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Red Versus White Wine as a Nutritional Aromatase Inhibitor in Premenopausal Women: A Pilot Study

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Abstract

Background: An increased risk of breast cancer is associated with alcohol consumption; however, it is controversial whether red wine increases this risk. Aromatase inhibitors (AIs) prevent the conversion of androgens to estrogen and occur naturally in grapes, grape juice, and red, but not white wine. We tested whether red wine is a nutritional AI in premenopausal women.

Methods: In a cross-over design, 36 women (mean age [SD], 36 [8] years) were assigned to 8 ounces (237 mL) of red wine daily then white wine for 1 month each, or the reverse. Blood was collected twice during the menstrual cycle for measurement of estradiol (E2), estrone (E1), androstenedione (A), total and free testosterone (T), sex hormone binding globulin (SHBG), luteinizing hormone (LH), and follicle stimulating hormone (FSH).

Results: Red wine demonstrated higher free T vs. white wine (mean difference 0.64 pg/mL [0.2 SE], $p = 0.009$) and lower SHBG (mean difference −5.0 nmol/L [1.9 SE], $p = 0.007$). E2 levels were lower in red vs. white wine but not statistically significant. LH was significantly higher in red vs. white wine (mean difference 2.3 mIU/mL [1.3 SE], $p = 0.027$); however, FSH was not.

Conclusion: Red wine is associated with significantly higher free T and lower SHBG levels, as well as a significant higher LH level vs. white wine in healthy premenopausal women. These data suggest that red wine is a nutritional AI and may explain the observation that red wine does not appear to increase breast cancer risk.

Introduction

Breast cancer remains the leading cancer in U.S. women. Epidemiologic studies have consistently reported an increased risk of breast cancer associated with alcohol consumption, including wine; however, it is controversial whether red wine raises risk.[1–4] While aromatase inhibitors (AIs) play a pivotal role in the management and treatment of estrogen receptor–positive breast cancer in postmenopausal women, their role in premenopausal women is still being investigated. AIs prevent the conversion of androstenedione (A) and testosterone (T) into estrogen, leading to increases in blood T and decreases in estradiol (E2), estrone (E1), and sex hormone binding globulin (SHBG) levels.[5]

Naturally occurring AIs have been identified in grapes, grape juice, grape seed extract, and red wine, but not white wine.[6,7] The AI activity in red wine has been attributed to the phytochemicals and not to the alcohol content.[8,9] More than one chemoprotective chemical has been identified in wine, including isoflavone phytoestrogens, flavones, and procyanidin B dimers.[6,7] All of these chemicals have AI activity on the cytochrome P450 aromatase enzyme in both *in vitro* and *in vivo* studies.[6,7,10,11] Other chemicals in wine, such as resveratrol, rutin, and quercetin, have not been clearly established as inhibiting aromatase, even at higher doses than those found in wine.[6,8,12]

In a randomized cross-over study, we tested whether red wine is a nutritional AI in healthy, premenopausal women. We tested whether red vs. white wine produced serum hormone levels in a pattern consistent with an AI.

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Methods

Subjects

Premenopausal women reporting regular ovulatory cycles for 12 months were eligible to participate. Subjects were recruited by the medical center's broadcast e-mails and letters sent by mass mailing using a commercial agency. Inclusion criteria included body mass index (BMI) between 18.5 and 30, normal serum liver function testing, and a regular, unrestricted diet. Exclusion criteria included irregular menstrual cycles or vasomotor symptoms within the last 12 month; pregnancy or breastfeeding; any hormone therapy, including phytoestrogens, oral contraceptives, selective estrogen receptor modulators, or androgens (or precursors) currently or within 3 months; a history of alcohol abuse; a history of estrogen-dependent neoplasia; any chronic health condition; and age <21 years. Participants were screened for alcohol abuse using the AUDIT questionnaire[13] prior to randomization. Study participants agreed to use nonhormonal contraception while they participated in this study. All subjects gave written and informed consent, and the study was approved by the Cedars-Sinai Medical Center Institutional Review Board.

Study protocol

In a randomized cross-over design, participants were assigned to either red wine (Cabernet Sauvignon, BV Coastal 2003) or white wine (Chardonnay BV Coastal 2003) in the first cycle, and the other wine for the second cycle. Participants were instructed not to drink any other alcoholic beverage or grape products during the study period. The wine was obtained in a single batch and stored at room temperature prior to use. Participants were provided with the wine in bottles and asked to consume 8 ounces (237 mL) of the assigned wine in the evening with food from day 1 to 21 and not drive or operate machinery after ingesting the wine for at least 3 hours. Each participant received all the bottles for the arm of the study they were randomized to at the beginning of each cycle.

The duration of each treatment was one menstrual cycle. During the baseline menstrual cycle, the participants abstained from all alcoholic and grape products. Serum hormone levels were assayed by a core laboratory that was blinded to the treatment randomization. Serum was collected at early follicular (day 5–8) and mid-luteal (day 17–21) phases during baseline, treatment 1, and treatment 2 cycles for measurement of E1, E2, A, T, SHBG, luteinizing hormone (LH), and follicle stimulating hormone (FSH) using previously described assay methods.[14,15] Free T and E2 were calculated as described previously.[14,15] A wash-out period between the two wine treatments, during which women again abstained from all alcohol and grape products, occurred after the mid-luteal serum collection and day 0 of the next menstrual cycle.

Statistical analysis

The values from the follicular and luteal phases of each cycle were combined and averaged for analysis. Absolute differences between treatments were assessed in a mixed effect model with random subject effect on logarithm transformed variables. All the estimated treatment effects have been adjusted for period effects. Goodness-of-fit of the mixed model was assessed by investigating the distribution of the residuals. No carryover effect was found for any variable. The "intention-to-treat' analysis was used. All tests were two-sided with a type I error rate of 0.05. All statistical analyses were done using SAS 9.1 (SAS Institute Inc.). Based on an estimated effect size of 25% difference in E2 levels in the red vs. white wine and a standard deviation of 76 pg/mL, a sample size of 35 women provided 85% power to detect a difference between red vs. white wine at a statistical significance level of 0.05.

Results

Overall, 55 women were screened, and 36 participants were enrolled and completed the study protocol. The majority of nonenrolled subjects were excluded due to being over or under the BMI criteria. The baseline characteristics of the study participants are summarized in Table 1. There were no statistical differences according to treatment order assignment (red vs. white wine first, see Table 1) for baseline characteristics or menstrual cycle length at baseline.

Mean differences in E1, free and total E2, A, free and total T, SHBG, FSH, and LH between the red vs. white wine treatments are presented in Table 2. Red wine was associated with a significantly higher free T vs. white wine (mean difference 0.64 pg/mL [0.2 SE], $p = 0.009$) and lower SHBG (mean difference −5.0 nmol/L [1.9 SE], $p = 0.007$). While overall total E2 levels trended toward being lower with the red vs. white wine treatment, this was not statistically significant. LH was significantly higher with red than white wine (mean difference 2.3 mIU/mL [1.3 SE], $p = 0.027$). FSH levels were higher with red vs. white wine, but not statistically significant.

Discussion

To our knowledge, this is the first report of a controlled clinical trial testing the hypothesis that red wine is a nutritional AI in healthy premenopausal women. Our results demonstrate that red vs. white wine has higher free T and lower SHBG levels. Serum estrogens were not significantly different, possibly due to the large standard error of the measurements. However LH was higher with red vs. white wine, suggestive of hypothalamic up-regulation in response to lower estrogen levels. These data suggest that red wine is a nutritional AI.

The strengths of this study include our 100% patient completion rate and core laboratory outcome measures. Our randomized cross-over trial design eliminated intersubject variability because each individual served as her own control.

Epidemiologic studies consistently report increased breast cancer risk associated with alcohol consumption, including wine.[1–3] Two meta-analyses plus the most recent largest study estimate a 12% increased risk with a daily alcoholic drink.[1,16,17] Some studies have evaluated breast cancer risk separately for red and white wine and have suggested no risk with red wine; however, the results are varied, possibly due to the methodological limitations related to mixed use of red and white wine, as well recent versus chronic use recall errors.[18–21]

Our results in a controlled setting provide a potential mechanistic pathway whereby red wine may serve as a nutritional AI. They provide further evidence that red wine, through the hormonal shift patterns, may not elevate breast cancer risk like other alcoholic beverages. Further work aimed at carefully measuring specific alcohol consumption and risk

Table 1. Baseline Characteristics

Variable[a]	*All women* (n = *36*)	*Red wine to white wine* (n = *20*)	*White wine to red wine* (n = *16*)	p *value*
Age, mean ± SD	36 ± 7.9	36.8 ± 8.5	35 ± 7.3	0.50
History of smoking, *n* (%)	25 (69%)	15 (75%)	10 (63%)	0.48
BMI, mean ± SD	26.3 ± 4.6	27.4 ± 4.6	24.9 ± 4.3	0.12
Systolic blood pressure, mean ± SD (mm Hg)	108.6 ± 9.1	109.7 ± 8.8	107.3 ± 9.5	0.39
Ethnicity, *n* (%)[b]				
White	15 (43%)	7 (37%)	8 (50%)	0.80
Asian	3 (9%)	2 (11%)	1 (6%)	
African American	8 (23%)	4 (21%)	8 (50%)	
Hispanic	9 (26%)	6 (32%)	3 (19%)	
Employed full time	28 (80%)	15 (79%)	13 (81%)	0.52
Menstrual cycle length, mean ± SD (days)	29.5 ± 4.82	30.5 ± 9.8	28.8 ± 4.2	0.34
Baseline hormone levels	Combined mean ± SD			
SHBG (nmol/L)	57 ± 39			
Free testosterone (pg/mL)	6.0 ± 2.5			
Total testosterone (ng/dL)	31.8 ± 8.7			
Free estradiol (pg/mL)	2.7 ± 1.1			
Total estradiol (pg/mL)	112 ± 52			
Estrone (pg/mL)	85 ± 27			
Androstenedione (ng/mL)	1.3 ± 0.50			
FSH (mIU/mL)	7.2 ± 5.8			
LH (mIU/mL)	8.6 ± 13.4			

[a]BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; SD, standard deviation; SHBG, sex hormone binding globulin.
[b]One missing value.

is needed. Prior epidemiologic studies have also indicated a positive relation between breast density, which is a risk factor for breast cancer, and alcohol consumption in both premenopausal women and postmenopausal women.[22–24] Notably, the Minnesota Breast Cancer Cohort Study found that red wine consumption, but not white wine, had an inverse relationship with breast density in postmenopausal women after adjusting for other sources of alcohol.[19]

Other potential chemoprotective factors have been identified in red wine. Procyanidin B dimers found in grape seed extract competitively bind to aromatase, which downregulates growth factor receptor signaling and has also been shown to be cytotoxic to cancer cells but not normal cells.[7,10] Phytoestrogens such as flavones and isoflavones have been found to bind to the active site of aromatase, leading to inhibition.[11] Grape polyphenols have been shown to induce mammary cellular apoptosis *in vitro* and decrease tumor growth metastasis.[25] Animal models have also demonstrated decreased rates of cancers with red wine. After ingesting dealcoholized red wine solids, transgenic mice had delayed spontaneous tumor onset.[26]

Table 2. Serum Hormone Level Differences in Red vs White Wine

Variable	*Difference (SE)*[a]	p *value*
SHBG (nmol/L)	−5.0 (1.9)	0.007
Free testosterone (pg/mL)	0.64 (0.2)	0.009
Total testosterone (ng/dL)	1.79 (1.0)	0.20
Free estradiol (pg/mL)	−0.21 (0.30)	0.52
Total estradiol (pg/mL)	−11.7 (11.2)	0.39
Estrone (pg/mL)	3.9 (6.9)	0.83
Androstenedione (ng/mL)	0.05 (0.04)	0.14
FSH (mIU/mL)	1.8 (1.7)	0.25
LH (mIU/mL)	2.3 (1.3)	0.027

[a]SE, standard error.

Limitations of our study include mid-luteal phase sampling, which resulted in relatively high serum estrogen level variability due to ovulation, resulting in greater statistical noise in this variable and possibly precluding an ability to see a relatively small estrogen level decrease. We were powered to detect a "clinically relevant" estimated 25% mean difference in E2, yet saw a 14% mean difference, so we were unpowered to detect this relatively smaller mean difference. Additionally, because AIs result in reduction of estrogen levels related to inhibition of the last step of estrogen synthesis, it is possible that lower serum estrogen levels may have resulted in increased gonadotropin levels that stimulated ovarian estrogen production, overriding the AI effects. Although we asked participants to maintain a stable diet, we did not measure nutritional intake or have our participants maintain food diaries.

In conclusion, red vs. white wine treatment is associated with changes in serum hormones consistent with an AI in healthy premenopausal women studied in a controlled clinical trial. These results combined with prior observational and laboratory data suggest that red wine may serve as a nutritional AI, which may ameliorate the elevated breast cancer risk associated with alcohol intake. Larger scale studies are needed to determine the safety and efficacy of red wine as an AI for breast conditions. Furthermore, because wine consumption has increased in the general population,[27] particularly among young women, further work to determine the relative safety and therefore advisability of red and white wine consumption in women is needed.

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Disclosure Statement

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References

1. Allen NE, Beral V, Casabonne D, et al. Moderate alcohol intake and cancer incidence in women. J Natl Cancer Inst 2009;101:296–305.
2. Smith-Warner SA, Spiegelman D, Yaun SS, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535–540.
3. World Cancer Research Fund/American Institute for Cancer Research (WCRF/AIRC). Alcoholic drinks. In: Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington DC: AICR, 2007:157–171.
4. Chen WY, Rosner B, Hankinson SE, et al. Moderate alcohol consuption during adult life, drinking patterns, and breast cancer risk. JAMA 2011;306:1884–1890.
5. Miller WR. Biological rationale for endocrine therapy in breast cancer. Best Pract Res Clin Endocrinol Metab 2004; 18:1–32.
6. Chen S, Sun X, Kao Y-C, Kwon A, Zhou D, Eng E. Suppression of breast cancer cell growth with grape juice. Pharm Biol 1998;36:53–61.
7. Eng ET, Ye J, Williams D, et al. Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. Cancer Res 2003;63:8516–8522.
8. Eng ET, Williams D, Mandava U, Kirma N, Tekmal RR, Chen S. Anti-aromatase chemicals in red wine. Ann N Y Acad Sci 2002;963:239–246.
9. Eng ET, Williams D, Mandava U, Kirma N, Tekmal RR, Chen S. Suppression of aromatase (estrogen synthetase) by red wine phytochemicals. Breast Cancer Res Treat 2001;67: 133–146.
10. Kijima I, Phung S, Hur G, Kwok S-L, Chen S. Grape seed extract is an aromatase inhibitor and a suppressor of aromatase expression. Cancer Res 2006;66:5960–5967.
11. Kao YC, Zhou C, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: a site-directed mutagenesis study. Environ Health Perspect 1998; 106:85–92.
12. Wang Y, Lee KW, Chan FL, Chen S, Leung LK. The red wine polyphenol resveratrol displays bilevel inhibition on aromatase in breast cancer cells. Toxicol Sci 2006;92: 71–77.
13. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. J Stud Alcohol 1995; 56:423–432.
14. Braunstein GD, Johnson BD, Stanczyk FZ, et al. Relations between endogenous androgens and estrogens in postmenopausal women with suspected ischemic heart disease. J Clin Endocrinol Metab 2008;93:4268–4275.
15. Anderson DC, Hopper BR, Lasley BL, Yen SS. A simple method for the assay of eight steroids in small volumes of plasma. Steroids 1976;28:179–196.
16. Ellison RC, Zhang Y, McLennan CE, Rothman KJ. Exploring the relation of alcohol consumption to risk of breast cancer. Am J Epidemiol 2001;154:740–747.
17. Key J, Hodgson S, Omar RZ, et al. Meta-analysis of studies of alcohol and breast cancer with consideration of the methodological issues. Cancer Causes Control 2006;17:759–770.
18. Newcomb PA, Nichols HB, Beasley JM, et al. No difference between red wine or white wine consumption and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2009;18: 1007–1010.
19. Vachon CM, Kushi LH, Cerhan JR, Kuni CC, Sellers TA. Association of diet and mammographic breast density in the Minnesota breast cancer family cohort. Cancer Epidemiol Biomarkers Prev 2000;9:151–160.
20. Viel JF, Perarnau JM, Challier B, Faivre-Nappez I. Alcoholic calories, red wine consumption and breast cancer among premenopausal women. Eur J Epidemiol 1997;13:639–643.
21. Zhang SM, Lee IM, Manson JE, Cook NR, Willett WC, Buring JE. Alcohol consumption and breast cancer risk in the Women's Health Study. Am J Epidemiol 2007;165:667–676.
22. Boyd NF, Martin LJ, Li Q, et al. Mammographic density as a surrogate marker for the effects of hormone therapy on risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2006; 15:961–966.
23. Byrne C, Webb PM, Jacobs TW, et al. Alcohol consumption and incidence of benign breast disease. Cancer Epidemiol Biomarkers Prev 2002;11:1369–1374.
24. Flom JD, Ferris JS, Tehranifar P, Terry MB. Alcohol intake over the life course and mammographic density. Breast Cancer Res Treat 2009;117:643–651.
25. Castillo-Pichardo L, Martinez-Montemayor MM, Martinez JE, Wall KM, Cubano LA, Dharmawardhane S. Inhibition of mammary tumor growth and metastases to bone and liver by dietary grape polyphenols. Clin Exp Metastasis 2009;26: 505–516.
26. Clifford A, Ebeler S, Ebeler J, et al. Delayed tumor onset in transgenic mice fed an amino acid-based diet supplemented with red wine solids. Am J Clin Nutr 1996;64:748–756.
27. Wine Consumption in the U.S., Apr 19, 2011. Available at www.wineinstitute.org/resources/statistics/article86 Accessed April 19, 2011.

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