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FOR CLINICIANS WHO PROVIDE CARE FOR WOMEN

## Thoughts on the prevention and early detection of postmenopausal ovarian cancer

► DANIEL W. CRAMER, MD, AND ALLISON F. VITONIS, BA, SM

In 2010, approximately 21,880 women in the United States developed ovarian cancer and 13,850 died from the disease—about 80% of whom were postmenopausal (<http://seer.cancer.gov/statfacts/html/ovary>).

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The high case-to-fatality ratio (about 64%) is due to the tendency for this cancer to present at advanced stages and for resistance to develop after chemotherapy has been initially effective. While advances in understanding the molecular pathogenesis of ovarian cancer may ultimately lead to better treatments, preventing ovarian cancer in the first place or detecting it earlier is more desirable. This article reviews the risk factors for ovarian cancer and discusses strategies for prevention and early detection.

### Risk factors for ovarian cancer

A family history of breast cancer that occurred premenopausally or ovarian cancer that occurred at any age is a strong risk factor for ovarian cancer. The first gene associated with hereditary breast-ovarian cancer syndrome, termed *BRCA1*, was identified in 1994, and a second gene, *BRCA2*, was identified in 1995. Hundreds of mutations in these genes, which deleteriously affect their function and increase the risks for breast and ovarian cancer, have been identified.

Specific founder mutations (specific mutations that appear repeatedly

in ethnically defined groups because of a shared common ancestry) have been identified in several ethnic groups, including Ashkenazi Jews, French Canadians, and Icelanders.<sup>1</sup> People of Ashkenazi Jewish heritage have a 1 in 50 likelihood of carrying 1 of 3 *BRCA* founder mutations, compared with the 1 in 800 likelihood in the general population.

Cumulative risk up to age 70 for breast cancer is about 12% in noncarriers of *BRCA* mutations, but risk rises to 40% to 55% if a *BRCA1* or *BRCA2* mutation is present; similarly, the risk for ovarian cancer is approximately 1.6% in noncarriers but 20% to 40% in women with a *BRCA1* or *BRCA2* mutation.<sup>2</sup> Among all women who present with ovarian cancer, about 8% have a *BRCA* mutation,<sup>3</sup> while the figure is about 40% for women who are Ashkenazi Jewish.<sup>4</sup> An additional 2% of women with ovarian cancer will have mutations in 1 of the mismatch repair genes associated with hereditary non-polyposis colorectal cancer (Lynch) syndrome.<sup>3</sup>

Good epidemiologic data demonstrate that various reproductive and lifestyle factors alter risk for ovarian

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## Keep your eye on the moving targets

Despite the many breakthroughs in knowledge and treatments for various types of cancer, we remain relatively helpless with ovarian cancer once it is clinically detected. Our principal current pathway for reducing morbidity and mortality from this deadly disease centers on prevention in high-risk women. Yet prevention comes at a price: many premenopausal women will undergo oophorectomy and endure symptoms and increased risk for other diseases to avoid ovarian cancer. Nonetheless, the benefit-to-risk ratio of prophylactic oophorectomy is favorable.

Early detection of ovarian cancer is considerably more difficult and is often a losing battle, since the number of women who require screening and may undergo perhaps inappropriate intervention often exceeds the number who will receive curative surgery. Thus, prevention may be the best form of cure for ovarian cancer, at least until we better understand the biology of this disease and can intervene more effectively or develop a perfect screening test. In this issue of *Menopausal Medicine*, Daniel W. Cramer, MD, and Allison F. Vitonis, BA, SM, guide us through the logic for prevention and early detection strategies. They show us how criteria for preventive intervention by oophorectomy may be evolving.

The role of estrogen in vascular health is also a moving target. Kerrie L. Moreau, PhD, explains the progression in thinking about estrogen and the vascular system. Although we are all well acquainted with the fact that estrogen did not seem to effect cardioprotection and even led to harm in one subgroup of the Women's Health Initiative, the finding remains at odds with a large body of observational data, and the discrepancy has yet to be reconciled. While the "critical window" hypothesis is plausible, it has yet to be proven. The target in this case may be an initially healthy blood vessel, well endowed with estrogen receptors and having minimal plaque, that evolves into an inflamed, atherosclerotic, thrombus-prone structure in which estrogen is ineffective and possibly prothrombotic.

We look forward to learning more about these mechanisms over the coming year. Happy New Year to all. The final moving target for this issue is your Editor! It has been a pleasure to have served for the past 3 years. I now turn over the task to the capable hands of Cynthia K. Sites, MD.

**Nanette F. Santoro, MD**



cancer. Risk is reduced by a greater number of pregnancies and years of oral contraceptive (OC) use, having breast-fed, and having had tubal sterilization. A pooled analysis showed a 30% decrease in risk with first birth and an additional reduction of about 14% with each subsequent pregnancy.<sup>5</sup> OC use is associated with about a 30% reduction in risk,<sup>5-8</sup> which further declines with duration of OC use. Each month of breast-feeding results in a 1% to 2% decrease in ovarian cancer risk<sup>5,9</sup>; and tubal sterilization decreases risk by about 18% to 40%.<sup>8,10-13</sup> Older studies suggest that women who had mumps parotitis have a reduced ovarian cancer risk,<sup>14,15</sup> and a more recent study found that women who had mastitis during breast-feeding are also at decreased risk.<sup>16</sup>

Factors that increase risk for ovarian cancer include a greater number of ovulatory cycles *not* interrupted by pregnancies, breast-feeding, or OC use<sup>17-21</sup>; long-term use of talc powder in genital hygiene<sup>22-24</sup>; and endometriosis.<sup>25</sup> The odds ratios (relative risks) associated with some of these risk factors from our own case-control studies of ovarian cancer are listed in the **TABLE**.

### Potential mechanisms involved in risk

To explain these risk factors, the popular “incessant ovulation” theory proposes that repeated disruption and repair of ovarian epithelium leads to accumulated damage progressing to epithelial ovarian cancers.<sup>26</sup> Other theories emphasize hormonal pathways through high gonadotropin<sup>27</sup> or high androgen and low progesterone levels.<sup>28</sup> Chronic inflammation leading to oxidative DNA damage might explain risk associated with ovulation, talc use, and endometriosis.<sup>29</sup>

Recently, we hypothesized that

risk for ovarian cancer is determined by factors affecting the immune system.<sup>16</sup> These include acute events in younger life, such as mumps, mastitis, tubal sterilization, and others, that affect tissues that express cell-surface glycoproteins known as mucins (MUC), especially MUC1. These events may prime the immune system to recognize and eliminate ovarian cancer precursors that also express an inflammatory form of MUC1. Conversely, events like incessant ovulation, talc use, and endometriosis also affect tissues that express MUC1 but lead to chronic, low levels of MUC1 and immune tolerance of an emerging cancer. This theory unites many seemingly diverse risk factors for ovarian cancer and provides a theoretical basis for risk algorithms that combine diverse factors, such as those illustrated in the Table.

**R**ecently, we hypothesized that risk for ovarian cancer is determined by factors affecting the immune system, including acute events in younger life that affect tissues that express cell-surface glycoproteins known as mucins.

### Preventive approaches

**Lifestyle factors.** Since the introduction of OCs 50 years ago, it is estimated that 100,000 deaths from ovarian cancer have been prevented worldwide by OC use.<sup>30</sup> When weighing the risks and benefits of OCs, protection against ovarian cancer should be considered, especially for women who may have a family history of ovarian cancer or painful periods suggestive of endometriosis. Breast-feeding should be encouraged after childbirth. Women should be cautioned to avoid using talc powders in genital hygiene. Gra-

dients in ovarian cancer risk by latitude suggest that sunlight and vitamin D may be important<sup>31</sup>; diets high in antioxidants may also be beneficial.<sup>32</sup> Although evidence is not definitive regarding the role of nutrition in preventing ovarian cancer, vitamin D supplements and a diet rich in colorful fruits and vegetables should be encouraged.

**Prophylactic surgery.** An obvious preventive measure is to remove the ovaries (and fallopian tubes) of women at high familial risk for disease. This is a weighty decision that ideally should involve a specialist who manages patients at genetic risk for cancer. Clearly, the process must begin with recognizing the woman at familial risk. Often, the primary care provider or gynecologist who takes a careful family history initiates a referral to the familial-risk clinic.

A history of 2 or more primary relatives (ie, mother, sister, daughter) with ovarian cancer or premenopausal breast cancer sets a red flag. It should also be appreciated that a Jewish woman who reports having only a single primary relative with ovarian cancer may have about a 20% chance herself of having a founder *BRCA* mutation. When possible, an affected relative should be tested first, since the results can confirm the link between cancer diagnosis and gene mutation, inform whether genetic testing in a relative is worthwhile, and allow testing for a particular mutation—which is less costly than a full screen. Reimbursement for testing varies among health insurance plans, but testing is generally covered for individuals with breast or ovarian cancer or if a mutation has been identified in the family. The Web site [www.geneclinics.org](http://www.geneclinics.org) educates physicians about cancer syndromes and genetic laboratories, while [www.facingourrisk.org](http://www.facingourrisk.org) is geared to patients.

**TABLE Conditions/exposures that increase the risk of ovarian cancer**

	<b>INVASIVE CASES N (%) (N=468)</b>	<b>CONTROLS N (%) (N=556)</b>	<b>ODDS RATIO (95% CI)</b>	<b>P VALUE</b>
Jewish ethnicity				
No	431 (92.1)	527 (94.8)	1.00	
Yes	37 (7.9)	29 (5.2)	1.62 (0.98, 2.70)	.06
OC use				
≥1 year	111 (23.7)	204 (36.7)	1.00	
<1 year or no use	357 (76.3)	352 (63.3)	1.90 (1.41, 2.57)	<.0001
Parity				
Parous	372 (79.5)	494 (88.8)	1.00	
Nulliparous	96 (20.5)	62 (11.2)	2.12 (1.50, 3.01)	<.0001
Breast-feeding				
Any	127 (27.1)	219 (39.4)	1.00	
None	341 (72.9)	337 (60.6)	1.72 (1.31, 2.24)	<.0001
Tubal ligation				
Yes	59 (12.6)	98 (17.6)	1.00	
No	409 (87.4)	458 (82.4)	1.45 (1.02, 2.08)	.04
Painful periods or endometriosis				
No	293 (62.6)	374 (67.3)	1.00	
Yes	175 (37.4)	182 (32.7)	1.26 (0.97, 1.63)	.08
Long-term genital talc use (≥10 years)				
No	380 (81.2)	475 (85.4)	1.00	
Yes	88 (18.8)	81 (14.6)	1.39 (1.00, 1.93)	.05
Total number of risk factors				
0-1	32 (6.8)	87 (15.6)	1.00	
2	100 (21.4)	161 (29.0)	1.64 (1.02, 2.66)	.04
3	152 (32.5)	174 (31.3)	2.31 (1.44, 3.70)	.0005
4	112 (23.9)	93 (16.7)	3.19 (1.93, 5.25)	<.0001
≥5	72 (15.4)	41 (7.4)	4.78 (2.72, 8.40)	<.0001

This table illustrates risk factors for ovarian cancer from 2 phases of data collection from the New England Case Control Study of Ovarian Cancer. The analysis excludes premenopausal women, those with a personal history of breast cancer or family history of ovarian cancer or early-onset breast cancer, and women with tumors of borderline malignancy. Painful periods were defined as periods that usually required analgesics and frequently led to missed work or school. Talc use was defined as use of cosmetic powders for at least 2 weeks per month for 10 or more years. Although Jewish ethnicity and painful periods were of borderline significance in a univariate analysis, they contributed significantly to the risk factor score. Odds ratios are adjusted for study center, reference age, and study phase. A detailed description of this data set can be found in: Terry KL, De Vivo I, Titus-Ernstoff L, et al. Androgen receptor cytosine, adenine, guanine repeats, and haplotypes in relation to ovarian cancer risk. *Cancer Res.* 2005;65:5974-5981.

Pathologic study of tissue specimens from women who had prophylactic surgery has produced new lines of thinking about the origin of ovarian cancer. Although atypical epithelium is occasionally observed in the ova-

ries, abnormalities within the fallopian tubes, especially in the fimbriae, are more common. A spectrum of lesions can be found, including serous tubal intraepithelial neoplasia (STIC), which has all the features of serous carcinoma

of the ovary.<sup>33</sup> These findings regarding the distal fallopian tubes have several ramifications: they (1) necessitate that ovaries *and tubes* be removed at prophylactic surgery; (2) may explain why women with serous cancer usually



present at stage III or greater if cancer cells “dribble” from the tubes into the pelvis; (3) limit pelvic ultrasound as a screening tool, since STIC may not present an ultrasound signal; and (4) raise the possibility that salpingectomy alone might suffice for risk reduction for ovarian cancer.

An opportunity for prevention occurs at the time of hysterectomy for benign disease, when a decision must be made about bilateral salpingo-oophorectomy (BSO). Physicians commonly recommend incidental BSO for postmenopausal women presenting for hysterectomy. However, citing evidence that postmenopausal ovaries may still be hormonally active, Parker et al recently concluded that BSO should not be routinely offered to women without a family history who are at “average risk” of ovarian cancer when undergoing hysterectomy for benign disease, since overall mortality may be poorer and only risk of death from ovarian cancer markedly reduced for those who had BSO.<sup>34</sup> Forcing a simple dichotomy between women with a family history of ovarian cancer and women at “average risk” does not serve patients well when a more personalized profile is possible.

### Tools for early detection of ovarian cancer

A major obstacle to early detection of ovarian cancer is the relative rarity of the disease, with only about 1/2000 postmenopausal women being diagnosed in any 1 year. The low incidence requires a test with very high specificity to reduce false positives, but good sensitivity as well to increase the predictive value of a positive test.

**Screening tests.** Recently, the results of 2 trials of ovarian cancer screening in postmenopausal women were published and reached different conclusions. In the Prostate, Lung,

Colorectal, and Ovarian (PLCO) cancer screening trial, approximately 39,000 women were enrolled between 1993 and 2001.<sup>35</sup> Subjects were randomized to receive annual screens with either CA125 and transvaginal ultrasound or conventional care. This trial showed that, after 4 rounds of screening, the combination screening regimen produced a high ratio of surgeries to detected cancers (20 to 1) without a clear shift toward earlier-stage disease. The authors concluded that screening for ovarian cancer in the general population could not be recommended, advice reiterated in an editorial and clinical opinion piece.<sup>36,37</sup>

In contrast, Menon et al reported results of the prevalence screen in the large United Kingdom Collabora-

**We are concerned with the assumption that women fall into only 2 categories: those with a family history of ovarian or breast cancer and those at “average risk.”**

tive Trial of Ovarian Cancer Screening (UKCTOCS), in which 2 screening modalities were compared: CA125 used as the primary screen with referral for ultrasound if necessary versus ultrasound alone.<sup>38</sup> The trajectory of serial CA125 values was considered in interpreting screening results. The sensitivity, specificity, and positive predictive values for ovarian and tubal cancers were all higher with CA125 followed by ultrasound (89.4%, 99.8%, and 43.3%) than with ultrasound alone (84.9%, 98.2%, and 5.3%). The ratio of operations per malignancy found within 1 year of the prevalence screen was 2.3 for CA125 but 18.8 for transvaginal ultrasound (similar to that seen in the PLCO trial). The authors

concluded that using CA125 as an initial triaging tool leads to fewer false positives compared with ultrasound alone (or simultaneously with CA125) and that general population screening using CA125 is “feasible.”<sup>38</sup>

It should be emphasized that only CA125 has been tested in randomized screening trials. The far more costly OVA1 test (an in vitro diagnostic multivariate index assay), which was recently FDA approved for use in distinguishing benign from malignant pelvic masses for referral decisions, cannot be assumed to have better performance than CA125 in the early detection setting.<sup>39</sup>

In a recent article, Clarke-Pearson presented a scenario that may resonate with clinicians.<sup>37</sup> He described a 56-year old woman with no family history of ovarian or breast cancer and a normal physical examination (including pelvic and rectal examination). The patient had requested screening after a friend died from ovarian cancer. After providing an excellent review of the current state of the art for ovarian cancer screening, the author advised against “screening in a patient who is at average risk, such as the woman described in the vignette.”

**Presence of risk factors.** We are concerned with the assumption that women fall into only 2 categories: those with a family history of ovarian or breast cancer and those at “average risk.” From the vignette, this woman at “average” risk could: be Jewish, never had children or breast-fed, never have used birth control pills or had a tubal ligation, have a history of endometriosis, and have used cosmetic talc in her hygiene. Our data in the Table suggest that each of these factors may increase risk for ovarian cancer some 1.3- to 2.1-fold and, importantly, may cumulate to increase risk further. If our patient had 5 of these risk factors,

she may be at about 5-fold greater risk for ovarian cancer than a woman with none or only 1 of these factors. If this were our patient with the factors described, we would order a CA125 (but not an ultrasound or OVA1 panel) and caution her that insurance may not cover it. A CA125 above the upper limit of normal for the laboratory used would necessitate at least a repeat CA125 test in a month or follow-up ultrasound. Cancer-associated CA125 will continue to rise, whereas the level may fall or stay the same in the benign disease setting.

Clearly, we cannot offer an absolute rule about what number of risk factors warrants consideration of ordering a CA125 test (or recommending BSO for a woman presenting for hysterectomy for benign disease). Our point is that women do not fall into only 2 categories, average risk and those with familial history. Discussing the various risk factors may help patients be comfortable with whatever

**General population screening for ovarian cancer is discouraged by all official organizations, but that should not dissuade physicians from selectively ordering CA125 for an individual postmenopausal woman who may score high in her number of risk factors or symptom index.**

recommendation is offered.

**Symptom frequency and duration.** There has also been work with a symptom index to trigger thinking about a possible diagnosis of ovarian cancer. Women with ovarian cancer were more likely to report bloating, increased abdominal size, and urinary urgency and frequency that was more likely to have new onset within a 6-month period and greater frequency of occurrence (20 to 30 times per month).<sup>40</sup>

## Conclusion

Use of OCs, breast-feeding after pregnancy, and avoiding use of talc in genital hygiene are simple ways to reduce risk for ovarian cancer. Ovarian cancer can largely be prevented in women who have strong family histories or known genetic risk for the disease by carefully timed prophylactic BSO.<sup>41</sup> Incidental BSO in women presenting for hysterectomy for benign disease (or at least salpingectomy) should be considered *if they are at elevated risk*.

General population screening for ovarian cancer is discouraged by all official organizations, but that should not dissuade physicians from selectively ordering CA125 for an individual postmenopausal woman who may score high in her number of risk factors or symptom index. Ovarian cancer is not silent but does speak quietly, requiring the practitioner to listen harder. ■

## References

- Lindor NM, McMaster ML, Lindor CJ, Greene MH. Concise handbook of familial cancer susceptibility syndromes—second edition. *J Natl Cancer Inst Monogr.* 2008;1-93.
- Chen S, Iversen ES, Friebel T, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. *J Clin Oncol.* 2006;24:863-871.
- Rubin SC, Blackwood MA, Bandera C, et al. BRCA1, BRCA2, and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic testing. *Am J Obstet Gynecol.* 1998;178:670-677.
- Robles-Diaz L, Goldfrank DJ, Kauff ND, et al. Hereditary ovarian cancer in Ashkenazi Jews. *Fam Cancer.* 2004;3:259-264.
- Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. *Am J Epidemiol.* 1992;136:1184-1203.
- Kumle M, Weiderpass E, Braaten T, et al; Norwegian-Swedish Women's Lifestyle and Health Cohort Study. Risk for invasive and borderline epithelial ovarian neoplasias following use of hormonal contraceptives: the Norwegian-Swedish Women's Lifestyle and Health Cohort Study. *Br J Cancer.* 2004;90:1386-1391.
- Purdie DM, Siskind V, Bain CJ, et al. Reproduction-related risk factors for mucinous and nonmucinous epithelial ovarian cancer. *Am J Epidemiol.* 2001;153:860-864.
- Tworoger SS, Fairfield KM, Colditz GA, et al. Association of oral contraceptive use, other contraceptive methods, and infertility with ovarian cancer risk. *Am J Epidemiol.* 2007;166:894-901.
- Danforth KN, Tworoger SS, Hecht JL, et al. Breast-feeding and risk of ovarian cancer in two prospective cohorts. *Cancer Causes Control.* 2007;18:517-523.
- Green A, Purdie D, Bain C, et al. Tubal sterilisation, hysterectomy and decreased risk of ovarian cancer. Survey of Women's Health Study Group. *Int J Cancer.* 1997;71:948-951.
- Hankinson SE, Hunter DJ, Colditz GA, et al. Tubal ligation, hysterectomy, and risk of ovarian cancer: a prospective study. *JAMA.* 1993;270:2813-2818.
- Ness RB, Grisso JA, Cottreau C, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. *Epidemiology.* 2000;11:111-117.
- Purdie D, Green A, Bain C, et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group. *Int J Cancer.* 1995;62:678-684.
- Newhouse ML, Pearson RM, Fullerton JM, et al. A case control study of carcinoma of the ovary. *Br J Prev Soc Med.* 1977;31:148-153.
- West RO. Epidemiologic study of malignancies of the ovaries. *Cancer.* 1966;19(7):1001-1007.
- Cramer DW, Titus-Ernstoff L, McKolanis JR, et al. Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2005;14:1125-1131.
- Casagrande JT, Louie EW, Pike MC, et al. "Incessant ovulation" and ovarian cancer. *Lancet.* 1979;2:170-173.
- Pelucchi C, Galeone C, Talamini R, et al. Lifetime ovulatory cycles and ovarian cancer risk in 2 Italian case-control studies. *Am J Obstet Gynecol.* 2007;196:83.e1-7.
- Rosner BA, Colditz GA, Webb PM, Hankinson SE. Mathematical models of ovarian cancer incidence. *Epidemiology.* 2005;16:508-515.
- Schildkraut JM, Bastos E, Berchuck A. Relationship between lifetime ovulatory cycles and overexpression of mutant p53 in epithelial ovarian cancer. *J Natl Cancer Inst.* 1997;89:932-938.
- Terry KL, Titus-Ernstoff L, McKolanis JR, et al. Incessant ovulation, mucin 1 immunity, and risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2007;16:30-35.
- Cramer DW, Liberman RF, Titus-Ernstoff L, et al. Genital talc exposure and risk of ovarian cancer. *Int J Cancer.* 1999;81:351-356.



23. Cramer DW, Welch WR, Scully RE, Wojciechowski CA. Ovarian cancer and talc: a case-control study. *Cancer*. 1982;50:372-376.
24. Gates MA, Tworoger SS, Terry KL, et al. Talc use, variants of the GSTM1, GSTT1, and NAT2 genes, and risk of epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev*. 2008;17:2436-2444.
25. Vlahos NF, Kalampokas T, Fotiou S. Endometriosis and ovarian cancer: a review. *Gynecol Endocrinol*. 2010;26:213-219.
26. Fathalla MF. Incessant ovulation--a factor in ovarian neoplasia? *Lancet*. 1971;2:163.
27. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. *J Natl Cancer Inst*. 1983;71:717-721.
28. Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J Natl Cancer Inst*. 1998;90:1774-86.
29. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. *J Natl Cancer Inst*. 1999;91:1459-1467.
30. Beral V, Doll R, Hermon C, et al; Collaborative Group on Epidemiological Studies of Ovarian Cancer. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. *Lancet*. 2008;371:303-314.
31. Garland CF, Mohr SB, Gorham ED, et al. Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. *Am J Prev Med*. 2006;31:512-514.
32. Jeong NH, Song ES, Lee JM, et al. Plasma carotenoids, retinol and tocopherol levels and the risk of ovarian cancer. *Acta Obstet Gynecol Scand*. 2009;88:457-462.
33. Crum CP, Drapkin R, Miron A, et al. The distal fallopian tube: a new model for pelvic serous carcinogenesis. *Curr Opin Obstet Gynecol*. 2007;19:3-9.
34. Parker WH, Broder MS, Chang E, et al. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the Nurses' Health Study. *Obstet Gynecol*. 2009;113:1027-1037.
35. Partridge E, Kreimer AR, Greenlee RT, et al; PLCO Project Team. Results from four rounds of ovarian cancer screening in a randomized trial. *Obstet Gynecol*. 2009;113:775-782.
36. Mutch DG. Ovarian cancer: to screen or not to screen. *Obstet Gynecol*. 2009;113:772-774.
37. Clarke-Pearson DL. Clinical practice. Screening for ovarian cancer. *N Engl J Med*. 2009;361:170-177.
38. Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). *Lancet Oncol*. 2009;10:327-340.
39. Muller CY. Doctor, should I get this new ovarian cancer test—OVA1? *Obstet Gynecol*. 2010;116(2 Pt 1):246-247.
40. Goff BA, Mandel LS, Melancon CH, Muntz HG. Frequency of symptoms of ovarian cancer in women presenting to primary care clinics. *JAMA*. 2004;291:2705-2712.
41. Kauff ND, Barakat RR. Risk-reducing salpingo-oophorectomy in patients with germline mutations in BRCA1 or BRCA2. *J Clin Oncol*. 2007;25:2921-2927.

# The modulatory influence of estrogen on vascular endothelial function in women: Is it all about timing?

► **KERRIE L. MOREAU, PHD**

**T**he vascular endothelium, a single layer of cells that line the inner surface of blood vessels, plays a key role in maintaining vascular homeostasis and vascular health. A normal functioning vascular endothelium regulates vascular tone, coagulation, proliferation, and inflammation through the synthesis and release of various factors in response to physical and chemical stimuli. Loss of normal endothelial function is thought to be a critical step in the initiation and progression of atherosclerosis.<sup>1</sup>

Indeed, endothelial dysfunction is a predictor of future cardiovascular events.<sup>2</sup> Notably, premenopausal women have a lower incidence of cardiovascular disease (CVD) compared with age-matched men.<sup>3</sup> This apparent female advantage has long been attributed to the protective effects of estrogen. Overwhelming data from experimental studies show that estrogen deficiency plays a significant role in vascular endothelial dysfunction and atherosclerotic

progression.<sup>4,5</sup> Despite this, recent clinical trials investigating the cardioprotective benefits of menopausal hormone therapy (MHT) in postmenopausal women (with conjugated equine estrogens [CEE] alone and combined with medroxyprogesterone acetate [MPA]), demonstrate no cardiovascular benefit of either regimen.<sup>6-8</sup> Several explanations have been proposed for the discordant findings between clinical trials and experimental evidence, including the type of MHT, age of the postmenopausal women, and use of MHT by women with pre-existing coronary artery disease.

## Estrogen deficiency and vascular endothelial function

Reduced vascular endothelial-dependent vasodilation, characterized by the loss of endothelial-derived nitric oxide (NO) production, is a key feature of endothelial dysfunction.<sup>9</sup>

Aging is associated with a decline in endothelial-dependent vasodilation in both men and women.<sup>10</sup> In men, this age-related decline begins at an earlier age; in women, however, the impaired endothelial function is evident only after the menopause transition, when it steadily declines and becomes similar to that of older men by the sixth decade of life.<sup>10</sup> These data suggest a beneficial effect of estrogen on the vascular endothelium.

Results of studies in both experimental animals and in postmenopausal women are consistent with the notion that estrogen improves vascular endothelial function and increases NO

bioavailability. Endothelial-dependent dilation, plasma nitrate/nitrite concentrations (markers of NO production), and vasoconstrictor responses to acute blockade of endothelial nitric oxide synthase (eNOS, the enzyme necessary for the production of NO) are all augmented in the peripheral vasculature after, compared with before, estrogen exposure.<sup>11-13</sup>

Moreover, in premenopausal women, endothelial-dependent dilation is higher during the late follicular (higher estrogenic milieu) phase compared to the early follicular (low estrogen) phase of the menstrual cycle.<sup>14</sup> Because of the variations in circulating levels of sex hormones, the menstrual cycle provides a natural physiologic setting in which to isolate the independent effect of estrogen on endothelial function without the confounding effects of changes due to menopause, aging, or other pathophysiologic influences. Collectively, these data support the idea that estrogen exerts protective effects on vascular endothelial function.

## The timing hypothesis

Although the recent findings from the Women's Health Initiative (WHI) clinical trials studying the cardiovascular effects of MHT do not support the cardioprotective benefits of CEE alone or combined with MPA when initiated 10 to 20 years after the onset of menopause (and for CEE plus MPA, there is increased harm),<sup>8,15</sup> it is plausible that initiating MHT during a "critical window" of time may offer cardioprotective

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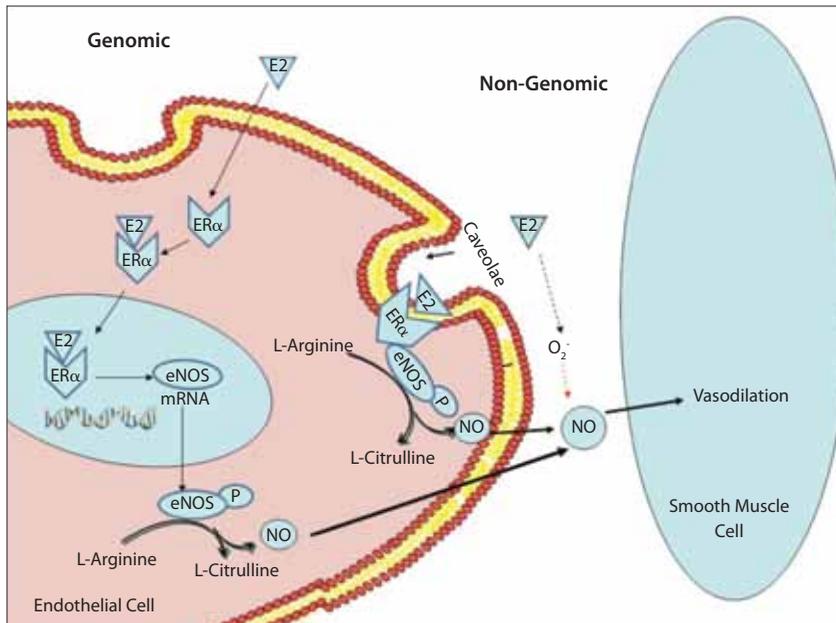
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**FIGURE** Genomic and nongenomic mechanisms of estrogen-induced nitric oxide (NO) production



In the genomic pathway, estrogen binds to endothelial cytosolic estrogen receptor (ER)- $\alpha$  and translocates to the nucleus, where it turns on gene transcription and stimulates and increases endothelial nitric oxide synthase (eNOS). In the nongenomic pathway, estrogen binds to ER $\alpha$  located in the endothelial cell membrane, which activates eNOS in the caveola. Activated eNOS promotes the transformation of L-arginine to L-citrulline and nitric oxide (NO) production. NO diffuses into the smooth muscle cells and causes vasodilation. Estrogen may also inhibit the production of reactive oxygen species ( $O_2^-$ ), thereby preventing the inactivation of NO.

tion. This critical window may occur during early estrogen withdrawal (ie, perimenopause to the early postmenopausal period), as suggested in animal models of atherosclerosis. Studies in postmenopausal women indicate that age appears to modulate the effects of MHT on vascular endothelial function: women aged 50 to 59 years have a marked improvement in endothelial function in response to MHT, whereas 60- to 79-year-old women show no evidence of improvement.<sup>16</sup>

Additionally, both acute and long-term MHT improves endothelial function more in women within 5 or fewer years of menopause onset compared with those who are remote from the last menstrual period by more than 5 years, indicating that the time since menopause influences the response of

endothelial function to MHT.<sup>17</sup>

Recent reanalyses of the WHI study support the timing hypothesis in that CEE alone was associated with fewer CV events (and total mortality) in women aged 50 to 59 years or within 10 years since menopause onset, while there was no protective effect in women older than 60 years of age or in those who were treated with CEE alone more than 10 years after menopause onset. Moreover, in women who were 20 or more years beyond menopause or 70 years of age or older, there was increased CV risk, particularly in women randomized to CEE plus MPA.<sup>18</sup>

These data are also consistent with those of the observational Nurses' Health Study, which showed a significant decrease in coronary heart dis-

ease (CHD) risk in women who initiated MHT between ages 50 and 59 years, but no beneficial effect of MHT on CHD risk in women who were 60 years of age or older at the time of MHT initiation.<sup>19</sup> The lack of benefit (and harmful effects) of MHT in older postmenopausal women may be due to alterations in the vascular wall that no longer enable the endothelium to respond to MHT, and in arteries with advanced atherosclerotic lesions, MHT may up-regulate plaque inflammatory processes, leading to plaque instability and increased CV events in the older women.<sup>20</sup>

### Type of HT and route of administration

The exact formulation and/or combination of hormones as well as the route of administration may influence vascular endothelial function. Key findings in these areas include:

**Oral vs transdermal route.** Oral MHT has been associated with an increase in some inflammatory markers, including C-reactive protein (CRP) and matrix metalloproteinase (MMP)-9 because of first-pass metabolism in the liver.<sup>21</sup> In a healthy artery this is not an issue because of estrogen's other anti-inflammatory effects; in an older and/or unhealthy artery with existing atherosclerotic lesions, an inflammatory cascade could cause plaque instability and CV events. Transdermal MHT is associated with a decrease in venous thromboembolism.<sup>22</sup> However, there was no decrease in acute CHD events (nonsignificant increase in event rates) in postmenopausal women with angiographically proven ischemic heart disease who were randomized to transdermal MHT.<sup>23</sup>

**Estrogen alone vs combined with progestin.** Estrogen plus progestin has been shown to minimize or abolish the

improvements in endothelial function seen with estrogen alone, although not consistently.<sup>24</sup> Inconsistencies may be related to progesterone type, dose, and/or continuous vs cyclic administration.

**Progesterin formulation.** Androgenic progestins reduce the beneficial effects of estrogens more than progesterone and such other progestins as cyproterone and dydrogesterone.<sup>25</sup> It is possible, however, that some negative effects are dose-dependent and that lower doses of androgenic progestins might not antagonize estrogen's effect.<sup>25</sup>

**CEE vs estradiol.** In vitro studies suggest that, compared with estradiol, CEE may be less effective in increasing nitric oxide.<sup>26</sup> In contrast, several in vivo studies have shown an improvement in endothelial-dependent dilation with CEE.<sup>24,27</sup>

### Mechanisms of estrogen action

The mechanisms by which estrogen modulates vascular endothelial function are not completely understood. In part, estrogen modulates vascular endothelial function by enhancing NO release and promoting vasodilation.<sup>28</sup> Estrogen triggers the release of NO through estrogen receptor (ER)- $\alpha$ -mediated nongenomic and genomic mechanisms (FIGURE). This occurs via activation of eNOS as well as by increasing eNOS protein via transcriptional regulation of the eNOS gene.<sup>28</sup>

Functional ER $\alpha$  appears to be required for normal vascular function and influences the bioavailability

of NO independent of the concentration of circulating estrogen.<sup>28</sup> Prolonged estrogen deprivation results in a marked reduction in ER $\alpha$  protein levels, resulting in a functional impairment of the ER $\alpha$ /eNOS signaling complex.<sup>29</sup> ER protein levels are lower in postmenopausal compared with premenopausal women and are strongly related to endothelial-dependent dilation.<sup>30</sup> Thus, altered endothelial ER $\alpha$  expression could explain the negative findings from the WHI study in that most of the women were approximately 10 to 20 years postmenopause. Sustaining estrogen levels during the menopause transition may preserve vascular endothelial health and may prevent or attenuate the decrease in vascular endothelial function by maintaining the expression and functioning of ER $\alpha$ .

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Other areas of ongoing investigation include estrogen's antioxidant and anti-inflammatory effects. Oxidative stress (imbalance between the production and destruction of reactive oxygen species [ROS]) and vascular

inflammation are key mechanisms mediating endothelial dysfunction with aging and, possibly, estrogen deficiency, although most of this evidence comes from animal studies.<sup>31-33</sup> The increases in ROS and inflammatory cytokines impair endothelial function through scavenging and inactivating NO, resulting in decreased NO bioavailability and vasodilation. Estrogen has direct antioxidant and anti-inflammatory effects in vitro and in vivo and is thought to play an inhibitory role in either the production and/or scavenging of ROS and proinflammatory cytokines. Thus, the impairment in vascular endothelial function with estrogen deficiency may be related to oxidative stress and vascular inflammation. However, this mechanism has yet to be clarified in women.

### Summary

CVD in women is a major public health concern. Vascular endothelial dysfunction is a critical factor involved in the etiology of CVD. Women endure a "double whammy" in that their arteries are exposed to adverse changes in other risk factors (ie, aging, cholesterol, adiposity) during a time (menopause) of vulnerability to damage mediated by changes in the hormonal environment. Because hormonal dysregulation may contribute to the initiation and progression of CVD, a better understanding is needed of the mechanisms mediating endothelial dysfunction in women as they transition through menopause and lose the vascular protection afforded by estrogen. ■

### References

1. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004;109:III-27-32.
2. Yeboah J, Crouse JR, Hsu F-C, et al. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation*. 2007;115:2390-2397.
3. Writing Group Members, Lloyd-Jones D, Adams RJ, Brown TM, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46-e215.
4. Holm P. Effect of estrogen on development of atherosclerosis: a review of experimental animal studies. *Dan Med Bull*. 2001;48:146-160.
5. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med*. 1999;340:1801-1811.
6. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA*. 1998;280:605-613.



7. Manson JE, Hsia J, Johnson KC, et al. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med.* 2003;349:523-534.
8. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA.* 2004;291:1701-1712.
9. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 1980;288:373-376.
10. Celermajer DS, Sorensen KE, Spiegelhalter DJ, et al. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol.* 1994;24:471-476.
11. Best PJ, Berger PB, Miller VM, Lerman A. The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Ann Intern Med.* 1998;128:285-288.
12. Kawano H, Motoyama T, Kugiyama K, et al. Gender difference in improvement of endothelium-dependent vasodilation after estrogen supplementation. *J Am Coll Cardiol.* 1997;30:914-919.
13. Lieberman EH, Gerhard MD, Uehata A, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med.* 1994;121:936-941.
14. Hashimoto M, Akishita M, Eto M, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation.* 1995;92:3431-3435.
15. Rouseau JE, Anderson GL, Prentice RL, et al; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288:321-333.
16. Sherwood A, Bower JK, McPetridge-Durdle J, et al. Age moderates the short-term effects of transdermal 17beta-estradiol on endothelium-dependent vascular function in postmenopausal women. *Arterioscler Thromb Vasc Biol.* 2007;27:1782-1787.
17. Vitale C, Mercurio G, Cerquetani E, et al. Time since menopause influences the acute and chronic effect of estrogens on endothelial function. *Arterioscler Thromb Vasc Biol.* 2008;28:348-352.
18. Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA.* 2007;297:1465-1477.
19. Grodstein F, Manson JE, Stampfer MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. *J Womens Health (Larchmt).* 2006;15:35-44.
20. Clarkson TB. Estrogen effects on arteries vary with stage of reproductive life and extent of subclinical atherosclerosis progression. *Menopause.* 2007;14:373-384.
21. Vehkavaara S, Silveira A, Hakala-Ala-Pietila T, et al. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. *Thromb Haemost.* 2001;85:619-625.
22. Canonico M, Oger E, Plu-Bureau G, et al; Estrogen and Thromboembolism Risk (ESTHER) Study Group. Hormone therapy and venous thromboembolism among postmenopausal women: impact of the route of estrogen administration and progestogens: the ESTHER Study. *Circulation.* 2007;115:840-845.
23. Clarke SC, Kelleher J, Lloyd-Jones H, et al. A study of hormone replacement therapy in postmenopausal women with ischaemic heart disease: the Papworth HRT Atherosclerosis Study. *BJOG.* 2002;109:1056-1062.
24. Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Effect of medroxyprogesterone acetate on endothelium-dependent vasodilation in postmenopausal women receiving estrogen. *Circulation.* 2001;104:1773-1778.
25. Rosano GMC, Vitale C, Silvestri A, Fini M. Metabolic and vascular effect of progestins in postmenopausal women: implications for cardioprotection. *Maturitas.* 2003;46:17-29.
26. Novensa L, Selent J, Pastor M, et al. Equine estrogens impair nitric oxide production and endothelial nitric oxide synthase transcription in human endothelial cells compared with the natural 17{beta}-estradiol. *Hypertension.* 2010;56:405-411.
27. Wakatsuki A, Ikenoue N, Shinohara K, et al. Effect of lower dosage of oral conjugated equine estrogen on inflammatory markers and endothelial function in healthy postmenopausal women. *Arterioscler Thromb Vasc Biol.* 2004;24:571-576.
28. Mendelsohn ME, Karas RH. HRT and the young at heart. *N Engl J Med.* 2007;356:2639-2641.
29. Pinna C, Cignarella A, Sanvito P, et al. Prolonged ovarian hormone deprivation impairs the protective vascular actions of estrogen receptor alpha agonists. *Hypertension.* 2008;51:1210-1217.
30. Gavin KM, Seals DR, Silver AE, Moreau KL. Vascular endothelial estrogen receptor alpha is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women. *J Clin Endocrinol Metab.* 2009;94:3513-3520.
31. Taddei S, Virdis A, Ghiadoni L, et al. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension.* 2001;38:274-279.
32. Virdis A, Ghiadoni L, Pinto S, et al. Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. *Circulation.* 2000;101:2258-2263.
33. Arenas IA, Armstrong SJ, Xu Y, Davidge ST. Chronic tumor necrosis factor- $\alpha$  inhibition enhances NO modulation of vascular function in estrogen-deficient rats. *Hypertension.* 2005;46:76-81.