 vs. T1: OR=1.51, 95% CI 0.99-2.31; *p*-value for linear trend=0.004). The association between BMI and breast cancer risk was attenuated toward the null after controlling for C-peptide (from OR=1.43 to OR=1.25 for BMI ≥30 kg/m^2 compared to <25 kg/m^2). The elevated risk of postmenopausal breast cancer associated with higher circulating levels of C-peptide is consistent with a role of hyperinsulinemia in breast carcinogenesis, and might account for some of the higher risk associated with obesity.

Keywords: Breast cancer, obesity, C-peptide, risk

Introduction

High body mass index (BMI) is an established risk factor for postmenopausal breast cancer [1-4]; however, the biological mechanisms mediating this association are not completely elucidated. Elevated circulating estrogen concentrations due to higher levels of aromatase from adipocytes only partially explain the association between large body size and postmenopausal breast cancer. Indeed, two large prospective studies showed that the BMI-postmenopausal breast cancer link persists even after accounting for levels of blood estrogen concentrations [5, 6]. Increasingly, investigations have explored inflammation and hyperinsulinemia as possible mediators of the relationship between obesity and breast cancer risk [7]. The inflammation pathway and the insulin/insulin-like growth factor (IGF) axis are characterized by numerous proteins that can be readily measured in serum. These investigations focused primarily on obesity-related biomarkers, including total adiponectin [8] and C-reactive protein (CRP) [9] to characterize the inflammation pathway and C-peptide [10] in lieu of insulin and IGF-1 [11] to characterize the insulin/IGF-axis.

Epidemiologic studies consistently show that the association between obesity and breast cancer is limited to women who did not use postmenopausal hormones at the time BMI is assessed [12]. The excess estrogens from postmenopausal hormone use likely masks an association in the current users. A major limitation of some of the published studies that explored the mediating effects of obesity markers on the relationship between obesity and postmenopausal breast cancer risk is the inclu-

sion of women who were using postmenopausal hormones at the time of blood collection. Therefore, it is possible that some studies might have missed important relationships with these markers.

To better understand the associations of obesity-related markers and breast cancer risk, we measured plasma levels of total adiponectin, CRP, C-peptide, and total IGF-1 in a nested case-control study of women who did not use postmenopausal hormone in the American Cancer Society's Cancer Prevention Study-II (CPS-II) Nutrition Cohort. In this study, we examined the associations of each marker with risk, and whether these markers mediated associations of BMI and waist circumference with risk.

Materials and methods

Study population

Women in this analysis were drawn from the 97,786 women in the CPS-II Nutrition Cohort, a prospective study of cancer incidence and mortality established by the American Cancer Society in 1992 as a subgroup of a larger mortality study initiated in 1982 [13]. At enrollment in 1992, most of the CPS-II Nutrition Cohort participants were 50-74 years of age. Participants completed a mailed baseline questionnaire on demographic, medical, behavioral, environmental, and occupational factors. Follow-up questionnaires were sent to cohort members every two years starting in 1997 to update exposure information and to ascertain newly diagnosed cancer outcomes. Waist circumference was ascertained once as part of the 1997 follow-up survey. With the 1997 survey, study participants were provided with a tape measure and instructed to measure their waist circumference just above the navel to the nearest quarter inch, while standing, and to avoid measuring over bulky clothing. BMI was calculated from weight reported on the 1997 survey and height reported on the 1982 survey. Incident cancers diagnosed through June 30, 2007 were self-reported on follow-up questionnaires and subsequently verified by obtaining medical records or through linkage with state registries when complete medical records could not be obtained [21]. Interval deaths between the biennial questionnaires were obtained through linkage of the cohort with the National Death Index [14].

From 1998 to 2001, participants in the CPS-II Nutrition Cohort were invited to provide a blood sample at a medical facility in their community. All participants provided informed consent and completed a brief questionnaire about risk factors and parameters related to the blood collection. Non-fasting blood samples, including two 15-ml tubes containing EDTA and a 13-ml serum separator tube, were provided by 21,956 female participants. Blood samples were shipped chilled overnight to a central repository where they were fractionated and placed in liquid nitrogen freezers for long-term storage.

Subject selection

Of the 21,956 women that provided a blood sample, we excluded those who reported a history of cancer prior to blood draw (except non-melanoma skin cancer, n=3,274), were lost to follow-up (n=17), were diagnosed with another cancer prior to their breast cancer diagnosis (n=17), were not postmenopausal (n=102), and were using postmenopausal hormones at the time of blood draw (n=9,637). From this population, a total of 302 postmenopausal breast cancer cases diagnosed between blood draw and end of follow-up as of June 30, 2007 were available for analysis. Each case was matched to a randomly selected control who was alive and cancer-free at the time of the case's diagnosis and matched on race, birth date within 6 months, and blood draw date within 6 months. The latter criteria had to be relaxed to match four cases: two controls were matched to cases with birth dates within one year, one control was matched to a case with the birth date within one year and the draw date within one year, and one control was matched to a case with the birth date and draw date within one and one-half years.

Laboratory measurement

The plasma fraction of the blood samples was used for these analyses. Circulating levels of total adiponectin, C-peptide, total IGF-1, and CRP were measured in the laboratory of Dr. Michael Pollak at McGill University (Montreal, Quebec, Canada) using analyte-specific, commercially-available ELISA-based assays with the exception of total IGF-1, which was measured using an immunoassay with chemiluminescence. The identity of the samples, including quality control (QC) replicates and

Table 1. Relationships of circulating levels of analytes with blood collection parameters and select breast cancer risk factors among 302 controls, Cancer Prevention Study-II Nutrition Cohort

Characteristics	N	%	Total Adiponectin (ng/ml)		Total Insulin-like Growth Factor-1 (ng/ml)		C-Reactive Protein (ng/ml)		C-peptide (ng/ml)	
			Median	*p*-value[1]	Median	*p*-value[1]	Median	*p*-value[1]	Median	*p*-value[1]
All controls	302		13,239		99		1,801		3.9	
Age (years) at blood draw										
<65	59	19.5	12,773		106		1,808		3.3	
65-69	64	21.2	11,825		109		1,597		3.8	
70-74	106	35.1	13,281		94		1,655		4.0	
75+	73	24.2	14,015	0.08	96	0.04	1,957	0.85	4.6	0.07
Hours since last meal										
<2	156	51.7	13,273		98		1,909		4.4	
2-4	118	39.1	13,290		98		1,793		3.6	
5+	17	5.6	11,493	0.73	114	0.52	1,741	0.88	2.6	0.01
Unknown	11	3.6	12,115		116		708		4.8	
Any alcohol consumed in 24 hours prior to blood draw										
No	216	71.5	12,616		98		1,869		4.1	
Yes	75	24.8	14,423	0.02	103	0.19	1,587	0.55	3.7	0.06
Missing	11	3.6	12,115		116		708		4.8	
Usual alcohol consumption										
Not current drinker	150	49.7	12,434		97		1,841		3.9	
<1 drink/day	113	37.4	13,397		103		1,642		4.1	
1+ drink(s)/day	22	7.3	14,010	0.46	102	0.08	1,502	0.32	3.9	0.93
Missing	17	5.6	12,583		92		2,323		3.9	
Diagnosis of diabetes										
No	273	90.4	13,303		99		1,655		3.8	
Yes	19	6.3	8,999	0.01	112	0.27	2,697	0.01	5.3	0.01
Missing	10	3.3	13,422		74		2,359		4.4	
Family history of breast cancer										
Yes	56	18.5	15,185		97		1,565		3.5	
No	246	81.5	12,493	0.005	99	0.73	1,818	0.42	4.1	0.14
Body mass index (kg/m^2)										
<25	141	46.7	14,551		104		1,046		3.4	
25-<30	71	23.5	11,650		94		2,219		4.5	
≥30	55	18.2	10,440	0.001	96	0.21	4,161	<0.001	4.6	0.02
Missing	35	11.6	14,279		98		1,597		4.4	
Weight change (lbs) from age 18 to 1997										
Loss >5	21	7.0	18,560		92		609		2.8	
Loss 5 - Gain 5	31	10.3	15,635		96		844		3.0	
Gain 6-20	50	16.6	14,263		113		1,003		3.7	
Gain 21-40	92	30.5	12,118		101		1,801		4.4	
Gain 41-60	29	9.6	9,812		112		3,024		4.0	
Gain 61+	42	13.9	10,263	<0.001	98	0.47	4,222	<0.001	5.2	<0.001
Missing	37	12.3	14369		96		1,830		4.4	
Waist circumference (cm)										
<80	94	31.1	15,428		102		907		3.2	
80-88	67	22.2	13,273		102		1,912		3.9	

>89	116	38.4	11,185	<0.001	98	0.45	2,792	<0.001	5.1	<0.001
Missing	25	8.3	13,278		96		1,660		4.0	
Recreation physical activity (MET hours/week)										
0	18	6.0	12,835		91		1,815		5.1	
0.1-6.9	65	21.5	12,773		109		2,476		4.1	
7.0-17.5	141	46.7	13,443		97		1,601		3.9	
17.6-24.4	27	8.9	15,656		110		1,801		2.8	
≥24.5	27	8.9	10,065	0.05	97	0.74	1,501	0.15	4.2	0.11
Missing	24	7.9	13,909		98		1,724		3.9	

[1]*p*-value calculated using Wilcoxon rank sum test if the risk factor had two categories and using the Kruskal-Wallis test if the risk factor had three or more categories.

case-control status, were blinded to the laboratory personnel. Each batch contained 37 samples including three QCs of which two were identical and one was a replicate of QC samples included in another batch. The between-batch coefficient of variation (CVs) and intraclass correlation coefficients (ICCs) for each analyte were <10% and >98%, respectively. Each case and her matched control were assayed in the same batch.

Statistical approach

Five sets of cases and controls were excluded from analysis of CRP (297 cases and 297 controls remained in the analytical dataset for CRP) because at least one subject in the pair had CRP values exceeding 40,000 ng/ml, the clinical cut-point for an acute inflammatory response [15]. Included in analyses of total adiponectin, total IGF-1, and C-peptide were 302 case-control pairs. Total adiponectin, C-peptide, total IGF-1, and CRP values were categorized based on tertiles of the control distribution.

Odds ratios (OR) and 95% confidence intervals (CI) were estimated using multivariable logistic regression models, conditional on the matched sets. Potential confounders that were related to at least one analyte in the control population and associated with breast cancer risk in the nested case-control study were included in the parsimonious multivariable model, including time from last meal to blood draw, alcohol in the 24 hours before blood draw, prior diagnosis of diabetes, and family history of breast cancer. Other covariates considered but not included in the parsimonious model were measures of time of blood draw (season of blood draw and hour of blood draw) and known or suspected breast cancer risk factors (history of benign breast disease, former use of postmenopausal hormones, age at menopause, age at first birth and parity, age at menarche, use of oral contraceptives, and usual alcohol consumption). We additionally considered whether these biomarkers were associated with breast cancer risk independent of potential upstream determinants of the biomarkers, by adding recreational physical activity, waist circumference, BMI (calculated as weight (kg) / height (m) squared), and adult weight change individually to the parsimonious multivariable model. Lastly, we examined the final multivariable model with BMI and the four biomarkers under study simultaneously. To examine associations of breast cancer risk with BMI and waist circumference, unconditional logistic regression models were adjusted for age, education, age at menarche, age at first birth, parity, age at menopause, active smoking status, family history of breast cancer in a sister or mother, history of breast cysts, alcohol consumption, and recent mammogram. Linear tests of trend for the analytes were conducted by modeling the continuous variables.

Sensitivity analyses were conducted to assess whether undiagnosed breast cancer might affect analyte values by excluding breast cancer cases (and matched controls) diagnosed less than one year after blood collection (n=47 pairs) and to assess the influence of insulin usage by excluding women who reported taking insulin at the time of blood draw (n=10 pairs).

Results

Relationships with analytes among controls

Among controls, the mean and standard deviation of the biomarkers were 14,273 ± 7,094

Table 2. Multivariable-adjusted[1] odds ratios (OR) and 95% confidence intervals (CI) of the association of biomarkers with breast cancer risk among postmenopausal women who did not use postmenopausal hormones at the time of blood collection, Cancer Prevention Study-II Nutrition Cohort

Analytes	Case N (%)	Control N (%)	Multivariable-adjusted[1] OR (95% CI)	Body Mass Index + Multivariate-adjusted[1] OR (95% CI)
Total adiponectin (ng/ml)				
3,896-10,095	97 (32.1)	100 (33.2)	1.00	1.00
>10,095-15,627	112 (37.1)	101 (33.4)	1.02 (0.67, 1.55)	1.01 (0.66, 1.53)
>15,627-51,376	93 (30.8)	101 (33.4)	0.81 (0.52, 1.24)	0.84 (0.54, 1.30)
p for trend[2]			0.26	0.38
Total insulin-like growth factor-1 (ng/ml)				
39.4-88.5	93 (30.8)	101 (33.4)	1.00	1.00
>88.5-114.1	93 (30.8)	100 (33.2)	0.98 (0.64, 1.51)	1.07 (0.69, 1.66)
>114.1-198	116 (38.4)	101 (33.4)	1.21 (0.81, 1.82)	1.28 (0.85, 1.93)
p for trend[2]			0.12	0.08
C-reactive protein (ng/ml)				
107-1,076	91 (30.6)	99 (33.3)	1.00	1.00
>1,076-2,669	100 (33.7)	99 (33.3)	1.08 (0.71, 1.64)	1.03 (0.67, 1.60)
>2,669-28,647	106 (35.7)	99 (33.3)	1.19 (0.79, 1.79)	1.09 (0.70, 1.70)
p for trend[2]			0.10	0.16
C-peptide (ng/ml)				
0.90-3.09	82 (27.2)	102 (33.8)	1.00	1.00
>3.09-5.15	92 (30.6)	99 (32.8)	1.22 (0.80, 1.85)	1.17 (0.77, 1.80)
>5.15-13.55	127 (42.2)	101 (33.4)	1.63 (1.08, 2.45)	1.51 (0.99, 2.31)
p for trend[2]			0.001	0.004

[1]The conditional logistic regression models included cases and controls matched on age and race and adjusted for time from last meal to blood draw, alcohol in the 24 hours before blood draw, prior diagnosis of diabetes, and family history of breast cancer. [2]p for trend was calculated using the continuous variable.

ng/ml for adiponectin, 103.7 ± 30.6 ng/ml for total IGF-1, 2,912 ± 3,408 ng/ml for CRP, and 4.56 ± 2.43 ng/ml for C-peptide. There were weak to no pairwise correlations for total adiponectin, total IGF-1, CRP, and C-peptide (Pearson partial correlation coefficients ranged from -0.30 to 0.16; results not shown). IGF-1 levels were lowest among older women (p=0.04) but age was not related to levels of total adiponectin, CRP, or C-peptide (**Table 1**). C-peptide levels significantly varied by time since last meal with lowest levels in women fasting for five hours (p=0.01). Women who consumed alcohol 24 hours prior to blood collection had higher levels of adiponectin compared to women who abstained (p=0.02); however, usual alcohol consumption was not associated with adiponectin levels (p=0.46). Women with type 2 diabetes had lower levels of adiponectin (p=0.01) and higher levels of CRP (p=0.01) and C-peptide (p=0.01) than women without diabetes. Women with a family history of breast cancer had higher levels of adiponectin (p=0.005). BMI at baseline, weight gain from age 18 to age in 1997, and waist circumference were inversely associated with adiponectin, but positively associated with CRP and C-peptide. IGF-1 was not associated with any anthropometric factor. MET-hours per week of physical activity was not associated with any of the analytes.

Associations of breast cancer with obesity markers

In the multivariable-adjusted model, there were suggestions of associations of low levels of total adiponectin and high levels of total IGF-1, and CRP, with breast cancer risk but results were not statistically significant (**Table 2**). Women with C-peptide levels in the highest tertile compared to the lowest tertile had a 63% higher risk of breast cancer (OR=1.63, 95% CI 1.08-2.45; p for trend=0.001).

Table 3. Multivariable-adjusted odds ratios (OR) and 95% confidence intervals (CI) of the association of body size variables with breast cancer risk among postmenopausal women who did not use postmenopausal hormones at the time of blood collection, Cancer Prevention Study-II Nutrition Cohort

Body Size Variables	Case N (%)	Control N (%)	Multivariable-adjusted[1] OR (95% CI)	C-peptide + Multivariable-adjusted[1] OR (95% CI)
Body mass index (kg/m^2)				
<25.0	121 (44.3)	141 (52.8)	1.00	1.00
25.0-29.9	94 (34.4)	71 (26.6)	1.62 (1.07, 2.46)	1.48 (0.97, 2.26)
≥30.0	58 (21.2)	55 (20.6)	1.43 (0.88, 2.32)	1.25 (0.76, 2.06)
p for trend[2]			0.21	0.51
Per 1 kg/m^2 unit			1.02 (0.99, 1.06)	1.01 (0.98, 1.05)
Waist circumference (cm)				
<80.0	78 (28.0)	94 (33.9)	1.00	1.00
80.0-88.9	73 (26.2)	67 (24.2)	1.38 (0.86, 2.23)	1.24 (0.76, 2.01)
≥89.0	128 (45.9)	116 (41.9)	1.56 (1.02, 2.37)	1.31 (0.84, 2.05)
p for trend[2]			0.02	0.13
Per 10 cm			1.17 (1.02, 1.34)	1.12 (0.97, 1.29)

[1]The unconditional logistic regression models were adjusted for age, education (≤ high school graduate, some college, college graduate, missing), age at menarche (<12, 12, 13, ≥14 years, missing), age at first birth and parity (nulliparous, age <25 and 1-2 births, age <25 and 3+ births, age ≥25 and 1-2 births, age ≥25 and 3+ births, missing) age at menopause (<50, 50-54, 55+, missing), active smoking (never, current, former, missing), family history of breast cancer in a sister or mother (yes, no), history of breast cysts (yes, no), alcohol consumption (not current drinkers, <1 drink/day, 1+ drinks/day, missing), and recent mammogram (never or not recent, in last 2 years, unknown). [2]p for trend was calculated using the continuous variable.

Associations of adiponectin, IGF-1, and CRP with breast cancer risk still did not reach the level of statistical significance after controlling for BMI. Additional control for BMI slightly attenuated the association between C-peptide levels and breast cancer risk, but the association was still statistically significant (p for trend=0.004; **Table 2**). Further adjustment for the other markers under study only slightly attenuated the association between C-peptide levels and breast cancer risk (T3 vs. T1: OR=1.44, 95% CI 0.90-2.29; *p*-value for linear trend=0.10; data not otherwise shown).

The association between C-peptide levels and breast cancer risk appeared slightly stronger among women aged 70 years or older (T3 vs. T1: OR=2.06, 95% CI 1.16-3.65) compared to women younger than 70 years (T3 vs. T1: OR=1.49, 95% CI 0.75-2.96), although this difference was not statistically significant (p for interaction: 0.43; data not otherwise shown).

Results were similar to those in **Table 2** after removal of women diagnosed with breast cancer within one year of blood collection and after removal of women using insulin at the time of blood collection (data not shown).

Associations of breast cancer with anthropometric factors

In this analytical dataset, BMI in a range of 25.0 to 29.9 kg/m^2 and ≥30.0 kg/m^2, compared to BMI <25.0 kg/m^2, were associated with higher risk of breast cancer, although the results for BMI ≥30.0 kg/m^2 were not statistically significant (**Table 3**). The highest tertile of waist circumference, compared to the lowest, was positively associated with risk of breast cancer. Controlling for C-peptide levels attenuated the magnitude and strength of the associations with BMI and waist circumference (**Table 3**).

Discussion

In our study of postmenopausal women who were not using exogenous hormones at the time of blood collection, circulating C-peptide levels were statistically significantly positively associated with subsequent risk of breast cancer. Although controlling for BMI slightly attenuated the breast cancer association, the magnitude of the relative risk with C-peptide still suggested a moderate relationship. C-peptide only explained a portion of the associations of BMI and waist circumference with breast can-

cer risk. Low levels of total adiponectin and high levels of total IGF-1 and CRP also were associated, albeit not statistically significantly, with breast cancer risk.

Although our results for adiponectin, IGF-1, and CRP were not statistically significant, the magnitude of the relative risks was consistent with previous findings. In the largest prospective cohort study published to date, including approximately 226 postmenopausal cases and 501 controls who never used postmenopausal hormones, the BMI-adjusted relative risk for the highest versus lowest quartile of serum levels of adiponectin was 0.57 (95% CI 0.35-0.93); no association was detected in ever postmenopausal hormone users [8]. Although two other prospective studies failed to detect an association, their results were not stratified by postmenopausal hormone use [16, 17]. Given that adiponectin has potent insulin-sensitizing effects [18] and is expressed with its receptors in breast epithelial cells [19-22], additional epidemiology studies with prospectively collected blood are needed to further evaluate its association with postmenopausal breast cancer risk. Consistent with its mitogenic and anti-apoptotic activity [23, 24], a recent pooled analysis of 17 prospective studies showed a positive association between IGF-I and the risk of breast cancer in postmenopausal women (Q5 vs. Q1: RR=1.33, 95% CI 1.14-1.55, p for trend=0.0002) [11]. Obese women have higher levels of CRP than normal weight women [25]. In a meta-analysis of five prospective studies with 1,240 cases, a log unit increase in CRP levels, controlled for BMI, was associated with a borderline, 1.10-fold increase in postmenopausal breast cancer risk (OR=1.10, 95% CI 0.97-1.26); none of the studies included in the meta-analysis excluded postmenopausal hormone users [9].

Unlike the positive association between breast cancer risk and C-peptide levels observed in the CPS-II Nutrition Cohort, most other prospective studies showed no association overall [10, 16, 26, 27]. However, results from two studies that specifically examined older postmenopausal women showed high C-peptide levels were associated with greater risk of breast cancer [10, 16]. In the largest study to date, which was conducted within the European Prospective Investigation into Cancer and Nutrition cohort and included 1,141 breast cancer cases and 2,204 matched controls, high C-peptide levels were not associated with breast cancer risk overall; whereas, a positive association was observed among women over age 60 years (Q5 vs. Q1: OR=1.69, 95% CI 0.97-2.95) [10]. Similarly, in a Swedish cohort, C-peptide levels were not associated with risk among women <55 years, but were associated with a statistically non-significant higher risk among women 55 years or older (T3 vs. T1: OR=1.32, 95% CI 0.84-2.05) [16]. These observations suggest that the association of breast cancer with C-peptide might be limited to older women. Alternatively, the association might only be observable in women who do not use postmenopausal hormones as our study was restricted to non-users of postmenopausal hormones, while in other studies older women would be less likely than younger women to be using hormones at blood collection.

Although C-peptide has no biological function of its own [28, 29], it has a longer half-life than insulin [30], and therefore provides a better measure of insulin secretion from the pancreas when specimens were not collected under fasting conditions [31-33]. Insulin is a growth factor for a wide range of tissues, including the breast, and plays a significant role in normal breast development [34]. Specifically, binding of insulin to its receptor can promote cell proliferation by activation of the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI-3K) pathways [23, 24]. In addition, insulin increases the bioavailability of estrogen by down-regulating hepatic sex hormone binding protein synthesis and can sensitize the estrogen receptor to estrogen thereby enhancing its activity [35]. The results for C-peptide are consistent with the moderate association between diabetes and postmenopausal breast cancer risk [36], and particularly with recent evidence that women are at highest risk of breast cancer in the years leading up to diabetes mellitus diagnosis [37], when insulin and C-peptide levels would be the highest.

Although smaller cohort studies failed to detect an association [38-40], in the Women's Health Initiative, the largest study to-date with prospectively-collected specimens from fasting women not using postmenopaual hormones, high levels of insulin were associated with a two-fold higher in breast cancer risk compared to the lowest level, independent of estradiol

[41]. In aggregate, results from studies with prospectively collected blood samples suggest that hyperinsulinemia, measured directly by circulating levels of fasting insulin or indirectly by C-peptide, is associated with postmenopausal breast cancer.

In our study, the association between BMI and breast cancer risk was only modestly attenuated by adjustment for C-peptide levels. This result supports the hypothesis that excess weight might increase risk of postmenopausal breast cancer partly through hyperinsulinemia and partly through other mechanisms.

Our study benefitted from a restricted study population of non-hormone users at the time of blood collection, which might have provided additional power compared to similarly sized studies. While we only used a single baseline measurement, prior evidence indicates that the markers of interest exhibit sufficient temporal stability to be reliable indicators of long-term exposure. For example, we previously found adiponectin varied only by 11% over a 2-3 year period, based on data from a cohort of postmenopausal women, most aged 51 to 70 years, with blood samples collected and stored in a manner similar to the CPS-II samples [17]. Also, several other studies have shown that levels of IGF-1 [38, 42, 43], CRP [44], and C-peptide [38, 45] in women are stable over several time points, suggesting that their measurement at one time point should not impact our observed results. In our study, we measured total IGF-1 and total adiponectin; it is possible that stronger associations might be observed for the biologically-active fractions of these molecules, namely free IGF-1 [46] and high molecular weight adiponectin [47].

The results from the CPS-II Nutrition Cohort provide additional evidence that hyperinsulinemia, as measured by circulating C-peptide, is associated with risk of postmenopausal breast cancer. Interventions to reduce hyperinsulinemia might be effective strategies for lowering risk of postmenopausal breast cancer. However, our results also suggest that additional research is needed to identify other metabolic perturbations caused by obesity that increase risk of postmenopausal breast cancer.

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References

[1] Morimoto LM, White E, Chen Z, Chlebowski RT, Hays J, Kuller L, Lopez AM, Manson J, Margolis KL, Muti PC, Stefanick ML and McTiernan A. Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control 2002; 13: 741-751.

[2] Macinnis RJ, English DR, Gertig DM, Hopper JL and Giles GG. Body size and composition and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev 2004; 13: 2117-2125.

[3] Lahmann PH, Hoffmann K, Allen N, van Gils CH, Khaw KT, Tehard B, Berrino F, Tjonneland A, Bigaard J, Olsen A, Overvad K, Clavel-Chapelon F, Nagel G, Boeing H, Trichopoulos D, Economou G, Bellos G, Palli D, Tumino R, Panico S, Sacerdote C, Krogh V, Peeters PH, Bueno-de-Mesquita HB, Lund E, Ardanaz E, Amiano P, Pera G, Quiros JR, Martinez C, Tormo MJ, Wirfalt E, Berglund G, Hallmans G, Key TJ, Reeves G, Bingham S, Norat T, Biessy C, Kaaks R and Riboli E. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer 2004; 111: 762-771.

[4] Feigelson HS, Jonas CR, Teras LR, Thun MJ and Calle EE. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2004; 13: 220-224.

[5] Rinaldi S, Key TJ, Peeters PH, Lahmann PH, Lukanova A, Dossus L, Biessy C, Vineis P, Sacer-

dote C, Berrino F, Panico S, Tumino R, Palli D, Nagel G, Linseisen J, Boeing H, Roddam A, Bingham S, Khaw KT, Chloptios J, Trichopoulou A, Trichopoulos D, Tehard B, Clavel-Chapelon F, Gonzalez CA, Larranaga N, Barricarte A, Quiros JR, Chirlaque MD, Martinez C, Monninkhof E, Grobbee DE, Bueno-de-Mesquita HB, Ferrari P, Slimani N, Riboli E and Kaaks R. Anthropometric measures, endogenous sex steroids and breast cancer risk in postmenopausal women: a study within the EPIC cohort. Int J Cancer 2006; 118: 2832-2839.

[6] Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson HE Jr, Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB and Miller SR. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst 2003; 95: 1218-1226.

[7] Renehan AG, Frystyk J and Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol Metab 2006; 17: 328-336.

[8] Tworoger SS, Eliassen AH, Kelesidis T, Colditz GA, Willett WC, Mantzoros CS and Hankinson SE. Plasma adiponectin concentrations and risk of incident breast cancer. J Clin Endocrinol Metab 2007; 92: 1510-1516.

[9] Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, Ben-Shlomo Y, Ebrahim S and Lawlor DA. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control 2009; 20: 15-26.

[10] Verheus M, Peeters PH, Rinaldi S, Dossus L, Biessy C, Olsen A, Tjonneland A, Overvad K, Jeppesen M, Clavel-Chapelon F, Tehard B, Nagel G, Linseisen J, Boeing H, Lahmann PH, Arvaniti A, Psaltopoulou T, Trichopoulou A, Palli D, Tumino R, Panico S, Sacerdote C, Sieri S, van Gils CH, Bueno-de-Mesquita BH, Gonzalez CA, Ardanaz E, Larranaga N, Garcia CM, Navarro C, Quiros JR, Key T, Allen N, Bingham S, Khaw KT, Slimani N, Riboli E and Kaaks R. Serum C-peptide levels and breast cancer risk: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 2006; 119: 659-667.

[11] Endogenous Hormones and Breast Cancer Collaborative Group; Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010; 11: 530-42.

[12] Calle EE and Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004; 4: 579-591.

[13] Calle EE, Rodriguez C, Jacobs EJ, Almon ML, Chao A, McCullough ML, Feigelson HS and Thun MJ. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. Cancer 2002; 94: 2490-2501.

[14] Calle EE and Terrell DD. Utility of the National Death Index for ascertainment of mortality among cancer prevention study II participants. Am J Epidemiol 1993; 137: 235-241.

[15] Clyne B and Olshaker JS. The C-reactive protein. J Emerg Med 1999; 17: 1019-1025.

[16] Cust AE, Stocks T, Lukanova A, Lundin E, Hallmans G, Kaaks R, Jonsson H and Stattin P. The influence of overweight and insulin resistance on breast cancer risk and tumour stage at diagnosis: a prospective study. Breast Cancer Res Treat 2009; 113: 567-576.

[17] Gaudet MM, Falk RT, Gierach GL, Lacey JV Jr, Graubard BI, Dorgan JF and Brinton LA. Do adipokines underlie the association between known risk factors and breast cancer among a cohort of United States women? Cancer Epidemiol 2010; 34: 580-586.

[18] Rabe K, Lehrke M, Parhofer KG and Broedl UC. Adipokines and insulin resistance. Mol Med 2008; 14: 741-751.

[19] Martin LJ, Woo JG, Geraghty SR, Altaye M, Davidson BS, Banach W, Dolan LM, Ruiz-Palacios GM and Morrow AL. Adiponectin is present in human milk and is associated with maternal factors. Am J Clin Nutr 2006; 83: 1106-1111.

[20] Weyermann M, Brenner H and Rothenbacher D. Adipokines in human milk and risk of overweight in early childhood: a prospective cohort study. Epidemiology 2007; 18: 722-729.

[21] Takahata C, Miyoshi Y, Irahara N, Taguchi T, Tamaki Y and Noguchi S. Demonstration of adiponectin receptors 1 and 2 mRNA expression in human breast cancer cells. Cancer Lett 2007; 250: 229-236.

[22] Treeck O, Lattrich C, Juhasz-Boess I, Buchholz S, Pfeiler G and Ortmann O. Adiponectin differentially affects gene expression in human mammary epithelial and breast cancer cells. Br J Cancer 2008; 99: 1246-1250.

[23] Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008; 8: 915-928.

[24] Pollak MN, Schernhammer ES and Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004; 4: 505-518.

[25] Yudkin JS, Stehouwer CD, Emeis JJ and Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999; 19: 972-978.

[26] Eliassen AH, Tworoger SS, Mantzoros CS, Pollak MN and Hankinson SE. Circulating insulin and c-peptide levels and risk of breast cancer among predominately premenopausal women. Cancer Epidemiol Biomarkers Prev 2007; 16: 161-164.

[27] Toniolo P, Bruning PF, Akhmedkhanov A, Bonfrer JM, Koenig KL, Lukanova A, Shore RE and Zeleniuch-Jacquotte A. Serum insulin-like growth factor-I and breast cancer. Int J Cancer 2000; 88: 828-832.

[28] Johansson BL, Kernell A, Sjoberg S and Wahren J. Influence of combined C-peptide and insulin administration on renal function and metabolic control in diabetes type 1. J Clin Endocrinol Metab 1993; 77: 976-981.

[29] Tillil H, Frank BH, Pekar AH, Broelsch C, Rubenstein AH and Polonsky KS. Hypoglycemic potency and metabolic clearance rate of intravenously administered human proinsulin and metabolites. Endocrinology 1990; 127: 2418-2422.

[30] Faber OK, Hagen C, Binder C, Markussen J, Naithani VK, Blix PM, Kuzuya H, Horwitz DL, Rubenstein AH and Rossing N. Kinetics of human connecting peptide in normal and diabetic subjects. J Clin Invest 1978; 62: 197-203.

[31] Bonser AM and Garcia-Webb P. C-peptide measurement: methods and clinical utility. Crit Rev Clin Lab Sci 1984; 19: 297-352.

[32] Hovorka R and Jones RH. How to measure insulin secretion. Diabetes Metab Rev 1994; 10: 91-117.

[33] Clark PM. Assays for insulin, proinsulin(s) and C-peptide. Ann Clin Biochem 1999; 36: 541-564.

[34] Rosenfeld RG. Insulin-like growth factors and the basis of growth. N Engl J Med 2003; 349: 2184-2186.

[35] Pasquali R, Vicennati V, Bertazzo D, Casimirri F, Pascal G, Tortelli O and Labate AM. Determinants of sex hormone-binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. Virgilio-Menopause-Health Group. Metabolism 1997; 46: 5-9.

[36] Larsson SC, Mantzoros CS and Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer 2007; 121: 856-862.

[37] Onitilo AA, Stankowski RV, Berg RL, Engel JM, Glurich I, Williams GM and Doi SA. Breast cancer incidence before and after diagnosis of type 2 diabetes mellitus in women: increased risk in the prediabetes phase. Eur J Cancer Prev 2013; [Epub ahead of print].

[38] Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, Ram M, Freudenheim JL, Sieri S, Trevisan M and Berrino F. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2002; 11: 1361-1368.

[39] Kaaks R, Lundin E, Rinaldi S, Manjer J, Biessy C, Soderberg S, Lenner P, Janzon L, Riboli E, Berglund G and Hallmans G. Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden. Cancer Causes Control 2002; 13: 307-316.

[40] Mink PJ, Shahar E, Rosamond WD, Alberg AJ and Folsom AR. Serum insulin and glucose levels and breast cancer incidence: the atherosclerosis risk in communities study. Am J Epidemiol 2002; 156: 349-352.

[41] Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, Li J, Ho GY, Xue X, Anderson GL, Kaplan RC, Harris TG, Howard BV, Wylie-Rosett J, Burk RD and Strickler HD. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2009; 101: 48-60.

[42] Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN and Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev 2006; 15: 972-978.

[43] Chia VM, Newcomb PA, White E, Zheng Y, Potter JD and Lampe JW. Reproducibility of serum leptin, insulin-like growth factor-I, and insulin-like growth factor-binding protein-3 measurements. Horm Res 2008; 69: 295-300.

[44] Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G and Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem 2001; 47: 444-450.

[45] Stocks T, Lukanova A, Rinaldi S, Biessy C, Dossus L, Lindahl B, Hallmans G, Kaaks R and Stattin P. Insulin resistance is inversely related to prostate cancer: a prospective study in Northern Sweden. Int J Cancer 2007; 120: 2678-2686.

[46] Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Muller J and Skakkebaek NE. Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. J Clin Endocrinol Metab 1997; 82: 2497-2502.

[47] Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M,

Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA and Scherer PE. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004; 279: 12152-12162.