Short Communication

Overweight and Obese Perimenopausal and Postmenopausal Women Exhibit Increased Abnormal Mammary Epithelial Cytology

Victoria L. Seewaldt,[1,2,3] Vanessa Goldenberg,[1] Lee W. Jones,[3,4] Charlotte Peace,[1] Gloria Broadwater,[3] Victoria Scott,[1] Gregory R. Bean,[1] Lee Gravit Wilke,[4] Carola M. Zalles,[6] and Wendy Demark-Wahnefried[3,4,5]

[1]Department of Medicine, [2]Department of Pharmacology and Cancer Biology, [3]Duke University Comprehensive Cancer Center, [4]Department of Surgery, and [5]School of Nursing, Duke University Medical Center, Durham, North Carolina; and [6]Department of Pathology, Yale-New Haven Medical Center, New Haven, Connecticut

Abstract

High body mass index (BMI $\geq$ 25 kg/m^2) is associated with increased postmenopausal breast cancer incidence and mortality. However, few studies have explored associations between BMI and direct measures on target tissue. Epithelial cytology was assessed in 62 high-risk perimenopausal and postmenopausal women using random periareolar fine needle aspiration. Masood cytology index scores were significantly higher among women with BMIs $\geq$25 kg/m^2 than in women with BMIs <25 kg/m^2 (13.9 $\pm$ 0.42 versus 12.7 $\pm$ 0.29 kg/m^2; P = 0.017). Overweight or obese women also had significantly higher random periareolar fine needle aspiration epithelial cell counts compared with those who were normal weight (1,230 $\pm$ 272 versus 521 $\pm$ 185; P = 0.028). These data suggest that overweight in perimenopausal and postmenopausal women is associated with direct cytologic abnormalities within the breast. Further research is needed to confirm these findings and to determine if this potential biomarker is responsive to changes in body weight resulting from diet and/or exercise interventions. (Cancer Epidemiol Biomarkers Prev 2007;16(3):613–6)

Introduction

It is estimated that roughly 10 million U.S. women are at high risk for breast cancer (1). Excessive body weight, as reflected by a body mass index (BMI $\geq$ 25 kg/m^2), is consistently associated with postmenopausal breast cancer and increases the risk of dying from this disease (2-6). Whereas previous studies have explored associations between body weight and indirect biomarkers associated with disease risk, such as circulating hormonal levels or mammographic density, few have explored associations between BMI and direct measures on target tissue (7, 8).

Random periareolar fine needle aspiration (RPFNA) is a research technique developed to sample mammary cells from the whole breast of asymptomatic women at high risk for development of breast cancer to assess both (*a*) breast cancer risk and (*b*) response to chemoprevention (9, 10). RPFNA can be done successfully in a majority of high-risk women (82-89% cell yield; refs. 9-11). RPFNA has been used successfully to predict short-term breast cancer risk; the presence of cellular atypia in a breast RPFNA specimen has been prospectively validated to confer a 5.6-fold increase in breast cancer risk in high-risk women (9).

The purpose of this brief communication is to relay findings obtained from an exploratory study aimed at investigating whether perimenopausal and postmenopausal women who are overweight or obese exhibit increased abnormal Masood Cytology as compared with women of normal weight. The rationale for undertaking this exploration was driven by the hypothesis that these direct measures on the target tissue would indeed be associated with BMI.

Materials and Methods

Informed Consent. This study was conducted among 62 women who underwent RPFNA under an Institutional Review Board–approved protocol at Duke University Medical Center (March 2003-August 2005) and who presented sufficient cells for analysis.

Eligibility. Women were required to have at least one of the following major risk factors for breast cancer: (*a*) 5-year Gail risk calculation $\geq$1.7% (12); (*b*) prior biopsy exhibiting atypical hyperplasia, lobular carcinoma *in situ*, or ductal carcinoma *in situ*; (*c*) known BRCA1/2 mutation carrier; or (*d*) prior history of contralateral breast cancer. Women were required to be perimenopausal or postmenopausal, defined as <6 menstrual periods/y in the absence of pregnancy, polycystic ovarian syndrome, or thyroid disorder or no menses for >12 months in the absence of pregnancy and/or status postsurgical removal of both ovaries, respectively. Sociodemographic variables (age, race), hormone replacement therapy (HRT) use, and family history of breast cancer were collected. Given the potential for HRT to serve as a confounder of an investigation focused on body weight and breast cancer risk, all women currently using HRT on a routine basis were excluded from the analyses.

Received 10/13/06; revised 12/12/06; accepted 1/8/07.

Grant support: NIH/National Cancer Institute grants CA68438-AV13 [AVON/National Cancer Institute Partners in Progress], 2P30CA14236, R01CA088799, R01CA98441, and R01CA114068 (V.L. Seewaldt); Susan G. Komen Breast Cancer Award BCTR0402720 (V.L. Seewaldt); V-Foundation Award (V.L. Seewaldt); and the American Institute for Cancer Research (W. Demark-Wahnefried).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Victoria L. Seewaldt, Duke University Medical Center, Box 2628, Durham, NC 27710. Phone: 919-668-2455; Fax: 919-668-2458. E-mail: seewa001@mc.duke.edu

doi:10.1158/1055-9965.EPI-06-0878

Table 1. Characteristics of study sample

	Total sample (n = 62)	Normal weight (BMI < 25 kg/m^2; n = 27)	Overweight/obese (BMI ≥ 25 kg/m^2; n = 35)	P
BMI, kg/m^2				
Mean (SE)	26.1 (0.6)	22.0 (0.3)	29.3 (0.6)	<0.001
Range	18.8-40.7	18.8-24.5	25.2-40.7	
Age, y				
Mean (SE)	52.4 (0.8)	53.1 (1.3)	51.8 (0.9)	0.41
Range	39-68	40-65	39-68	
Race, % (n)				
Caucasian	87 (54)	89 (24)	86 (30)	0.51
African-American	13 (8)	11 (3)	14 (5)	
Menopausal status, % (n)				
Perimenopausal	32 (20)	30 (8)	34 (12)	0.71
Postmenopausal	68 (42)	70 (19)	66 (23)	
Bilateral vs unilateral RPFNA				
Bilateral	29 (18)	30 (8)	29 (10)	0.94
Unilateral	71 (44)	70 (19)	71 (25)	
HRT, % (n)				
Current	0 (0)	0 (0)	0 (0)	0.56
Ever	29 (18)	30 (8)	29 (10)	
Never	71 (44)	70 (19)	71 (25)	
Prior HRT type, % (n)				
Estrogen only	11 (8)	15 (4)	11 (4)	*
Estrogen + progesterone	16 (10)	15 (4)	17 (6)	*
Family history of breast cancer, % (n)	40 (25)	37 (10)	43 (15)	0.65
Prior abnormal biopsy, % (n)				
Atypia	16 (9)	11 (3)	17 (6)	0.59
LCIS	8 (5)	7 (2)	9 (3)	*
Contralateral DCIS	8 (5)	7 (2)	9 (3)	*
History of contralateral breast cancer	21 (13)	22 (6)	20 (7)	0.95
BRCA1 mutation, % (n)	2 (1)	0 (0)	3 (1)	*
BRCA2 mutation, % (n)	3 (2)	0 (0)	3 (2)	*

NOTE: BMI was compared using the Satterthwaite t test. Age was compared using the pooled t test. Race, menopausal status, bilateral versus unilateral RPFNA, HRT, and family history of breast cancer were compared using the test of proportions. Prior abnormal biopsy was compared using the χ^2 test.
Abbreviations: LCIS, lobular carcinoma *in situ*; DCIS, ductal carcinoma *in situ*.
*Too few cases to make statistical comparisons.

BMI Calculation. Body weights and heights were clinically measured on a platform scale with a fixed stadiometer; BMIs were calculated and the standard cutoff point of 25 kg/m^2 was used to dichotomize normal weight from overweight women (13).

RPFNA. RPFNA was done as previously published (11). In subjects with prior invasive cancer or ductal carcinoma *in situ*, only the contralateral breast was aspirated. Slides for cytology were prepared in the laboratory of Dr. Carol Fabian (9, 10, 14). Morphologic assessment, Masood cytology index scores, and cell count were assigned by a single dedicated pathologist who was blinded to BMI (C.M.Z.; refs. 9, 15). Cells were given a score of 1 to 4 for each of six morphologic characteristics including cell arrangement, pleomorphism, number of myoepithelial cells, anisonucleosis, nucleoli, and chromatin clumping; the sum of these points computed the Masood score: ≤10, nonproliferative (normal); 11 to 13, hyperplasia; 14 to 16, atypia; and ≥17, suspicious cytology (9, 15). The number of epithelial cells was quantitated per breast and classified as <10 cells (insufficient quantity for analysis); 10 to 99 cells; 100 to 499 cells; 500 to 999 cells; 1,000 to 4,999 cells; and ≥5,000 cells.

Statistics. Differences in mean cell counts and Masood cytology index scores between women with BMIs <25 kg/m^2 versus those with BMIs ≥25 kg/m^2 were tested using the pooled and Satterthwaite t tests, respectively.

Results

Subject Demographics. This study was conducted among 62 women who underwent RPFNA from March 2003 to August 2005. Table 1 lists the characteristics of the total study sample, as well as their distribution according to BMI. Most women were Caucasian and postmenopausal and none were currently taking HRT.

Increased BMI Is Associated with Increased Masood Cytology Index. The distribution of Masood cytology index score and RPFNA cell counts was reported as a function of BMI. RPFNA aspirates were stratified using the Masood cytology index. Figure 1 depicts the distribution of Masood Cytology and RPFNA cell count as a function of BMI. Masood cytology index scores were significantly higher (P = 0.017) in women with BMIs ≥25 kg/m^2 (13.9 ± 0.42 kg/m^2) than in women with BMIs <25 kg/m^2 (12.7 ± 0.29 kg/m^2). Women with BMIs ≥25 kg/m^2 also had significantly higher (P = 0.028) RPFNA epithelial cell counts (1,230 ± 272) than women whose BMIs were lower (521 ± 185).

These observations show that high BMI is associated with increased RPFNA cytologic abnormalities as measured by the Masood cytology index. No significant differences were detected between groups defined by BMI including race, prior abnormal biopsy, and age. Importantly, no women were using hormone replacement at the time of undergoing RPFNA. These data show that high BMI is associated with increased RPFNA cytologic abnormalities as measured by the Masood cytology index.

Discussion

These findings represent the first reported data to show that BMI is associated with direct cytologic abnormalities within the breast. These results are noteworthy given the mean Masood Index score among overweight women was 13.9, which approaches the Masood cutoff point of 14 established for atypia. The presence of atypia in RPFNA from high-risk

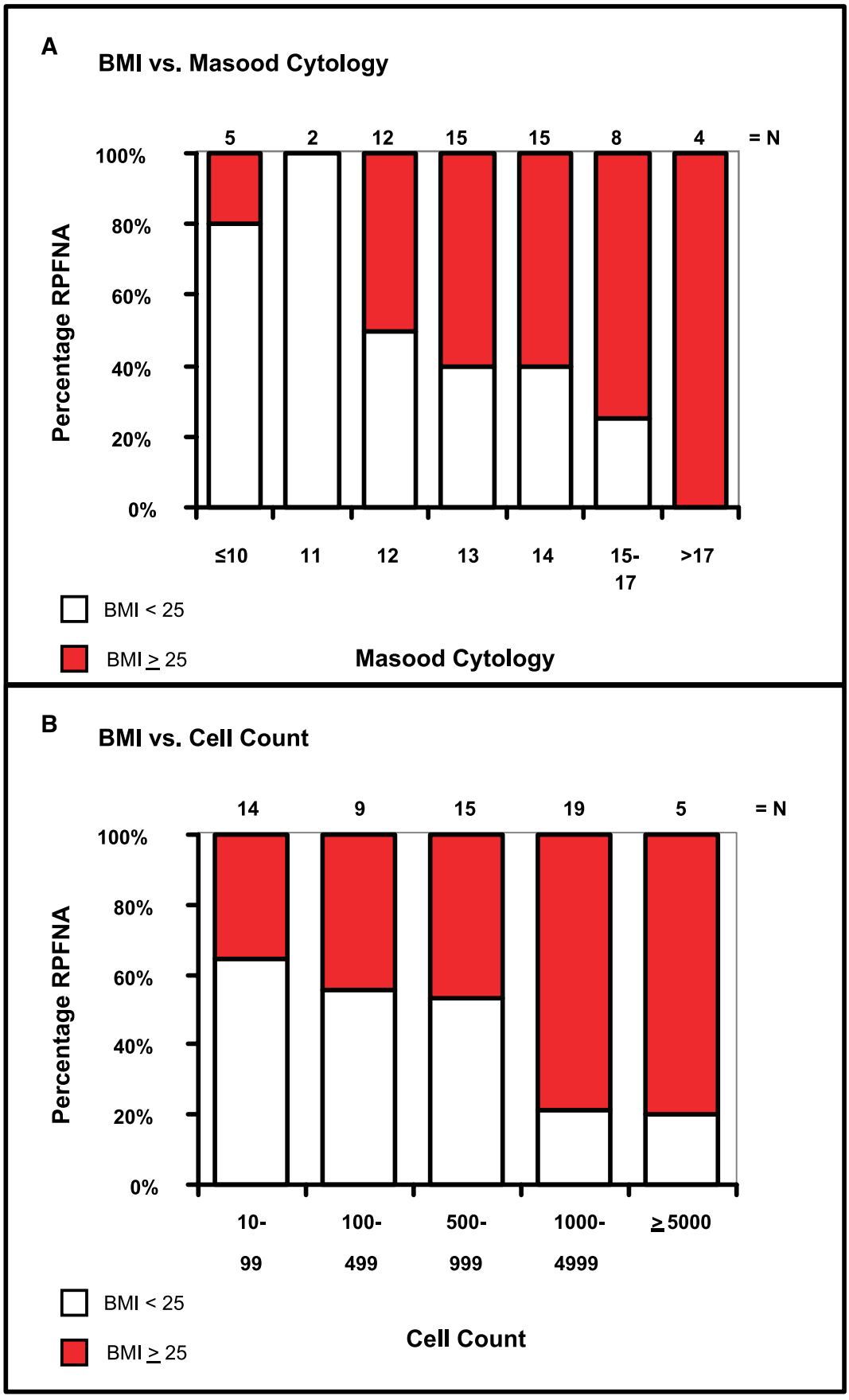

Figure 1. RPFNA cytology and cell counts in overweight/obese versus normal weight women. **A,** distributions of Masood cytology index scores for RPFNA specimens obtained for women with BMI $\geq$ 25 kg/m^2 versus BMI < 25 kg/m^2. **B,** cell count distributions of women with BMI $\geq$25 kg/m^2 versus BMI < 25 kg/m^2. *N,* number of subjects for each variable subclassification.

women has been previously shown to be an independent short-term risk factor for breast cancer (9). Importantly, none of our 62 subjects currently used HRT (Table 1), thus eliminating a potential confounding variable.

The limitations of this study are that (*a*) we have not tested for the stability of RPFNA cytology in all of our subjects over time and (*b*) we have only one single dedicated cytopathologist. Currently, there are no published studies documenting the interobserver and intraobserver variability of RPFNA over time. Repeat RPFNA cytology was done in 32 of our 62 subjects as part of ongoing studies: (*a*) 23 of 32 (72%) subjects had the same RPFNA score on repeat testing; (*b*) 7 of 32 (22%) had a RPFNA score that differed by one Masood Index point; and (*c*) 2 of 32 (6%) subjects had RPFNA scores that differed by two points. Multi-institutional studies are under way to test for the interobserver and intraobserver variability and reproducibility of RPFNA cytology in a multi-institutional cohort.

Given the exploratory nature of this study, such findings require further validation. Additional studies are also needed to test whether these RPFNA markers are responsive to changes in body weight. If indeed they are, then these biomarkers may serve as useful intermediate end points that could be used in diet and exercise interventions that are aimed at reducing weight as a means of breast cancer prevention. The discovery of such biomarkers would have tremendous potential import because they would allow prevention studies to be accomplished in far less time, with far fewer participants and using far fewer resources.

References

1. Freedman AN, Graubard BI, Rao SR, McCaskill-Stevens W, Ballard-Barbash R, Gail MH. Estimates of the number of U S. women who could benefit from tamoxifen for breast cancer chemoprevention. J Natl Cancer Inst 2003;95:526–32.
2. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U S. adults. N Engl J Med 2003;348:1625–38.
3. Kroenke CH, Chen WY, Rosner B, Holmes MD. Weight, weight gain, and survival after breast cancer diagnosis. J Clin Oncol 2005;23:1370–8.
4. Lahmann PH, Hoffmann K, Allen N, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer 2004;111:762–71.
5. Morimoto LM, White E, Chen Z, et al. Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Contr 2002;13:741–51.
6. World Health Organization (200). IARC Handbook of Cancer Prevention (ISSN 1027–5622) Vol. 6 Lyon, France: IARC Press.
7. Boyd NF, Rommens JM, Vogt K. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6:798–808.
8. McTiernan A. Obesity and cancer: the risks, science, and potential management strategies. Oncology (Huntingt) 2005;19:871–81.
9. Fabian CJ, Kimler BF, Zalles CM, et al. Short-term breast cancer prediction by random preiareolar fine-needle aspiration cytology and the Gail risk model. J Natl Cancer Inst 2000;92:1217–27.
10. Fabian CJ, Kimler BF, Brady DA, et al. A phase II breast cancer chemoprevention trial of oral difluoromethylornithine: breast tissue, imaging, and serum and urine biomarkers. Clin Cancer Res 2002;8:3105–17.
11. Bean GR, Scott V, Yee L, et al. Retinoic acid receptor-β2 promoter methylation in random periareolar fine needle aspiration. Cancer Epidemiol Biomarkers Prev 2005;14:790–8.
12. National Cancer Institute. Breast cancer assessment tool. Avaialble from: http://bcra.nci.nih.gov.
13. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Am J Clin Nutr 1998;68:899–917.
14. Zalles CM, Kimler BF, Kamel S, McKittrick R, Fabian CJ. Cytology patterns in random aspirates from women at high and low risk for breast cancer. Breast J 1995;1:343–9.
15. Masood S, Frykberg ER, McLellan GL, Scalapino MC, Mitchum DG, Bullard JB. Prospective evaluation of radiologically directed fine-needle aspiration biopsy of nonpalpable breast lesions. Cancer 1990;66:1480–7.

Overweight and Obese Perimenopausal and Postmenopausal Women Exhibit Increased Abnormal Mammary Epithelial Cytology

Victoria L. Seewaldt, Vanessa Goldenberg, Lee W. Jones, et al.

Cancer Epidemiol Biomarkers Prev 2007;16:613-616.