Short Communication

The Interaction between Alcohol Consumption and GSTM1 Genotype on Polycyclic Aromatic Hydrocarbon-DNA Adduct Levels in Breast Tissue[1]

Andrew Rundle, Deliang Tang, LaVerne Mooney, Surah Grumet, and Frederica Perera[2]

Departments of Epidemiology [A. R.] and Environmental Health Sciences [D. T., L. M., S. G., F. P.], Mailman School of Public Health, Columbia University, New York, New York 10032

Abstract

We investigated the association between alcohol consumption, GSTM1 genotype, and polycyclic aromatic hydrocarbon (PAH)-DNA adduct levels in breast tissue. Women referred for breast surgery were enrolled prior to surgery, responded to an interview, and gave a blood sample. Women diagnosed with ductal carcinoma *in situ* and invasive ductal or lobular cancer were defined as cases, and women with benign conditions without atypia were defined as controls. Paraffin-embedded tumor and nontumor tissue from cases and benign tissue from controls were retrieved from the pathology samples. *GSTM1* genotype status was determined by PCR using WBC DNA, and PAH-DNA adduct levels were measured in breast tissue using immunohistochemistry. In tumor and nontumor tissue from cases, the *GSTM1*-null genotype was associated with increased adduct levels among current alcohol consumers but not among nondrinkers. In nontumor tissue, the interaction between genotype and alcohol consumption was significant ($P = 0.02$), but in tumor tissue, the interaction did not achieve statistical significance ($P = 0.10$). In benign tissue from controls, there was no association between genotype and adducts, regardless of drinking status. Among subjects with the null genotype who drank alcohol, adduct levels were significantly higher in tumor and nontumor tissue from cases than in benign tissue from controls. These results indicate the presence of a novel gene-lifestyle interaction that influences PAH-DNA adduct levels in breast tissue from cases but not controls. This apparent difference in PAH metabolism in response to alcohol may be an important clue as to how alcohol influences breast cancer risk.

Introduction

We have been investigating the genetic and lifestyle determinants of PAH[3]-DNA adduct levels in breast tissue and the association between increased levels and breast cancer case-control status (1–3). When adduct levels in tumor tissue from cases were compared with adduct levels in benign tissue from controls, high adduct levels were associated with breast cancer case-control status (1). The *GSTM1*-null genotype was associated with increased adduct levels in tumor and nontumor tissue from cases, but it was not associated with adduct levels in benign tissue from controls (2). Case-control differences in adduct levels were stronger among those with the *GSTM1*-null genotype.

As in many other studies of breast cancer (4, 5), we found current alcohol consumption to be associated with breast cancer case-control status (3). The mechanisms through which alcohol may cause breast cancer are unknown, although several have been have proposed (4). We have hypothesized that alcohol consumption may influence breast cancer risk by inducing and suppressing genes responsible for the metabolism of xenobiotics (6, 7). Several animal and cell culture studies have demonstrated that ethanol exposure alters PAH metabolism and increases adduct formation (7–11). Recent experiments with MCF-10F breast cells have shown that cotreatment of cells with B[a]P and ethanol produced higher adduct levels than treatment with B[a]P alone (7). Ethanol treatment was shown to reduce the expression of *GSTP1*, and the authors attributed the increased adduct levels to ethanol-induced reductions in B[a]P metabolism by *GSTP1* (7). *GSTP1* and *GSTM1* both detoxify PAH, creating water-soluble conjugates that are less reactive than the PAH diol-epoxide metabolites (12, 13). Thus, the GST enzymes are thought to prevent reactive xenobiotics from damaging DNA (2, 12). Here we extend our earlier findings on *GSTM1* and show that the *GSTM1* genotype is a stronger predictor of PAH-DNA adduct levels in subjects who were regular alcohol drinkers.

Materials and Methods

Study Population. Patient recruitment has been described previously in detail (1) and will only be described briefly here. From 1994 to 1998, women referred for breast surgery at Columbia-Presbyterian Medical Center were enrolled before surgery into a hospital-based case-control study. After informed consent had been obtained, during their preoperative tests, patients took part in a structured interview covering established reproductive breast cancer risk factors, active and passive smoking, dietary practices, other environmental and occupa-

Received 10/11/02; revised 5/5/03; accepted 5/22/03.
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.
[1] Supported by grants from the Department of the Army, United States Army Medical Research and Materiel Command, DAMD17-94-J-4251, Noreen T. Holland Foundation, and National Cancer Institute Grant K07 CA92348.
[2] To whom requests for reprints should be addressed, at Department of Environmental Health Sciences, Mailman School of Public Health, 60 Haven Avenue B1, New York, NY 10032. Phone: (212) 304-7280; Fax: (212) 544-1943; E-mail: fpp1@columbia.edu.

[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; B[a]P, benzo(*a*)pyrene; GST, glutathione *S*-transferase; BBD, benign breast disease.

Downloaded from http://aacrjournals.org/cebp/article-pdf/12/9/911/2262081/ce0903000911.pdf by guest on 21 February 2024

Table 1 Adduct levels in breast tissue by current drinking status and GSTM1 genotype

	Current drinker		Current non-drinker	
	GSTM1 +/+, +/−	*GSTM1* null	*GSTM1* +/+, +/−	*GSTM1* null
Tumor tissue	0.32 (1.72) $n = 25$	0.59 (2.09) $n = 21$[a]	0.35 (1.81) $n = 17$	0.41 (1.67) $n = 19$
Nontumor tissue	0.30 (1.55) $n = 25$	0.51 (1.87) $n = 20$[b]	0.38 (1.66) $n = 14$	0.34 (1.64) $n = 17$
Benign tissue	0.30 (1.45) $n = 9$	0.34 (1.59) $n = 28$[c,d]	0.34 (1.63) $n = 29$	0.43 (1.55) $n = 15$

[a] $P = 0.002$ comparing adduct levels in tumor tissue by *GSTM1* status.
[b] $P = 0.003$ comparing adduct levels in nontumor tissue by *GSTM1* status.
[c] In current drinkers who are *GSTM1* null, adduct levels in tumor tissue are significantly higher than in benign tissue ($P = 0.002$).
[d] In current drinkers who are *GSTM1* null, adduct levels in nontumor tissue are significantly higher than in benign tissue ($P = 0.01$).

tional exposures, and vitamin consumption. Patients whose confirmed diagnosis was of ductal carcinoma *in situ* or invasive ductal or invasive lobular cancer were defined as cases. Patients with rare tumors were not included because the small numbers precluded analyses by histological type. Patients diagnosed with BBD or BBD with hyperplasia were classified as controls. Because patients were enrolled before diagnosis, matching of BBD controls to cases was not possible. BBD patients whose diagnoses were other than these categories (*e.g.,* BBD with atypia or lobular carcinoma *in situ*) were excluded from analysis because of their elevated risk of future breast cancer. Breast cancer patients seen at Columbia-Presbyterian Medical Center for follow-up surgery (*e.g.,* mastectomy or re-excisions) after an initial surgical biopsy at another hospital were excluded from the study. Additional exclusion criteria included a prior history of cancer at any site except basal skin cancer, current pregnancy, recent bone fractures, or recent breastfeeding. The last two exclusion criteria were included because these factors were thought to interfere with biomarkers used in other aspects of this study. Study subjects responded to an interviewer-administered questionnaire and provided blood samples. Samples of paraffin-embedded tumor and nontumor tissue were retrieved from pathology blocks from cases, as were samples of benign tissue from BBD controls. Thus, two tissue samples, tumor and nontumor, were available from cases, and one tissue sample, benign, was available from the BBD controls.

A total of 119 cases and 108 BBD controls were enrolled with a response rate of 76%. Data on adduct levels, *GSTM1* status, and alcohol consumption were available from 82 cases for analyses in tumor tissue, 76 cases for analyses in nontumor tissue, and 81 controls for analyses in benign tissue.

Laboratory Methods. To assess individual *GSTM1* genotype, DNA was extracted from blood leukocytes and analyzed by PCR as described previously (14). The primers used in the PCR mix were G5 (5′-GAA-CTC-CCT-GAA-AAG-CTA-AAG-C) and G6 [5′-GTT-GGG-CTC-AAA-TAT-ACG-GTG-G (14)]. As a control to detect PCR failures, the assay included primers for the β-globin gene. Women who were homozygous (+/+) or heterozygous (−/+) for *GSTM1* were classified as *GSTM1* positive, and those who were homozygous deleted (−/−) were classified as *GSTM1* null.

The immunohistochemical assay for PAH-DNA adducts and its performance characteristics have been described extensively elsewhere (1, 3, 15). Briefly, using standard immunohistochemical techniques, tissue slides were incubated with anti-benzo(*a*)pyrene-diol epoxide-DNA monoclonal antibody 5D11, kindly provided by Dr. Regina Santella. Staining was accomplished using a biotinylated antimouse secondary antibody (Vector Laboratories, Burlingame, CA), ABC reagents, and diaminobenzidine. Methyl green was used as a counterstain. Nuclear staining was quantified using absorbance image analysis with the Cell Analysis System 200 microscope (Becton-Dickinson, San Jose, CA) running the Cell Measurement software. A total of 50 cells (5 fields with 10 cells/field scored) were measured on each tissue slide. Results are reported in optical density units as described previously (15). Serial tissue slices from laboratory control breast tissue specimens previously shown to have low and high staining for adducts were used as negative and positive control samples, respectively, and were run with every batch. As an additional negative control, in each batch, a laboratory control sample was run without the primary antibody.

Statistical Methods. Absorbance data reflecting the extent of staining for PAH-DNA adducts was log (ln) transformed for analyses to produce a more normal distribution. Results are reported as geometric means and geometric SDs (16). As part of the interview, subjects were asked to estimate the average number of drinks they consumed per week during the 12 months before the interview. Study subjects who responded that they drank 0 drinks/week were classified as nonconsumers, and subjects who responded that on average they drank more than 0 drinks/week were classified as regular current consumers of alcohol. Subjects were cross-classified into four groups based on their *GSTM1* genotype status and whether they were current alcohol consumers. For each tissue type (tumor, nontumor, and benign), geometric mean adduct levels were calculated for each of the four alcohol consumption/genotype strata. *t* tests were used to determine whether *GSTM1* status was associated with adduct levels within each stratum of alcohol consumption. Within each tissue type, linear regression analyses, with interaction terms for the joint effects of genotype and alcohol consumption, were used to determine whether the association between *GSTM1* and adduct levels differed by alcohol consumption status. *t* tests were used to determine whether, among subjects who were *GSTM1* null and drank alcohol, adduct levels were higher in cases *versus* controls.

Results

Among current alcohol consumers, the *GSTM1*-null genotype was significantly associated with higher adduct levels in tumor and nontumor tissue, but not in benign tissue from controls (see Table 1). Among nondrinkers, *GSTM1* genotype was not associated with adduct levels in any of the tissues (see Table 1). In tumor tissue, the apparent interaction between *GSTM1* status and current alcohol consumption on adduct levels approached but did not reach statistical significance ($P = 0.10$). However, in nontumor tissue from cases, the interaction term was significant ($P = 0.02$), indicating that the association between *GSTM1* and adduct levels significantly differs by alcohol consumption status. Within the *GSTM1*-null/alcohol consumer stratum, adducts levels were significantly higher in tumor tissue

Downloaded from http://aacrjournals.org/cebp/article-pdf/12/9/911/2262081/ce0903000911.pdf by guest on 21 February 2024

from cases than in benign tissue from controls and significantly higher in nontumor tissue from cases compared with benign tissue from controls (see Table 1).

Discussion

We report here on a gene-lifestyle interaction that influences PAH-DNA adduct levels in breast tissue from cases but not in tissue from BBD controls. This is an extension of our prior work showing that *GSTM1* was associated with PAH-DNA adduct levels in breast tissue from cases but not from BBD controls (2). The presence of the interaction in cases only provides further evidence that women with breast cancer differ from women with benign conditions in some as yet unknown manner that makes *GSTM1* a more important determinant of PAH-DNA adduct levels in breast tissue (2). Additionally, case-control differences in adduct levels were strongest among women who consumed alcohol and were *GSTM1* null.

The analyses presented here were motivated by the recent report of breast cell culture experiments showing that ethanol increases the extent of adduct formation in cells treated with B[a]P (7). The authors attributed the increased levels of adducts to ethanol-induced reductions in the expression of *GSTP1* that they observed in the cell cultures (7). *GSTP1* and *GSTM1* both metabolize PAH, creating water-soluble conjugates that are less reactive than the PAH diol-epoxide metabolites; however, neither gene is involved in the metabolism of ethanol (12, 13). This overlapping specificity may provide multiple layers of defense against genetic insult from PAH. Our data indicate that the *GSTM1* genotype is an important predictor of PAH-DNA adduct levels in subjects who were regular alcohol consumers but not among nonconsumers, suggesting that other detoxifying pathways were compromised among alcohol consumers.

One limitation of our research is that data are not available on *GSTM1* and *GSTP1* expression in the tissue sections. The literature on whether alcohol alters GST expression is inconsistent (7, 8, 17–21). There appear to be organ- and species-specific differences in whether alcohol exposure inhibits or induces *GST* expression, and the effects appear to differ by *GST* subclass. A recent study in rats found that long-term ethanol treatment induced *GST-*μ and *GST-*α but not *GST-*π class activity in liver cells, yet a study in hamsters observed no increase in *GST*-mediated metabolism of B[a]P in response to ethanol treatment (8, 17). A recent study using cDNA expression arrays found that chronic ethanol feeding was associated with increased *GSTM1* expression in C57BL/6J, ethanol-preferring mice but not in BALB/c, ethanol-avoiding mice (21). Our results suggest that alcohol consumption does influence PAH metabolism and that *GSTM1* is not inhibited by alcohol consumption. In addition to the possibility that alcohol influences *GSTP1* expression, alcohol may alter the expression of important phase 1 genes, such as *CYP1B1*, which activates PAH to the reactive diol-epoxide form (7). Increased availability of reactive metabolites would also be hypothesized to make *GSTM1* genotype a more important determinant of adduct levels among alcohol consumers.

If alcohol consumption is indeed reducing the expression of *GSTP1* in our subjects, and this accounts for the interaction between alcohol consumption and *GSTM1*, the presence of the interaction in cases but not controls suggests that the effect of alcohol on *GSTP1* expression is stronger in cases than BBD controls. Differences in responsiveness to alcohol in terms of gene inhibition or induction may, especially for genes related to PAH metabolism, represent an important determinant of breast cancer risk. *GSTP1* has a polymorphism that appears to influence adduct levels in a substrate-specific manner (22). However, given the evidence that alcohol consumption inhibits *GSTP1* expression, genotype analyses may not be useful in understanding the role of *GSTP1* in adduct formation.

Subjects were defined as current consumers if they reported any consumption on a regular weekly basis over the past year. Among the consumers, the average number of drinks/week was 4.82, with a range of 0.07–36 drink(s)/week. The interview question on current alcohol consumption asked about drinking patterns in the past year; however, many of the women who consumed alcohol reported that they had been drinking more since they had been told they needed breast surgery. Thus because our question on past year consumption may underestimate very recent consumption patterns, we did not categorize the women who reported the lowest alcohol consumption, for instance 0.07 drink/week, as nondrinkers. Because the interview was conducted before surgical biopsy, and none of the women knew whether they were cancer free, we do not expect changes in recent drinking patterns to be differential by case-BBD control status. Overall, the data suggest that these women were relatively light consumers of alcohol, and the effect appears to occur at low levels of alcohol consumption. This is consistent with the work of Barnes *et al.* (7), who treated MCF-10F cells with alcohol at concentrations consistent with blood alcohol levels achieved by drinking 1–3 drinks. The level of drinking seen in our study is consistent with the popularly held but unproven belief that a glass of red wine a day is protective against heart disease (23–25). Should these findings be replicated and prove to be causal, current recommendations regarding alcohol consumption may need to be reconsidered (23–25).

In noting the apparent case-control differences in the response to alcohol consumption, we hypothesize that there exists some constitutive difference between cases and controls in gene expression in response to alcohol. It is possible, however, that this differential response represents a local effect of alcohol on tumor tissue. It is possible that, in some manner, alcohol alters metabolism in tumor cells such that *GSTM1* is an important determinant of adduct levels in tumor tissue, but metabolism in normal breast cells is unaffected. The observed interaction between *GSTM1* and alcohol consumption in nontumor tissue from cases argues against the hypothesis that the effect is a result of alcohol influencing metabolism in tumor tissue only. However, because the nontumor tissue analyzed in this work was adjacent to the tumors *in situ*, the possibility of a field effect or that the tumor influenced metabolism in nearby tissues cannot be ruled out. Further work is needed to determine whether the apparent differential responses to alcohol represent a constitutive difference between cases and controls or a local effect of alcohol on metabolism in and around the tumor.

Another consideration in assessing the apparent case-control differences in adduct levels is the use of women with BBD as the control group. Although the BBD control group only included diagnoses with a low risk for future breast cancer, a concern is that the BBD controls may overly share risk factors, both measured and unmeasured, with the cases (26). To the extent that an exposure of interest is positively associated with another risk factor overrepresented in the controls, the use of BBD controls will cause risk estimates for the exposure to be attenuated to the null. However, in comparisons of odds ratios for known breast cancer risk factors calculated using a healthy control group and the BBD control group, we did not find that the use of BBD controls resulted in a consistent trend of attenuated risk estimates (3).

In conclusion, we report here on a novel gene-lifestyle

Downloaded from http://aacrjournals.org/cebp/article-pdf/12/9/911/2262081/ce0903000911.pdf by guest on 21 February 2024

interaction that influences PAH-DNA adduct levels in breast tissue and appears to occur in women with breast cancer but not in women with benign conditions. We suggest that cases and controls may differ in their metabolic responses to alcohol, and this may be an important clue as to how alcohol influences breast cancer risk. This work requires confirmation in a larger population and further investigations of how alcohol consumption influences xenobiotic metabolism and risk through gene induction and/or suppression.

Acknowledgments

We acknowledge the thoughtful comments of Stan Cho and Dr. Alex Halim.

References

1. Rundle, A., Tang, D., Hibshoosh, H., Estabrook, A., Schnabel, F., Cao, W., Grumet, S., Della Rocca, A., and Perera, F. The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. Carcinogenesis (Lond.), *21:* 1281–1289, 2000.

2. Rundle, A., Tang, D., Zhou, J., Cho, S., and Perera, F. The association between glutathione *S*-transferase M1 genotype and polycyclic aromatic hydrocarbon (PAH)-DNA adducts in breast tissue. Cancer Epidemiol. Biomark. Prev., *9:* 1079–1085, 2000.

3. Rundle, A., Tang, D., Hibshoosh, H., Schnabel, F., Kelly, A., Levine, R., Zhou, Z., Link, B., and Perera, F. Molecular epidemiologic studies of polycyclic aromatic hydrocarbon-DNA adducts and breast cancer. Environ. Mol. Mutagen., *39:* 201–207, 2002.

4. Smith-Warner, S., Spiegelman, D., Yaun, S., van den Brandt, P., Folsom, A., Goldbohm, R., Graham, S., Holmberg, L., Howe, G., Marshall, J., Miller, A., Potter, J., Speizer, F., Willett, W., Wolk, A., and Hunter, D. Alcohol and breast cancer in women: a pooled analysis of cohort studies. J. Am. Med. Assoc., *279:* 535–540, 1998.

5. Longnecker, M. P. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer Causes Control, *5:* 73–82, 1994.

6. Roberts, B., Shoaf, S., and Song, B. Rapid changes in cytochrome P4502E1 (*CYP2E1*) activity and other P450 isozymes following ethanol withdrawal in rats. Biochem. Pharmacol., *49:* 1665–1673, 1995.

7. Barnes, S., Singletary, K., and Frey, R. Ethanol and acetaldehyde enhance benzo[*a*]pyrene-DNA adduct formation in human mammary epithelial cells. Carcinogenesis (Lond.), *21:* 2123–2128, 2000.

8. Murphy, S., and Hecht, S. Effects of chronic ethanol consumption on benzo-[*a*]pyrene metabolism and glutathione *S*-transferase activities in Syrian golden hamster cheek pouch and liver. Cancer Res., *46:* 141–146, 1986.

9. Melikian, A., Goldin, B., Prahalad, A., and Hecht, S. Modulation of benzo-[*a*]pyrene-DNA adducts in hamster cheek pouch by chronic ethanol consumption. Chem. Res. Toxicol., *3:* 139–143, 1990.

10. Autrup, J., Hansen, C., and Autrup, H. Detection of tobacco smoke carcinogen-DNA adducts in cultured rat buccal mucosa cells following exposure to ethanol and total cigarette smoke condensate or chewing tobacco. Chem. Biol. Interact., *85:* 141–150, 1992.

11. Izzotti, A., Balansky, R., Blagoeva, P., Mircheva, Z., Tulimiero, L., Cartiglia, C., and De Flora, S. DNA alterations in rat organs after chronic exposure to cigarette smoke and/or ethanol ingestion. FASEB J., *12:* 753–758, 1998.

12. Rebbeck, T. R. Molecular epidemiology of the human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. Cancer Epidemiol. Biomark. Prev., *6:* 733–743, 1997.

13. Helzlsouer, K., Selmin, O., Huang, H., Strickland, P., Hoffman, S., Alberg, A., Watson, M., Comstock, G., and Bell, D. Association between glutathione *S*-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. J. Natl. Cancer Inst. (Bethesda), *90:* 512–518, 1998.

14. Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L., and Lucier, G. W. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. J. Natl. Cancer Inst. (Bethesda), *85:* 1159–1164, 1993.

15. Zhang, Y., Weksler, B., Wang, L., Schwartz, J., and Santella, R. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis, *140:* 325–331, 1998.

16. Flanders, W., DerSimonian, R., and Freedman, D. Interpretation of linear regression models that include transformations or interaction terms. Ann. Epidemiol., *2:* 735–744, 1992.

17. Vanhaecke, T., Lindros, K., Oinonen, T., Coecke, S., DeBast, G., Phillips, I., Shepard, E., Vercruysse, A., and Rogiers, V. Effect of ethanol on the expression of hepatic glutathione *S*-transferase: an *in vivo/in vitro* study. Biochem. Pharmacol., *60:* 1491–1496, 2000.

18. Munoz, M., Martin, M., Fermosa, J., Gonzalez, J., and Esteller, A. Effect of chronic ethanol feeding on glutathione and glutathione-related enzyme activities in rat liver. Drug Alcohol Depend., *20:* 221–226, 1987.

19. Gregus, Z., Madhu, C., and Klaassen, C. Inducibility of glutathione *S*-transferase in hamsters. Cancer Lett., *44:* 89–94, 1989.

20. Van de Wiel, J., Fijneman, P., Teeuw, K., Van Ommen, B., Noordhoek, J., and Bos, R. Influence of long-term ethanol treatment on rat liver biotransformation enzymes. Alcohol, *10:* 397–402, 1993.

21. Murphy, B., Chiu, T., Harrison, M., Uddin, R., and Singh, S. Examination of ethanol responsive liver and brain specific gene expression, in the mouse strains with variable ethanol preferences, using cDNA expression arrays. Biochem. Genet., *40:* 395–410, 2002.

22. Hu, X., Herzog, C., Zimniak, P., and Singh, S. Differential Protection against benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-induced DNA damage in HepG2 cells stably transfected with allelic variants of π class human glutathione *S*-transferase. Cancer Res., *59:* 2358–2362, 1999.

23. Goldberg, I., Mosca, L., Piano, M., and Fisher, E. AHA Science Advisory: wine and your heart: a science advisory for health care professionals from the Nutrition Committee, Council on Epidemiology and Prevention, and Council on Cardiovascular Nursing of the American Heart Association. Circulation, *103:* 472–475, 2001.

24. Mosca, L., Grundy, S., Judelson, D., King, K., Limacher, M., Oparil, S., Pasternak, R., Pearson, T., Redberg, R., Smith, S. J., Winston, M., and Zinberg, S. Guide to Preventive Cardiology for Women. AHA/ACC Scientific Statement Consensus Panel statement. Circulation, *99*: 2480–2484, 1999.

25. Campbell, N., Ashley, M., Carruthers, S., Lacourciere, Y., and McKay, D. Lifestyle modifications to prevent and control hypertension. 3. Recommendations on alcohol consumption. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Center for Disease Control at Health Canada, Heart and Stroke Foundation of Canada. Can. Med. Assoc. J., *160:* S13–S20, 1999.

26. Miller, A. Hospital or population controls? It depends on the question. Prev. Med., *23:* 263–266, 1994.

Downloaded from http://aacrjournals.org/cebp/article-pdf/12/9/911/2262081/ce0903000911.pdf by guest on 21 February 2024