Body mass and DNA promoter methylation in breast tumors in the Western New York Exposures and Breast Cancer Study[1–3]

Meng-Hua Tao, Catalin Marian, Jing Nie, Christine Ambrosone, Shiva S Krishnan, Stephen B Edge, Maurizio Trevisan, Peter G Shields, and Jo L Freudenheim

ABSTRACT

Background: The mechanism of the observed association between body mass, particularly centralized body fat, and postmenopausal breast cancer risk is not well understood.

Objective: We hypothesized that body mass may affect DNA methylation through increased estrogen and chronic inflammation. The association between body mass and promoter methylation in breast tumors was investigated in a population-based, case-control study.

Design: The promoter methylation of *E-cadherin*, *p16*, and *RAR-*β_2 genes was assessed in breast tumor blocks from 803 pre- and postmenopausal cases by using real-time methylation-specific polymerase chain reaction. Unconditional logistic regression was used to derive the adjusted OR and 95% CI for case-case comparisons of tumors with and without promoter methylation of the genes.

Results: The frequency of promoter methylation was 20% for *E-cadherin*, 25.9% for *p16*, and 27.5% for *RAR-*β_2. There was no difference in the prevalence of the DNA methylation of individual genes by BMI, waist-to-hip ratio (WHR), or lifetime weight change between the age of 20 y and the present. However, in a case-case comparison of postmenopausal breast cancer, a greater WHR was associated with an increased likelihood of $\geq$1 of the 3 genes being methylated (OR: 1.85; 95% CI: 1.10, 3.11; *P*-trend $<$ 0.02).

Conclusions: We showed that WHR was associated with DNA promoter methylation of $\geq$1 of 3 genes in postmenopausal breast tumors. It may be that the association of body fat composition and postmenopausal breast cancer is related to altered DNA methylation. However, future studies in other populations and with an examination of the methylation of more genes are needed. *Am J Clin Nutr* 2011;94:831–8.

INTRODUCTION

Body mass, particularly increased body weight or BMI, an elevated WHR,[4] and adult lifetime weight gain have been consistently shown to be associated with increased risk of postmenopausal breast cancer. However, the biological mechanisms underlying the observed associations are not fully clear (1–8). One possible mechanism is an effect of body fat on DNA methylation in the breast tissue.

For postmenopausal women, overweight and obesity are associated with increased amounts of estrogens, which have been shown to be associated with increased risk of subsequent breast cancer (9). There is some evidence that estrogens and estrogenic compounds alter DNA methylation in breast tissues (10–13). A high concentration of 17β-estradiol has been shown to induce hypermethylation in a human breast epithelial cell model (10) and cause the promoter hypermethylation of the *RASSF1A* gene in mammary glands of estrogen-exposed rats (13). In addition, overweight and obesity have been associated with chronic low-grade inflammation (14). Chronic inflammation has been shown to affect DNA methylation changes, which may contribute to carcinogenesis (15–20). The promoter methylation of *p16*, *E-cadherin*, *BRCA1*, and *MLH1* genes occurs more frequently in patients with chronic inflammatory diseases such as gastritis, pancreatitis, and colitis than in individuals without such diseases (15–18). Finally, there is evidence that body fat is associated with changes in methylation. In otherwise healthy individuals, Feinberg et al (21) reported 4 variably methylated regions across the genome that were in a consistent association with BMI over time.

CpG island promoter methylation is a widespread alteration in breast carcinogenesis (22, 23). The *p16* gene, which is a known tumor suppressor gene is involved in cell cycle regulation. *p16* promoter methylation is a common epigenetic alteration in carcinogenesis, including that of the breast (22). Overweight [BMI (in kg/m^2) $>$25] has been shown to be positively associated with the promoter methylation of the *p16* gene in esophageal cancers (24). In addition, the inactivation of other tumor suppressor genes involved in cell adhesion (*E-cadherin*), hormone, and receptor-mediated cell signaling (ie, *RAR-*β_2) by

[1] From the Department of Social and Preventive Medicine, School of Public Health and Health Professions, University at Buffalo, Buffalo, NY (M-HT, JN, MT, and JLF); the Institute for Translational Epidemiology, Mount Sinai School of Medicine, New York, NY (M-HT); the Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC (CM, SSK, and PGS); the Departments of Cancer Prevention and Control (CA) and Surgery (SBE), Roswell Park Cancer Institute, Buffalo, NY; and the University of Nevada Health Sciences System, Las Vegas, NV (MT).

[2] Supported in part by the Department of Defense (DAMD 179616202 and DAMD 17030446), the US Public Health Service National Cancer Institute (R01CA 092040), and the National Institute on Alcohol Abuse and Alcoholism (P50 AA09802).

[3] Address reprint requests and correspondence to M-H Tao, Institute for Translational Epidemiology, Mount Sinai School of Medicine, Box 1057, New York, NY 10029. E-mail: menghua.tao@mssm.edu.

[4] Abbreviations used: CpG, cytosine-guanine dinucleotides; ER, estrogen receptor; HT, hormone therapy; PCR, polymerase chain reaction; PR, progesterone receptor; WHR, waist-to-hip ratio.

Received December 3, 2010. Accepted for publication June 22, 2011.

First published online July 20, 2011; doi: 10.3945/ajcn.110.009365.

promoter methylation is a critical event in breast carcinogenesis (22). The promoter methylation of *E-cadherin*, *p16*, and *RAR-β_2* has also been shown to occur more frequently in breast cancer cell lines and breast tumors than in nonmalignant adjacent breast tissue or benign breast tissue from cancer patients (25–32) and is associated with clinical features of breast cancer, including distant metastasis and ER-positive tumors (32–34).

Therefore, there is evidence of an association of body weight with increased circulating estrogen and with inflammation and associations of estrogen and inflammation with aberrant methylation. We hypothesized that tumors from postmenopausal women with breast cancer with higher body mass would have a higher prevalence of methylation than tumors from women with lower body mass. We evaluated the association between being overweight and the promoter methylation of *E-cadherin*, *p16*, and *RAR-β_2* genes in breast tumors in a population-based study. In addition, we examined the potential effect modification of factors related to hormone exposures on the association of DNA methylation and body mass.

SUBJECTS AND METHODS

Subjects and study design

Detailed study methods have been published previously (5, 34). In brief, the Western New York Exposures and Breast Cancer Study included 1170 women with primary, histologically confirmed, incident breast cancer who were aged 35–79 y at diagnosis and 2115 randomly selected population control subjects who were frequency-matched to cases for age and race. Cases were interviewed in $\leq$1 y of diagnosis. All participants provided informed consent, and the study protocol was approved by the Institutional Review Boards of the University at Buffalo and of all participating institutions. These analyses were restricted to breast cancer cases.

Extensive in-person interviews and self-administered questionnaires were administered to participants including queries on potential confounding factors and breast cancer risk factors. Several anthropometric measures were made, including current height, weight, and central adiposity (abdominal height, waist circumference, and hip circumference). All anthropometric measurements were made by trained interviewers according to a standardized protocol. Waist circumference was measured by placing a tape around the smallest point between the top of iliac crest and the bottom of rib cage, hip circumference was measured by placing the tape around the hips at the biggest circumference point between the iliac crest and the crotch, and abdominal height was measured with a caliper on the participant in a recumbent position. All measures were to the nearest 0.1 cm. Three measurements were initially made for accuracy, and if necessary, these measurements were repeated until the 3 readings were all within 0.5 cm of each other. Participants were also asked to recall their body weight for each decade of their lives from age 20 until 12–24 mo before diagnosis for cases and until 12–24 mo before the interview for control subjects. BMI was calculated as body weight in kilograms divided by the square of height in meters. The reported weight 12–24 mo before the interview and measured height during the interview were used for our analyses. Reported weights 12–24 mo before the interview and measured weights were highly correlated ($r = 0.91$). Measured waist and hip circumferences were used to calculate the WHR.

Information on the tumor size, histologic grade, and cancer stage (as measured by tumor-node-metastasis stage) was abstracted from medical charts by trained research nurses by using a standard protocol. ER and PR status were determined by using immunohistochemistry at the Georgetown University Lombardi Comprehensive Cancer Center, and stained tumor slides were evaluated by a single certified pathologist as previously described (34).

Laboratory analysis

We successfully obtained archived tumor blocks from 78.6% of participant breast cancer cases. Tumor samples were microdissected from fixed microscope slides to minimize the inclusion of the normal surrounding tissue. Bisulfite modification was performed on 2μg tumor DNA isolated from the dissected tissue in accordance with methods described elsewhere (34, 35). To assess the promoter methylation of *E- cadherin*, *p16*, and *RAR-β_2*, a fluorescence-based version of qualitative methylation-specific PCR was applied by using a real-time PCR amplification of bisulfate-converted DNA in an ABI 7900HT (Applied Biosystems) real-time PCR system as previously described (34, 36). Briefly, each reaction contained 5 μL Taqman Universal Master Mix (37), 4.5 μL bisulfite-treated DNA, and 0.5 μL of a 60$\times$ assay in a design premix that contained primers and probes that were designed for each respective gene (Applied Biosystems); primers and probes sequences were previously published(34). The thermal cycling started with an initial 10-min denaturation at 95°C followed by 45 cycles at 95°C for 15 s and at 60°C for 1 min with a final extension of 5 min at 72°C. As a control to check for modified viable DNA, we used an assay for the *ACTB* gene with primers and probes specifically designed for CpG-free sites within the gene sequence, thus amplifying the modified DNA regardless of the methylation status. If the *ACTB* result was negative (ie, no amplification signal was detected), the DNA was not used in subsequent assays, and remodification was attempted; the other 3 genes were assayed only if *ACTB* was positive. Each individual DNA sample was assayed in triplicate for each gene for quality-control purposes. In addition, as a positive control, universally methylated DNA (CpGenome) was used along with water blanks as a negative control. We had successful promoter methylation results for 803 cases. We examined the replicability for the assay of the methylation status of *E- cadherin*, *p16*, and *RAR-β_2* genes in 142 randomly selected samples. Concordance rates for replicates were 100% for the methylation status of the 3 genes.

Statistical analysis

Characteristics of participating cases with and without the promoter methylation of specific genes and control subjects were compared by using ANOVA for continuous variables and the chi-square test for categorical variables. BMI was classified into 3 categories: $<$25.0, 25.0–29.9, and $\geq$30.0 for categories of normal, overweight, and obese, respectively, according to World Health Organization criteria (38). The lifetime adult weight change was calculated as the difference between the reported weight 1 y before the diagnosis or interview and weight at age 20 y. Quartile distributions in control subjects were used to categorize data on WHR, abdominal height, and lifetime adult

weight change. Unconditional logistic regression was used for case-case comparisons of those with and without promoter methylation to evaluate ORs and 95% 95% CIs for associations of BMI, WHR, abdominal height, and lifetime adult weight change with the likelihood of promoter methylation. Because menopausal HT is a major resource of exogenous hormones that can influence breast cancer risk (39), and ER status can have an effect on breast tissue responsiveness to estrogens, we also investigated interactions between anthropometric measures and HT, ER status, and age by constructing a multiplicative term in the logistic regression model. All analyses were adjusted for age, educational level, race, and other known breast cancer risk factors. Because we (34) had previously shown an association of methylation with ER and PR status, we also adjusted for ER status. ER status and PR status were highly correlated. We examined results with adjustment for both ER and PR; results were similar to those with adjustment for ER alone. The results presented in the current study were only adjusted for ER status. We also evaluated associations with stratification of menopausal status. All statistical tests were based on a 2-sided probability. Statistical analyses were conducting with SAS software (version 9.2; SAS Institute).

RESULTS

Comparisons of cases with and without the promoter methylation of *E -cadherin*, *p16*, and *RAR-β_2* genes for selected known breast cancer risk factors and anthropometric factors are shown in **Table 1**. The frequency of promoter methylation was 20% (n = 161) for *E-cadherin*, 25.9% (n = 208) for *p16*, and 27.5% (n = 221) for *RAR-β_2*. There were no differences in the methylation frequency by recent BMI, height, BMI at age 20 y,

TABLE 1
Descriptive characteristics of breast cancer cases (n = 803) by hypermethylation status of *E-cadherin*, *p16*, and *RAR-β2* in the Western New York Exposures and Breast Cancer Study, 1996–2001[1]

	E-cadherin		*p16*		*RAR-β_2*	
	Methylated (n = 161)	Unmethylated	Methylated (n = 208)	Unmethylated	Methylated (n = 221)	Unmethylated
Age (y)	58.0 ± 11.8[2]	57.4 ± 11.2	58.0 ± 11.2	57.4 ± 11.3	57.4 ± 11.3	57.6 ± 11.3
White race-ethnicity [n (%)]	148 (91.9)	594 (92.5)	192 (92.3)	550 (92.4)	210 (95.0)	532 (91.4)
Education [n (%)]						
<12 y	13 (8.1)	41 (6.4)	15 (7.2)	39 (6.5)	15 (6.8)	39 (6.7)
12 y	64 (39.7)	236 (36.8)	74 (35.6)	226 (38.0)	89 (40.3)	211 (36.3)
>12 y	84 (52.2)	365 (56.8)	119 (57.2)	330 (55.5)	117 (52.9)	332 (57.0)
Postmenopausal [n (%)]	111 (68.9)	455 (70.9)	147 (70.7)	419 (70.4)	157 (71.0)	409 (70.3)
Aspirin [n (%)]						
User	69 (43.4)	270 (42.7)	79 (38.4)	260 (44.4)	95 (44.0)	244 (42.4)
Nonuser	90 (56.6)	362 (57.35)	127 (61.6)	325 (55.6)	121 (56.0)	331 (57.6)
Hormone replacement therapy [n (%)][3]						
Never	60 (54.1)	209 (46.2)	72 (49.0)	197 (47.4)	73 (47.1)	196 (48.0)
Ever	51 (45.9)	243 (53.8)	75 (51.0)	219 (52.6)	82 (52.9)	212 (52.0)
TNM stage [n (%)]						
0	17 (12.3)	74 (12.8)	21 (11.5)	70 (13.1)	28 (13.9)	63 (12.3)
I	68 (49.3)	267 (46.3)	93 (51.1)	242 (45.4)	87 (43.3)	248 (48.3)
II_a/II_b	47 (34.1)	195 (33.8)	57 (31.3)	185 (34.7)	68 (33.8)	174 (33.8)
III/IV	6 (4.3)	41 (7.1)	11 (6.1)	36 (6.8)	18 (9.0)	29 (5.6)
ER status [n (%)]						
Positive	114 (71.3)	446 (70.0)	136 (65.7)	424 (71.9)	163 (74.8)	397 (68.6)
Negative	46 (28.7)	191 (30.0)	71 (34.3)	166 (28.1)	55 (25.2)	182 (31.4)
PR status [n (%)])						
Positive	93 (58.2)	411 (65.4)	128 (63.0)	375 (64.3)	142 (65.7)	361 (63.3)
Negative	66 (41.8)	217 (34.6)	75 (37.0)	208 (35.7)	74 (34.3)	209 (36.7)
Anthropometric factors[4]						
Recent BMI (kg/m^2)[5]	28.1 ± 5.6	28.5 ± 6.5	28.5 ± 6.3	28.4 ± 6.3	28.6 ± 6.1	28.4 ± 6.4
Height (m)	1.62 ± 0.07	1.62 ± 0.07	1.63 ± 0.06	1.62 ± 0.07	1.63 ± 0.06	1.62 ± 0.07
BMI at age 20 y (kg/m^2)	21.0 ± 3.0	21.0 ± 3.3	20.9 ± 3.1	21.0 ± 3.3	21.2 ± 3.9	20.8 ± 2.9
Waist (cm)	89.0 ± 14.0	89.8 ± 15.0	89.7 ± 14.9	89.6 ± 14.8	90.8 ± 14.8	89.1 ± 14.8
Waist-to-hip ratio	0.84 ± 0.08	0.83 ± 0.08	0.83 ± 0.08	0.83 ± 0.09	0.84 ± 0.09	0.83 ± 0.08
Abdominal height (cm)	21.1 ± 3.9	20.9 ± 3.8	20.6 ± 3.8	21.1 ± 3.8	21.2 ± 3.6	20.9 ± 3.9
Lifetime weight change (kg)	8.75 ± 6.39	8.66 ± 6.71	8.98 ± 6.76	8.57 ± 6.62	8.35 ± 6.62	8.80 ± 6.67

[1] Subjects with missing values were excluded from the analysis. ER, estrogen receptor; PR, progesterone receptor; TNM, tumor-node-metastasis. All continuous variables were analyzed with ANOVA, and all categorical variables were analyzed with the chi-square test. None of the differences were significant.

[2] Mean ± SD (all such values).

[3] In postmenopausal women.

[4] Comparisons between participating cases with and without promoter methylation of specific gene were adjusted for age.

[5] From 12 to 24 mo before the diagnosis or interview.

waist circumference, WHR, abdominal height, and lifetime weight change between cases with the promoter methylation of *E-cadherin*, *p16*, or *RAR-β_2* genes and unmethylated cases.

BMI was not associated with premenopausal breast cancer; in addition, there were no associations between the likelihood of the promoter methylation of each of the 3 genes and these anthropometric measures in premenopausal women (data not shown). Thus, additional analyses were limited to postmenopausal women.

Associations of BMI 12–24 mo before the interview, WHR, abdominal height, and adult weight change with the likelihood of promoter methylation and risk of postmenopausal breast cancer after multivariable adjustment were investigated, and results are shown in **Table 2**. The likelihood of promoter methylation did not differ for those anthropometric measures for any of the 3 genes. Additional adjustment for ever use of HT and aspirin use did not appreciably change the presented likelihood estimates.

We evaluated associations of BMI, WHR, abdominal height, and adult weight change with the likelihood of promoter methylation of $\geq$1 of the 3 genes in tumors in postmenopausal women (**Table 3**). The WHR was associated with increased likelihood of tumors with promoter methylation of at least one gene, the adjusted OR (95% CI) for the comparison of the highest compared with lowest quartile was 1.85 (1.10, 3.11) (*P*-trend = 0.02). BMI, abdominal height, and adult weight change were not associated with the likelihood of promoter methylation of at least one gene.

We conducted additional analyses stratified by ER status, age, and HT use in postmenopausal women. There were no significant interactions for ER status, age ,or history of HT with the WHR on the likelihood of promoter methylation of at least one gene in postmenopausal women. However, there was some evidence of differences in associations within strata of these variables. The association of the WHR with the likelihood of having tumors with promoter methylation of at least one gene appeared to be limited to ER-positive tumors [the OR (95% CI) for the highest compared with lowest quartile of WHRs was 2.06 (95% CI: 1.12, 3.80) (*P*-trend = 0.03)]; there was no association in ER-negative tumors (*P*-interaction = 0.93). There was no significant interaction between the WHR and age in the association with the promoter methylation of at least one gene (*P*-interaction = 0.21). The association appeared to be stronger in younger postmenopausal women (aged <63 y) (OR: 2.68; 95% CI: 1.25, 5.75) than older postmenopausal women (aged $\geq$63 y) (OR: 1.10; 95% CI: 0.81, 2.39). Finally, although we did not observe a multiplicative interaction between the WHR and HT on the likelihood of the promoter methylation of at least one gene (*P*-interaction = 0.14), there was some evidence of an effect modification. Compared with the lowest quartile of WHR, the highest quartile of WHR was associated with an increased likelihood of the promoter methylation of at least one gene (OR: 2.31; 95% CI: 1.09, 4.90) in women who used HT after menopause, whereas no association was shown in never users. There were no differences for the associations of other anthropometric measurements with the likelihood of the methylation of at least one gene for either age strata or HT strata (data not shown).

DISCUSSION

In this population-based study, there was no overall association between BMI, WHR, abdominal height, or adult weight change and the likelihood of the promoter methylation of the individual genes *E-cadherin*, *p16*, and *RAR-β_2*. We showed that a greater WHR was positively associated with the likelihood of the promoter methylation of at least one gene in postmenopausal breast tumors. Although no significant interaction was observed, our results suggested that the association between the WHR and likelihood of promoter methylation of at least one gene differed across strata of ER status, age, or history of HR in postmenopausal women.

To our knowledge, there are few studies that have explored the relation between aberrant DNA methylation and overweight and obesity in human cancers of any site (24, 40–43). In normal autopsy kidney tissues, it was reported that the promoter methylation of the *RASSF1A* gene was significantly increased in the obese group (BMI $\geq$30) than in the normal-weight group (BMI of 20–25) (41). Another recent study of esophageal cancer showed a positive relation of BMI with the promoter methylation of the *p16* gene (24). Ye et al (43) also showed a significantly higher frequency of *hMLH1* methylation in non-neoplastic rectal mucosa of overweight or obese (BMI $\geq$25) subjects. However, in a study of endometrial cancer cases with microstatellite instability–positive tumors, BMI did not differ significantly by *MLH1* promoter methylation status (40). In a large colon cancer study, Slattery et al (42) examined the DNA methylation status of 5 genes for the CpG island methylator phenotype, and their case-case comparisons showed that obesity (BMI $\geq$30) was inversely associated with the prevalence of a CpG island methylator phenotype–high tumor. In our study, although no associations between BMI and the promoter methylation of *E-cadherin*, *p16*, and *RAR-β_2* genes was observed in breast tumors, we showed that a greater WHR was associated with increased risk of the promoter methylation of at least one gene in postmenopausal breast tumors.

As an anthropometric indicator for central obesity, there was evidence that a smaller WHR was inversely associated with risk of postmenopausal breast cancer, with 24% lower risk in women with the smallest WHR (7, 8). After menopause, the adipose tissue is a major resource for converting androstenedione to estrone. Compared with women with a small WHR, women with a large WHR have higher free testosterone and possibly higher estrogen concentrations (7). Growing in vivo and in vitro evidence showed that higher estrogen exposure could induce aberrant DNA methylation and silence genes in breast carcinogenesis (10, 13). A recent study demonstrated aberrant and progressive *RASSF1A* promoter methylation in the early stage of breast carcinogenesis in rats exposed to 17β-estradiol for 12 wk (13). In the current study, we showed that the increased likelihood of the promoter methylation of at least one gene with the WHR was restricted to women who used HT after menopause. Moreover, we also showed that the association of promoter methylation of at least one gene with the WHR was limited to postmenopausal women with hormone-sensitive ER-positive tumors, which strengthened the hypothesis of an estrogen contribution to aberrant DNA methylation. More studies that examine a larger number of methylation sites are needed to replicate our findings and to further assess the association of circulating estrogen concentrations with aberrant DNA methylation, particularly in tumors from postmenopausal women who are overweight or obese.

Inflammation is another important mechanism that may explain the observed association between the WHR and promoter methylation in postmenopausal breast tumors. There is also

TABLE 2
Association between body size and promoter methylation of *E-cadherin*, *p16*, and *RAR-β_2* genes in postmenopausal women: case-case comparisons

	E-cadherin			*p16*			*RAR-β_2*		
	Methylated	Unmethylated	OR (95% CI)[1]	Methylated	Unmethylated	OR (95% CI)[1]	Methylated	Unmethylated	OR (95% CI)[1]
	n	*n*		*n*	*n*		*n*	*n*	
BMI	—								
<25 kg/m^2	33	131	1.0	42	120	1.0	42	122	1.0
25–29 kg/m^2	43	145	1.16 (0.68, 1.98)	48	140	1.04 (0.63, 1.73)	53	135	1.14 (0.70, 1.88)
≥30 kg/m^2	35	179	0.68 (0.38, 1.19)	57	157	1.14 (0.69, 1.88)	62	152	1.17 (0.71, 1.91)
P-trend	—	—	0.14	—	—	0.61	—	—	0.55
Waist-to-hip ratio									
<0.78	17	105	1.0	28	90	1.0	32	90	1.0
0.78–0.81	25	84	1.65 (0.84, 3.25)	28	81	1.14 (0.61, 2.11)	26	80	0.91 (0.50, 1.67)
0.82–0.87	30	120	1.37 (0.72, 2.61)	43	107	1.48 (0.84, 2.60)	37	113	0.87 (0.49, 1.52)
≥0.88	36	137	1.40 (0.74, 2.67)	44	129	1.20 (0.68, 2.15)	59	114	1.48 (0.86, 2.53)
P-trend	—	—	0.41	—	—	0.38	—	—	0.24
Abdominal height									
<17.83 cm	12	52	1.0	20	44	1.0	14	50	1.0
17.83–19.94 cm	18	74	1.01 (0.43, 2.34)	25	67	0.71 (0.34, 1.50)	23	69	1.13 (0.52, 2.47)
19.95–22.74 cm	22	95	0.97 (0.43, 2.20)	33	84	0.86 (0.42, 1.75)	36	81	1.46 (0.70, 3.06)
≥22.75 cm	25	110	0.86 (0.38, 1.97)	32	103	0.62 (0.30, 1.28)	42	93	1.47 (0.70, 3.09)
P-trend	—	—	0.67	—	—	0.30	—	—	0.18
Weight change (from age 20 y to 1 y before the study)									
≤4.1 kg	23	94	1.0[2]	23	87	1.0[2]	33	87	1.0[2]
4.2–8.07 kg	32	106	1.22 (0.65, 2.27)	32	106	0.73 (0.41, 1.33)	35	107	0.94 (0.53, 1.67)
8.1–12.4 kg	32	132	0.92 (0.50, 1.72)	38	126	0.80 (0.45, 1.41)	50	114	1.14 (0.66, 1.98)
>12.4 kg	24	120	0.80 (0.41, 1.55)	44	100	1.20 (0.68, 2.14)	39	105	0.94 (0.53, 1.67)
P-trend	—	—	0.33	—	—	0.42	—	—	0.98

[1] Unless noted otherwise, values are ORs (95% CIs) that were computed by unconditional logistic regression and adjusted for age, race, education, age at menarche, age at first birth, age at menopause, family history of breast cancer in first-degree relatives, history of benign breast disease, energy, alcohol drinking status, smoking status, and estrogen receptor status.

[2] Additionally adjusted for BMI for weight-change analyses.

TABLE 3
Association between body size and promoter methylation of genes in postmenopausal women: case-case comparisons

	Methylation of ≥1 of 3 genes	Unmethylated	OR (95% CI)[1]
	n	*n*	
BMI			
<25 kg/m^2	107	57	1.0
25–29 kg/m^2	127	61	1.12 (0.70, 1.78)
≥30 kg/m^2	143	71	1.02 (0.64, 1.61)
P-trend	—	—	0.98
Waist-to-hip ratio			
<0.78	70	52	1.0
0.78–0.81	73	36	1.41 (0.82, 2.44)
0.82–0.87	100	50	1.37 (0.82, 2.27)
≥0.88	125	48	1.85 (1.10, 3.11)
P-trend	—	—	0.02
Abdominal height			
<17.83 cm	40	24	1.0
17.83–19.94 cm	62	30	1.10 (0.55, 2.21)
19.95–22.74 cm	82	35	1.35 (0.69, 2.65)
≥22.75 cm	90	45	0.98 (0.51, 1.91)
P-trend	—	—	0.96
Weight change (from age 20 y to 1 y before the study)			
≤4.1 kg	82	38	1.0[2]
4.2–8.0 kg	89	49	0.79 (0.46, 1.35)
8.1–12.4 kg	109	55	0.83 (0.49, 1.40)
>12.4 kg	97	47	0.91 (0.53, 1.58)
P-trend	—	—	0.83

[1] Unless noted otherwise, values are ORs (95% CIs) that were computed by unconditional logistic regression and adjusted for age, race, education, age at menarche, age at first birth, age at menopause, family history of breast cancer in first-degree relatives, history of benign breast disease, energy, alcohol drinking status, smoking status, and estrogen receptor status.

[2] Additionally adjusted for BMI for weight-change analyses.

evidence that suggested that central obesity was associated with chronic low-grade inflammation in adiposity tissues and an excessive production of proinflammatory molecules, including C-reactive protein, TNF-α, and IL-6 (14). Chronic inflammation has been shown to alter the DNA promoter methylation pattern and critical gene regulation in both human (15–18) and animal (44) studies. In a study in 24 obese subjects who participated in a balanced low-calorie diet intervention, Campion et al (45) showed that men with weight loss (≥5% of initial body weight) showed significantly lower concentrations of total TNF-α promoter methylation in the peripheral blood mononuclear cells, and moreover, baseline TNF-α circulation concentrations were positively associated with total promoter methylation.

Another possible mechanism for the observed positive relation was methyl bioavailability. In a study of healthy women between 15 and 45 y of age, BMI was shown to be a strong positive determinant of the *S*-adenosylmethioinine concentration (46), which is important in one-carbon metabolism as a methyl donor in the methylation of DNA (47). Decreased plasma homocysteine concentrations that accompany increased *S*-adenosylmethioinine have been observed in insulin-resistant rats (48). It is possible that overweight and obesity, in general, and central obesity, in particular, may be involved in the homeostatic interaction between methylation and endocrine pathways through its influence on insulin resistance.

In assessing these study results, it is important to consider strengths and weakness of the study. Strengths of our study included the population-based study design, large sample size, measurements of anthropometric factors by trained interviewers, and the detailed information on known risk factors for breast cancer and disease characteristics. However, several limitations should be considered when evaluating our results. Although the number of tumor blocks available for our study was relatively large, there were issues of statistical power. Chance must be considered as an explanation for our findings of an association with the WHR; with reduced power, there may have been an overestimation of the effect size. Furthermore, the statistical power in some subgroups of our study remained limited, which limited our ability to identify weak associations. This lack of power may also have explained the difference in null findings for other anthropometric measurements. As in all epidemiologic studies, additional studies in other populations are needed to replicate our finding and to elucidate the underlying biological mechanisms. Although we chose to examine 3 genes that have been identified as frequently methylated in breast tumors, other genes, including *RASSF1A* (41), may be more informative for examination, specifically with regard to differences by BMI or body weight. As was the case in other studies (24, 42, 43), the qualitative nature of the real-time methylation-specific PCR method limited our ability to detect quantitative changes in the methylation of genes. Furthermore, our inability to obtain archived tumor tissue for all breast cancer cases may have led to a selection bias. In comparisons of cases without available archived breast tumor tissue, cases with tissue were slightly younger at diagnosis and tended to have tumors of a more advanced stage. However, the 2 groups were similar in terms of BMI, WHR, abdominal height, adult weight change, tumor size, histologic grade, and ER and PR status (34). There may have

been a recall bias in the lifetime self-reported weight. However, the reported weight 12–24 mo before the interview was highly correlated with the measured weight (5); a recall bias would not likely have affected case-case comparisons because participants would not have been aware of their methylation status. Finally, our case-case comparisons suggested the different prevalence of promoter methylation in relation to obesity. Aberrant DNA methylation is a tissue-specific and dynamic process; our inability to measure DNA methylation patterns in breast tissue in control subjects limited our ability to determine differences of promoter methylation between cases and control subjects.

In conclusion, our findings suggest that the observed association of postmenopausal breast cancer with overweight and obesity, particularly centralized obesity, may be related, at least in part, to DNA methylation; replication in other populations and the exploration of methylation of a larger number of sites is necessary to better understand this finding.

This study would not have been possible without the support of all the study participants and the research staff of the Western New York Exposures and Breast Cancer Study.

The authors' responsibilities were as follows—M-HT: development of hypothesis and leadership of statistical analysis and on the writing of the manuscript; CM and SSK: promoter methylation assessment and manuscript preparation; JN, SBE, and MT: data collection, management, and manuscript preparation; CA: statistical analysis and manuscript preparation; PGS: direction of laboratory analyses, hypothesis conception, and manuscript preparation; and JLF: overall leadership of the Western New York Exposures and Breast Cancer Study design, conduct, and analysis, hypothesis conception, and manuscript preparation. None of the authors had a conflict of interest regarding the manuscript.

REFERENCES

1. Feigelson HS, Jonas CR, Teras LR, Thun MJ, Calle EE. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2004;13:220–4.
2. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371:569–78.
3. Bianchini F, Kaaks R, Vainio H. Overweight, obesity, and cancer risk. Lancet Oncol 2002;3:565–74.
4. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579–91.
5. Han D, Nie J, Bonner MR, McCann SE, Muti P, Trevisan M, Ramirez-Marrero FA, Vito D, Freudenheim JL. Lifetime adult weight gain, central adiposity, and the risk of pre- and postmenopausal breast cancer in the Western New York exposures and breast cancer study. Int J Cancer 2006;119:2931–7.
6. Adderley-Kelly B, Williams-Stephens E. The relationship between obesity and breast cancer. ABNF J 2003;14:61–5.
7. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obes Rev 2003;4:157–73.
8. Friedenreich CM. Review of anthropometric factors and breast cancer risk. Eur J Cancer Prev 2001;10:15–32.
9. Cleary MP, Grossmann ME. Minireview: Obesity and breast cancer: the estrogen connection. Endocrinology 2009;150:2537–42.
10. Fernandez SV, Russo J. Estrogen and xenoestrogens in breast cancer. Toxicol Pathol 2010;38:110–22.
11. Weng YI, Hsu PY, Liyanarachchi S, Liu J, Deatherage DE, Huang YW, Zuo T, Rodriguez B, Lin CH, Cheng AL, et al. Epigenetic influences of low-dose bisphenol A in primary human breast epithelial cells. Toxicol Appl Pharmacol 2010;248:111–21.
12. Hsu PY, Deatherage DE, Rodriguez BA, Liyanarachchi S, Weng YI, Zuo T, Liu J, Cheng AS, Huang TH. Xenoestrogen-induced epigenetic repression of microRNA-9-3 in breast epithelial cells. Cancer Res 2009;69:5936–45.
13. Starlard-Davenport A, Tryndyak VP, James SR, Karpf AR, Latendresse JR, Beland FA, Pogribny IP. Mechanisms of epigenetic silencing of the Rassf1a gene during estrogen-induced breast carcinogenesis in ACI rats. Carcinogenesis 2010;31:376–81.
14. Vona-Davis L, Rose DP. Angiogenesis, adipokines and breast cancer. Cytokine Growth Factor Rev 2009;20:193–201.
15. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007;121:2373–80.
16. Kang GH, Lee HJ, Hwang KS, Lee S, Kim JH, Kim JS. Aberrant CpG island hypermethylation of chronic gastritis, in relation to aging, gender, intestinal metaplasia, and chronic inflammation. Am J Pathol 2003;163:1551–6.
17. Liggett T, Melnikov A, Yi QL, Replogle C, Brand R, Kaul K, Talamonti M, Abrams RA, Levenson V. Differential methylation of cell-free circulating DNA among patients with pancreatic cancer versus chronic pancreatitis. Cancer 2010;116:1674–80.
18. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. Cancer Res 2001;61:3573–7.
19. Ushijima T, Asada K. Aberrant DNA methylation in contrast with mutations. Cancer Sci 2010;101:300–5.
20. Kanai Y. Genome-wide DNA methylation profiles in precancerous conditions and cancers. Cancer Sci 2010;101:36–45.
21. Feinberg AP, Irizarry RA, Fradin D, Aryee MJ, Murakami P, Aspelund T, Eiriksdottir G, Harris TB, Launer L, Gudnason V, et al. Personalized epigenomic signatures that are stable over time and covary with body mass index. Sci Transl Med 2010;2:49ra67.
22. Widschwendter M, Jones PA. DNA methylation and breast carcinogenesis. Oncogene 2002;21:5462–82.
23. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer 2003;3:253–66.
24. Mohammad Ganji S, Miotto E, Callegari E, Sayehmiri K, Fereidooni F, Yazdanbod M, Rastgar-Jazii F, Negrini M. Associations of risk factors obesity and occupational airborne exposures with CDKN2A/p16 aberrant DNA methylation in esophageal cancer patients. Dis Esophagus 2010;23:597–602.
25. Caldeira JR, Prando EC, Quevedo FC, Neto FA, Rainho CA, Rogatto SR. CDH1 promoter hypermethylation and E-cadherin protein expression in infiltrating breast cancer. BMC Cancer 2006;6:48.
26. Fackler MJ, McVeigh M, Evron E, Garrett E, Mehrotra J, Polyak K, Sukumar S, Argani P. DNA methylation of RASSF1A, HIN-1, RAR-beta, Cyclin D2 and Twist in in situ and invasive lobular breast carcinoma. Int J Cancer 2003;107:970–5.
27. Feng W, Shen L, Wen S, Rosen DG, Jelinek L, Hu X, Huan S, Huang M, Liu J, Sahin AA, et al. Correlation between CpG methylation profiles and hormone receptor status in breast cancers. Breast Cancer Res 2007;9:R57.
28. Herman JG, Merlo A, Mao L, Lapidus RG, Issa JP, Davidson NE, Sidransky D, Baylin SB. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res 1995;55:4525–30.
29. Krassenstein R, Sauter E, Dulaimi E, Battagli C, Ehya H, Klein-Szanto A, Cairns P. Detection of breast cancer in nipple aspirate fluid by CpG island hypermethylation. Clin Cancer Res 2004;10:28–32.
30. Lehmann U, Länger F, Feist H, Glöckner S, Hasemeier B, Kreipe H. Quantitative assessment of promoter hypermethylation during breast cancer development. Am J Pathol 2002;160:605–12.
31. Lewis CM, Cler LR, Bu DW, Zöchbauer-Müller S, Milchgrub S, Naftalis EZ, Leitch AM, Minna JD, Euhus DM. Promoter hypermethylation in benign breast epithelium in relation to predicted breast cancer risk. Clin Cancer Res 2005;11:166–72.
32. Mehrotra J, Vali M, McVeigh M, Kominsky SL, Fackler MJ, Lahti-Domenici J, Polyak K, Sacchi N, Garrett-Mayer E, Argani P, et al. Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung. Clin Cancer Res 2004;10:3104–9.
33. Lippone P, Saarelainen E, Ji H, Aaltomaa S, Syrjanen K. Expression of E-cadherin (E-CD) as related to other prognostic factors and survival in breast cancer. J Pathol 1994;174:101–9.
34. Tao MH, Shields PG, Nie J, Millen A, Ambrosone CB, Edge SB, Krishnan SS, Marian C, Xie B, Winston J, et al. DNA hypermethylation and clinicopathological features in breast cancer: the Western New York Exposures and Breast Cancer (WEB) Study. Breast Cancer Res Treat 2009;114:559–68.

35. Jeronimo C, Usadel H, Henrique R, Oliveira J, Lopes C, Nelson WG, Sidransky D. Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst 2001;93:1747–52.
36. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. MethyLight: a high-throughput assay to measure DNA methylation. Nucleic Acids Res 2000;28:E32.
37. Marsit CJ, McClean MD, Furniss CS, Kelsey KT. Epigenetic inactivation of the SFRP genes is associated with drinking, smoking and HPV in head and neck squamous cell carcinoma. Int J Cancer 2006;119:1761–6.
38. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. Obes Res 2001;9:228S–33S.
39. Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab 2010;95(suppl 1):s1–s66.
40. McCourt CK, Mutch DG, Gibb RK, Rader JS, Goodfellow PJ, Trinkaus K, Powell MA. Body mass index: relationship to clinical, pathologic and features of microsatellite instability in endometrial cancer. Gynecol Oncol 2007;104:535–9.
41. Peters I, Vaske B, Albrecht K, Kuczyk MA, Jonas U, Serth J. Adiposity and age are statisticaly related to enhanced *RASSF1A* tumor suppressor gene promoter methylation in normal autopsy kidney tissue. Cancer Epidemiol Biomarkers Prev 2007;16:2526–32.
42. Slattery ML, Curtin K, Sweeney C, Levin TR, Potter J, Wolff RK, Albertsen H, Samowitz WS. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. Int J Cancer 2007;120:656–63.
43. Ye C, Shrubsole MJ, Cai Q, Ness R, Grady WM, Smalley W, Cai H, Washington K, Zheng W. Promoter methylation status of the MGMT, hMLH1, and CDKN2A/p16 genes in non-neoplastic mucosa of patients with and without colorectal adenomas. Oncol Rep 2006;16:429–35.
44. Kamei Y, Suganami T, Ehara T, Kanai S, Hayashi K, Yamamoto Y, Miura S, Ezaki O, Okano M, Ogawa Y. Increased expression of DNA methyltransferase 3a in obese adipose tissue: studies with transgenic mice. Obesity (Silver Spring) 2010;18:314–21.
45. Campión J, Milagro FI, Goyenechea E, Martínez JA. TNF-alpha promoter methylation as a predictive biomarker for weight-loss response. Obesity (Silver Spring) 2009;17:1293–7.
46. van Driel LM, Eijkemans MJ, de Jonge R, de Vries JH, van Meurs JB, Steegers EA, Steegers-Theunissen RP. Body mass index is an important determinant of methylation biomarkers in women of reproductive ages. J Nutr 2009;139:2315–21.
47. Choi SW, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 2002;132:2413S–8S.
48. Wijekoon EP, Hall B, Ratnam S, Brosnan ME, Zeisel SH, Brosnan JT. Homocysteine metabolism in ZDF (type 2) diabetic rats. Diabetes 2005; 54:3245–51.