Association of lifestyle and demographic factors with estrogenic and glucocorticogenic activity in Mexican American women

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Abstract

Breast cancer risk is higher in US-born than in foreign-born Hispanics/Latinas and also increases with greater length of US residency. It is only partially known what factors contribute to these patterns of risk. To gain new insights, we tested the association between lifestyle and demographic variables and breast cancer status, with measures of estrogenic (E) and glucocorticogenic (G) activity in Mexican American women. We used Chemical-Activated Luciferase gene expression assays to measure E and G activity in total plasma from 90 Mexican American women, without a history of breast cancer at the time of recruitment, from the San Francisco Bay Area Breast Cancer Study. We tested associations of nativity, lifestyle and sociodemographic factors with E and G activity using linear regression models. We did not find a statistically significant difference in E or G activity by nativity. However, in multivariable models, E activity was associated with Indigenous American ancestry (19% decrease in E activity per 10% increase in ancestry, \( P = 0.014 \)) and with length of US residency (28% increase in E activity for every 10 years, \( P = 0.035 \)). G activity was associated with breast cancer status (women who have developed breast cancer since recruitment into the study had 21% lower G activity than those who have not, \( P = 0.054 \)) and alcohol intake (drinkers had 25% higher G activity than non-drinkers, \( P = 0.015 \)). These associations suggest that previously reported breast cancer risk factors such as genetic ancestry and alcohol intake might in part be associated with breast cancer risk through mechanisms linked to the endocrine system.

Introduction

Breast cancer risk in US Latina women, although lower than that of non-Latina Whites (1), is higher in those born in the USA, and risk increases with younger age at migration (2). Age-adjusted incidence rates for the period 1988–2004 showed 38% higher rates for US-born than for foreign-born Latinas (3). It is only partially known what factors contribute to these patterns of increasing risk (2,3). Previous studies have attempted to explain the changes in breast cancer incidence among Latina immigrants using measures of exposure obtained through questionnaires or through record linkage to census data to evaluate the effect of socioeconomic status (SES) or neighborhood effects (2,3). However, by themselves, these results are limited because they are bound to provide information about exposures that have already

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ER and either activate or suppress downstream signaling, includ-
through binding ER, which in turn leads to expression of ER tar-
top versus bottom 20–25% of plasma estradiol, estrone or estrone 
higher risk of breast cancer in postmenopausal women in the 
(21–24). Epidemiologic studies have documented about a 2-fold 
trials to prevent breast cancer, particularly ER-positive disease 
raloxifene, as well as aromatase inhibitors, are cornerstones of 
for the role of estrogens in breast cancer risk and prognosis. 
and estradiol may activate ER-mediated signaling pathways 
Singapore Chinese Health Study tested the associations between 
analysis conducted in samples collected prospectively from the 
that individuals with similar reported exposures are not equally 
susceptible to disease, due to inter-individual variation in the 
metabolism of endogenous and exogenous compounds (4,5).
Conducting analysis of endocrine disruptors by measuring 
elevated or reduced hormone activity in plasma is a novel way 
to understand the differences in breast cancer risk between 
Latina women born in the USA compared with foreign-born 
Latinas. Enzyme activation by exposure to hormone receptor 
binding compounds can lead to increased hormone catabolism 
and compromise hormone signaling (6). Breast cancer risk has 
been directly linked to hormone receptor disruptors in animal 
models (7,8) and in occupational exposure studies (9,10). There 
is also evidence linking endocrine disruptors to breast cancer 
risk through regulation of microRNAs’ expression (11), as well 
as through their involvement in the formation of reactive elec-
trolytes such as reactive oxygen species and subsequent DNA 
adduct formation (12).
Cell-based reporter bioassays have been commonly used to 
identify estrogenic (E) compounds present in the environment 
(13–16), but few studies have used them to test the association 
between overall E activity in human blood and breast cancer 
risk (17–19), as was originally proposed by Brouwers et al. (20). An 
analysis conducted in samples collected prospectively from the 
Singapore Chinese Health Study tested the associations between 
levels of estrogens and estrogen receptor (ER)-mediated bioac-
tivity and breast cancer risk among postmenopausal women 
and found results suggesting that factors other than estrone 
and estradiol may activate ER-mediated signaling pathways 
to increase breast cancer risk (19). There is extensive evidence 
for the role of estrogens in breast cancer risk and prognosis. 
Selective estrogen receptor modulators, such as tamoxifen and 
naloxifene, as well as aromatase inhibitors, are cornerstones of 
breast cancer treatment and have been shown in randomized 
trials to prevent breast cancer, particularly ER-positive disease 
(21–24). Epidemiologic studies have documented about a 2-fold 
higher risk of breast cancer in postmenopausal women in the 
top versus bottom 20–25% of plasma estradiol, estrone or estrone 
sulfate levels (25,26). Estrogens affect breast tissue largely 
through binding ER, which in turn leads to expression of ER tar-
genesis (27). However, multiple other compounds can also bind 
ER and either activate or suppress downstream signaling, includ-
ing metals (e.g. cadmium), chemicals for industrial or household 
use (e.g. bisphenol A, parabens and phthalates), natural food 
components (e.g. isoflavones) and endogenous compounds (e.g. 
Exposure to endogenous and exogenous glucocorticoid 
receptor (GR) modulators is also likely to contribute to breast 
cancer development. Glucocorticoids are adrenocortical ster-
oid hormones involved in several physiological and cellular 
processes, including cell differentiation, metabolism and pro-
grammed cell death by interacting with the GR (33). Reduced 
expression of the GR gene was observed in a panel of human 
liver, lung, prostate, colon and breast cancers and found to play 
an important role in promoting accurate chromosome segrega-
tion during mitosis, which highlights its role as a tumor sup-
pressor (34). In addition, GR expression in breast cancer tissue 
has been associated with smaller tumor size and lower grade 
(35). In addition, glucocorticogenic (G) activity might reflect cor-
tisol levels, which, given their link to stress (36), could be par-
ticularly relevant to this population of immigrant women.
In the present study, we used cell-based assays (37,38) to 
to measure overall E and G activity in plasma of 90 Mexican 
American women who participated as controls in the San 
Francisco Bay Area Breast Cancer Study (SFBCS), a population-
based case–control study of women aged 35–79 years. The 
specific goal of our study was to test if nativity (ref. US-born) and 
other breast cancer risk factors were associated with E and G 
activity in total plasma.

Materials and methods

Study samples
The San Francisco Bay Area Breast Cancer Study (SFBCS), described else-
where (2,39), is a multiethnic population-based case–control study of 
breast cancer initiated in 1995, and with biospecimen collection added 
for cases diagnosed between 1 April 1997 and 30 April 2002 and match-
ing controls. Briefly, participating women aged 35–79 years resided in the 
San Francisco Bay Area when diagnosed with a first primary histologi-
cally confirmed invasive breast cancer between April 1995 and April 2002. 
Controls identified by random-digit dialing were frequency matched to 
cases based on race/ethnicity and the expected 5 year age distribution 
of cases. Trained interviewers administered a structured questionnaire 
in English or Spanish at a home visit and took anthropometric measure-
ments. Trained phlebotomists collected a fasting blood sample. Since 
for some women, the blood was collected a few years after recruitment into 
the study, a phlebotomy questionnaire was administered at the time of 
blood draw to update some key variables.
For the present study, 90 women were selected from the set of 603 Latina 
controls with stored plasma if they had developed breast cancer since the 
time of blood collection or if they remained free of breast cancer and were 
Mexican origin. Through linkage with the California Cancer Registry in 
2013, 15 Latina women were identified who developed breast cancer after 
blood collection. The remaining 75 women were randomly selected within 
subsets according to age at migration to the USA if foreign-born (balancing 
the number of younger and older age at migration) and menopausal sta-
tus (balancing the number of premenopausal and postmenopausal women 
in each demographic category). The final sample included 60 foreign-
born women (8 cases and 52 controls) and 30 US-born women (7 cases and 
23 controls) (Supplementary Figure S1). Overall, 33 women were premeno-
pausal and 57 were postmenopausal. Since we included all Latina women 
who developed breast cancer after blood collection, some of them were not 
of Mexican origin. Of the 15 cases, one was from Colombia, one from Puerto 
Rico and two from Nicaragua. Given that 97% of the women included in 
the present analysis were of Mexican origin, we refer to them generally as 
Mexican American throughout the manuscript, despite the fact that three 
women had different national origins.

Measures
The questionnaire for the main study obtained data on demographic back-
ground (education in years, country of birth, and age at migration if not US 
born) and known or suspected breast cancer risk factors. For the present 
analysis, we selected specific risk factors that we hypothesized could be 
associated with E or G activity at the time of blood draw, such as use of 
menopausal hormone therapy (HT), alcohol intake, body mass index (BMI)
Cell culture and treatments for the ER bioassay

Similar to the ER bioassay, the GR bioassay also used methods previously described by Wilson et al. (47). However, the transfected breast cancer cell line MDA-Kb2 expresses two receptors. This cell line was used to measure androgens such as testosterone and dihydrotestosterone present in human plasma for premenopausal and postmenopausal women in our study. Because the androgen receptor and the GR have homologous DNA binding domains and act on the mouse mammary tumor virus promoter, this cell line also has the ability to measure glucocorticoids such as cortisol and aldosterone using the GR. To distinguish between A and G activities, the use of a potent androgen receptor inhibitor, hydroxyflutamide (OHF), was needed. Cells were cultured in Leibovitz’s L-15 (15) medium with 10% FBS until 1 week before plasma addition. External sources of androgens and glucocorticoids were removed by treating the cells for 1 week with ‘stripped’ medium composed of L-15 medium and 10% charcoal–dextran serum. The cells were seeded at 27,000 cells per well at 200 µl final volume in white, 96-well plates and incubated at 37°C for 24 hours. The 165 µl of plasma used per female sample was diluted in stripped medium and then added in quadruplicate directly onto cells, both in the presence and absence of 0.5 µM OHF. After 24 hours of incubation at 37°C, the cells were lysed and the microplate was read using the luminometer to obtain RLU readings. To get G activity measurements, RLUs from quadruplicate wells in the presence of OHF were averaged to get one measure per individual. The intra-assay and inter-assay CVs of this assay are 5–10%. The minimum detection limit for cortisol is 4.4 nM.

Statistical analysis

Differences in means and proportions for all analyzed variables between US-born and foreign-born Mexican women were assessed using two-sided t-tests and Fisher’s exact tests, respectively. E and G activity measures were ln-transformed in order to approximate the normal distribution. We used linear regression models with the receptor activity as the outcome and demographic and lifestyle factors as predictors. We also included a batch or plate variable to account for experimental variation. To facilitate interpretation of regression results, we calculated the percent change in RLUs per unit change of predictor variables using the formula \(\left(1 + e^{-\beta \cdot x}\right) - 1\). Analyses were conducted in R (48) or STATA (49).

The multivariable regression models included E or G activity (continuous, ln-transformed RLUs) as the outcome and height (continuous, in meters; m), BMI (continuous and ln-transformed, in kg/m²), proportion of Indigenous American ancestry (continuous, 10% ancestry unit), level of education (less than high school versus high school or more), neighborhood SES (continuous score, based on first component of principal components analysis), age at blood draw (continuous, 10 years unit), alcohol intake at first interview (yes, no), nativity (US-born, foreign-born) and menopausal status (premenopausal, postmenopausal) as predictors. We also conducted two additional analyses, one stratifying by menopausal status, which included use of HT (never, former or current), and the other by nativity, in which we were able to assess the association between E or G activity and years of residence in the USA (continuous, 10 years unit). Models included all predictors (based on the a priori hypothesis that they could all influence E and G activity). We did not include use of OCs because only one woman in the study was taking them at the time of blood draw and was therefore excluded from the analysis. African genetic ancestry is relatively low in women of Mexican origin and, therefore, estimates obtained are less reliable than for the major components (Indigenous American and European). As a result, the present analyses only included Indigenous American ancestry (which is the complement of the European ancestry and therefore collinear). Seventeen women had discordant alcohol intake answers during the calendar year prior to selection into the parent study versus during the 6 months prior to the blood draw. All 17 reported to drink some alcohol in the first interview (ranging from half a drink per week to approximately two drinks a day) and no alcohol at blood draw. The E activity analysis included education level, but not SES as predictor, and the G activity analysis included SES but not education level. Since SES and education were highly correlated, for each model we kept the variable that had the largest effect on the adjusted R². The multivariable regression analyses excluded individuals with missing data on genetic ancestry (one case from Puerto Rico and three Mexican American cases) and education (three controls), a breastfeeding woman and a woman currently using OCs. The final
sample set included 11 breast cancer cases and 70 controls for E activity and 9 cases and 50 controls for G activity.

**Ethical statement**

All participants provided written informed consent and the study was approved by the Institutional Review Boards at the University of California San Francisco and the Cancer Prevention Institute of California.

**Results**

In the present study, we tested if first-generation Mexican immigrants had different E and/or G activity in plasma, as measured using a CALUX cell-based assay, compared with US-born women of Mexican origin, and if those levels were associated with other known breast cancer risk factors. Median E activity of transfected cells after addition of plasma was 2925 RLUs (interquartile range [IQR]: 8226), and G was 178232 RLUs (IQR: 72207). Table 1 describes the levels of all considered variables by place of birth (US-born versus foreign-born). There were no differences in E or G activity, age at blood draw, height, BMI and menopausal status (the latter due to selection of similar number of postmenopausal and premenopausal women from the two migration groups during the study design). There was a statistically significant difference in the level of education and neighborhood SES, with US-born Latinas having higher levels for both variables, and suggestive differences in alcohol intake during the calendar year prior to selection into the study (higher intake among US-born

| Table 1. Sample characteristics by migration status in Mexican American women (N = 90) |
|---------------------------------|-----------------|------------------|-----------------|------------------|-----------------|
|                                 | US-born         | Foreign-born     | P value          |
|---------------------------------|-----------------|------------------|-----------------|-----------------|
| Estrogenic activity level (RLUs), median (IQR) | 2403 (8833) | 3437 (7000) | 0.546 |
| Glucocorticogenic activity level (RLUs), median (IQR) | 177443 (72473) | 183735 (68794) | 0.813 |
| Age at blood draw (years), mean (SD) | 54 (11) | 53 (11) | 0.624 |
| % Indigenous American ancestry, mean (SD) | 44 (12) | 42 (14) | 0.53 |
| History of breast cancer, n (%) | 23 (77) | 52 (87) | 0.245 |
| No                              | 7 (23) | 8 (13) | |
| Education, n (%) | 7 (23) | 40 (67) | <0.001 |
| <High school                     | 21 (70) | 15 (25) | |
| High school+                     | 2 (7) | 5 (8) | |
| Neighborhood SES (statewide quintiles), n (%) | 1st quintile (lowest) | 0 | 4 (7) | 0.011 |
| 2nd quintile                     | 2 (7) | 19 (32) | |
| 3rd quintile                     | 10 (33) | 16 (27) | |
| 4th quintile                     | 9 (30) | 15 (25) | |
| 5th quintile (highest)           | 7 (23) | 4 (7) | |
| Unknown                          | 2 (7) | 2 (3) | |
| Menopausal status at blood drawd, n (%) | Premenopausal | 11 (37) | 21 (35) | 1.000 |
| Postmenopausal                   | 19 (63) | 38 (63) | |
| Unknown                          | 0 | 1 (2) | |
| Height (cm), mean (SD)           | 157 (6) | 156 (6) | 0.632 |
| Body mass index (kg/m²), mean (SD) | 29 (6) | 30 (6) | 0.358 |
| OC use at blood drawd, n (%)     | Never | 8 (27) | 26 (43) | 0.157 |
| Former                          | 22 (73) | 32 (53) | |
| Current                         | 0 (0) | 1 (2) | |
| Unknown                          | 0 | 1 (2) | |
| HT use at blood drawd, n (%)     | Never | 8 (42) | 21 (55) | 0.186 |
| Former                          | 8 (42) | 16 (42) | |
| Current                         | 3 (16) | 1 (3) | |
| Alcohol intake during calendar year before selection into parent studyd, n (%) | None | 16 (53) | 44 (73) | 0.086 |
| Some                            | 12 (40) | 14 (23) | |
| Unknown                          | 2 (7) | 2 (3) | |
| Alcohol intake during the 6 months prior to blood drawd, n (%) | None | 25 (83) | 56 (93) | 0.154 |
| Some                            | 5 (17) | 4 (7) | |
| Unknown                          | 0 | 0 | |

*We used t-test (for continuous variables) or Fisher’s exact test (for categorical variables) to assess the significance of the difference in variable distribution between US-born and foreign-born women.

*Seven US-born and 20 foreign-born women did not have information on glucocorticogenic activity.

*Only among postmenopausal women.

*The participants answered two questionnaires, one at first interview and one at blood draw. The one at interview asked about behavior within the year prior to interview. The one at blood draw asked about behavior within the 6 previous months.
women), and menopausal HT (higher use in US-born women). A higher proportion of breast cancer cases were US-born.

### Estrogenic activity

We tested the association between E activity of transfected cells after addition of plasma from 86 Mexican American women and multiple anthropometric, lifestyle and demographic factors using univariate and multivariable regression models. In univariable analyses, we found a strong positive association with age at blood draw, where for every 10 years increase in age, E activity decreased 50% (P = 1 × 10⁻¹²) (Supplementary Figure S2). Mean E activity level among postmenopausal women was 79% lower than that of premenopausal women (P < 1 × 10⁻¹⁰) (Supplementary Figure S3). Variation in age at blood draw and menopausal status explained ~40% of the variation in E activity (adjusted R² = 0.43).

We did not find an association between E activity level and nativity (US-born versus foreign-born) in univariate or multivariable models.

The multivariable model suggested a negative association between E activity and proportion of Indigenous American genetic ancestry, where for every 10% increase in ancestry there was a 19% decrease in E activity (P = 0.014) (Table 2).

### Glucocorticogenic activity

Glucocorticogenic (G) activity was only obtained for 60 of the 86 women due to lack of plasma availability for 26 women. We found no association between G activity, age at blood draw, menopausal status or nativity (Table 4). There was an inverse association with breast cancer status, suggesting that women who had developed breast cancer after recruitment into the study had 21% lower G activity than those who did not (P = 0.054). We

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**Table 2.** Association between estrogenic activity, lifestyle and demographic factors in Mexican American women (N = 81)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>% Change in RLUsb</td>
</tr>
<tr>
<td>Nativity (ref. US-born)</td>
<td>0.12 (−0.46, 0.71)</td>
<td>13</td>
</tr>
<tr>
<td>Age at blood draw (per 10 years)</td>
<td>−0.69 (−0.89, −0.50)</td>
<td>−50</td>
</tr>
<tr>
<td>Indigenous American ancestry</td>
<td>−0.16 (−0.37, 0.06)</td>
<td>−15</td>
</tr>
<tr>
<td>(per 10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer (ref. No)</td>
<td>−0.57 (−1.30, 0.16)</td>
<td>−43</td>
</tr>
<tr>
<td>Education (ref. &lt;High school)</td>
<td>−0.02 (−0.60, 0.55)</td>
<td>−2</td>
</tr>
<tr>
<td>Postmenopausal (ref. Premenopausal)</td>
<td>−1.55 (−2.00, −1.09)</td>
<td>−79</td>
</tr>
<tr>
<td>Height (per 10 cm)</td>
<td>0.21 (−0.23, 0.65)</td>
<td>23</td>
</tr>
<tr>
<td>BMI (ln kg/m²)</td>
<td>−0.04 (−0.19, 0.11)</td>
<td>−4</td>
</tr>
<tr>
<td>Alcohol intake (ref. None)</td>
<td>0.10 (−0.52, 0.71)</td>
<td>11</td>
</tr>
</tbody>
</table>

CI, confidence interval.  
*The coefficients and 95% CI are based on the ln-transformed RLUs.  
*Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula [e^β − 1] * 100.  
*Alcohol intake during calendar year before selection into parent study.

**Table 3.** Association between estrogenic activity, lifestyle and demographic factors by place of birth (N = 81)

<table>
<thead>
<tr>
<th>Variable</th>
<th>US-born women (N = 28)</th>
<th>Foreign-born women (N = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>% Change in RLUsb</td>
</tr>
<tr>
<td>Age at blood draw (per 10 years)</td>
<td>−0.59 (−1.19, 0.03)</td>
<td>−45</td>
</tr>
<tr>
<td>Indigenous American ancestry</td>
<td>0.05 (−0.33, 0.44)</td>
<td>5</td>
</tr>
<tr>
<td>(per 10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer (ref. No)</td>
<td>0.62 (−0.58, 1.83)</td>
<td>86</td>
</tr>
<tr>
<td>Education (ref. &lt;High school)</td>
<td>−0.58 (−1.54, 0.41)</td>
<td>−44</td>
</tr>
<tr>
<td>Years in the USA (per 10 years)</td>
<td>−1.27 (−2.51, 0.02)</td>
<td>−72</td>
</tr>
<tr>
<td>Postmenopausal (ref. Premenopausal)</td>
<td>−0.14 (−1.01, 0.73)</td>
<td>−13</td>
</tr>
<tr>
<td>Height (per 10 cm)</td>
<td>0.00 (−0.22, 0.22)</td>
<td>0</td>
</tr>
<tr>
<td>BMI (ln kg/m²)</td>
<td>0.29 (−0.60, 1.19)</td>
<td>34</td>
</tr>
</tbody>
</table>

*The coefficients and 95% CI are based on the ln-transformed RLUs.  
*Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula [e^β − 1] * 100.  
*Alcohol intake during calendar year before selection into parent study.
also observed a positive association with alcohol intake during the year prior to the first interview (compared with non-drinkers, women who reported drinking at least some alcohol had a 25% higher G activity, \( P = 0.015 \)) and a positive association with height (per every 10 cm there was a 17% increase in G activity, \( P = 0.037 \)) (Table IV). Stratified analyses did not suggest heterogeneity by menopausal status (Supplementary Table S2) or nativity (Supplementary Table S3) regarding these two variables. The association between G activity and alcohol intake was not observed when alcohol intake at blood draw instead of interview was included in the model (Supplementary Table S4). However, we found a statistically significant association between discordant status for the two alcohol variables and G activity (Supplementary Table S5).

### Discussion

We presented the results of a semi-targeted analysis of E and G activity in plasma in Mexican American women from the San Francisco Bay Area. Our results suggest that E activity in plasma is associated with Indigenous American ancestry and length of US residence among foreign-born Mexican American women. We also observed an association between G activity and alcohol intake. Indigenous American ancestry has consistently been associated with breast cancer risk, with lower risk among women with high Indigenous American ancestry (50,51). A genome-wide association study in Latinas reported the existence of a single nucleotide polymorphism, only present in Indigenous American populations, and associated with protection against breast cancer (52). In addition to this protective variant, non-genetic factors are likely to contribute to the decreased breast cancer risk in highly Indigenous American women, given that genetic ancestry is correlated with sociodemographic, reproductive and lifestyle factors such as education, neighborhood SES, use of HT and alcohol intake (53).

Our results suggest that lower levels of E activity among women with high Indigenous American ancestry could partly contribute to the inverse association between Indigenous American ancestry and breast cancer risk. It is unclear from our results if the lower E activity is due to lower levels of endogenous estrogens or lower levels of endocrine disruptors, and further studies will need to be conducted to clarify these results. Targeted studies looking at the association between endogenous estrogens and breast cancer risk have already shown a positive relationship (25,26), and a previous study of E activity in Asian women strongly suggested increased activity among women who developed breast cancer, beyond the effect of endogenous estrogen levels (19). Analyses did not include variables such as age at menarche, number of live births and breastfeeding, which are important breast cancer risk factors related to variation in estrogen levels. We focused on factors that were likely to be acting on hormone levels near the time of sample extraction. Given the average age of women in the study, remote events such as age at menarche or breastfeeding were not thought as likely to be reflected in estrogen levels. In order to confirm our assumption, we ran a model that included these variables, which did not show any meaningful change in estimates compared with those in the model without those variables (data not shown). There were only four women in the study who reported using HT at the time of blood draw. In the model for postmenopausal women, it was clear that the difference in activity was between current users versus former or never users. To make sure that the current use of HT was not affecting the association between years of residence in the USA and E activity, we tested a model that included only the postmenopausal foreign-born women and information on hormone replacement therapy use, which confirmed that the association with years of residence in the USA was independent of HT (\( P \) value for years in the USA was slightly lower when including HT, \( P = 0.026 \)).

The analysis of G activity, even though limited by a small sample size, also provided interesting results that warrant replication. Despite there being only nine breast cancer cases in the sample, we observed a negative association with G activity (the average G activity was lower among the women who developed breast cancer compared with those who did not). This is consistent with the observation that GR stimulation decreases the risk of relapse in breast tumors that are ER positive (54) due to cross talk between ER and GR (54,55). We also observed an association between G activity and alcohol intake as reported during the first interview (which for some of the individuals was 4 years before the time of blood draw). Due to low levels of alcohol intake among Mexican American women, we decided to compare individuals who responded that they do not drink at all to those who responded that they drink some alcohol. Women who reported drinking at least some alcohol had higher G activity than those

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### Table 4. Association between glucocorticogenic activity, lifestyle and demographic factors (N = 59)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (95% CI)</th>
<th>% Change in RLUs</th>
<th>P value</th>
<th>Coefficient (95% CI)</th>
<th>% Change in RLUs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nativity (ref. US-born)</td>
<td>-0.4 (-0.20, 0.12)</td>
<td>-33</td>
<td>0.628</td>
<td>-0.05 (-0.22, 0.13)</td>
<td>-5</td>
<td>0.589</td>
</tr>
<tr>
<td>Age at blood draw (per 10 years)</td>
<td>0.04 (-0.03, 0.11)</td>
<td>4</td>
<td>0.238</td>
<td>0.03 (-0.06, 0.13)</td>
<td>3</td>
<td>0.468</td>
</tr>
<tr>
<td>Indigenous American ancestry (per 10%)</td>
<td>-0.03 (-0.09, 0.03)</td>
<td>-3</td>
<td>0.305</td>
<td>-0.03 (-0.09, 0.03)</td>
<td>-3</td>
<td>0.338</td>
</tr>
<tr>
<td>Breast cancer (ref. No)</td>
<td>-0.09 (-0.29, 0.11)</td>
<td>-9</td>
<td>0.359</td>
<td>-0.23 (-0.45, 0.00)</td>
<td>-21</td>
<td>0.054</td>
</tr>
<tr>
<td>Neighborhood SES (continuous score)</td>
<td>-0.07 (-0.17, 0.04)</td>
<td>-7</td>
<td>0.217</td>
<td>-0.09 (-0.20, 0.02)</td>
<td>-9</td>
<td>0.106</td>
</tr>
<tr>
<td>Postmenopausal (ref. Premenopausal)</td>
<td>0.15 (-0.01, 0.31)</td>
<td>16</td>
<td>0.073</td>
<td>0.16 (-0.06, 0.37)</td>
<td>17</td>
<td>0.157</td>
</tr>
<tr>
<td>Height (per 10cm)</td>
<td>0.11 (-0.04, 0.26)</td>
<td>12</td>
<td>0.149</td>
<td>0.16 (0.01, 0.32)</td>
<td>17</td>
<td>0.037</td>
</tr>
<tr>
<td>BMI (ln kg/m²)</td>
<td>-0.01 (-0.05, 0.03)</td>
<td>-1</td>
<td>0.604</td>
<td>0.01 (-0.04, 0.05)</td>
<td>1</td>
<td>0.781</td>
</tr>
<tr>
<td>Alcohol intake (ref. None)</td>
<td>0.16 (-0.00, 0.33)</td>
<td>17</td>
<td>0.057</td>
<td>0.22 (0.05, 0.40)</td>
<td>25</td>
<td>0.015</td>
</tr>
</tbody>
</table>

1 The coefficients and 95% CI are based on the ln-transformed RLUs.
2 Percent change in RLUs (untransformed) per unit change in predictor was estimated using the formula \([e^\beta - 1] \times 100\).
3 Alcohol intake during calendar year before selection into parent study.
who responded that they never drank. Stress-induced glucocorticoid secretion triggers changes in gene expression through activation of the GR, which might alleviate immediate negative feelings associated with stress but lead to behavioral pathologies such as addiction, anxiety and depression (56). Studies have shown that inactivation of GR decreases motivation to take alcohol or other drugs (57,58). Our finding that G activity is higher among drinkers is consistent with these results and suggests the possibility that stress-induced activation of the GR might lead to increased levels of alcohol intake among some women in this overall low alcohol consumption group.

The study had some limitations. One limitation was that the data analyzed only included 11 women who had developed breast cancer. Ideally, we would have analyzed a larger number of cases to test if the associations observed between E or G activity and breast cancer risk factors mediated the association between those factors and breast cancer risk. Due to the small number of cases, we focused our analysis on the relationship between E and G activity and other factors that have been associated with breast cancer risk and were likely to be correlated with this activity. Another limitation was the relatively small overall sample size. However, we were able to discover some interesting associations that warrant replication and further investigation in future studies including a larger number of Latina women as well as women from other race/ethnicities. In addition, we lacked measures of endogenous estrogen, which would have allowed us to estimate what proportion of the variability in E activity might be due to differences in the level of estrogen-like compounds of exogenous origin versus differences in endogenous levels of estrogen. Finally, endogenous hormone levels as well as exposure to other receptor antagonists and agonists vary on a daily basis and therefore a measure of E and G activity obtained from a single plasma sample might not represent the average level of exposure for the individual. However, we believe that, at the population level, observed associations are informative and should be further explored, while absence of association cannot be taken as conclusive given that it is possible that for certain exposures the time at which the biospecimen was collected could be crucial.

In summary, we have investigated the levels of plasma estrogenic and glucocorticogenic activity in Mexican American women born in and outside the USA and tested their association with lifestyle, demographic and anthropometric breast cancer risk factors. Despite the null association with the main predictor (nativity), the cell-based measure of E and G activity reflected the expected differences by age at blood draw and menopausal status and also suggested previously unknown associations with genetic ancestry, years of US residence and alcohol intake. Future research will use cutting edge mass spectrometry-based technology to further identify the specific chemicals, and their precursors, that contribute to the observed associations and possibly to breast cancer risk. If modifiable, these agents could be targets of prevention programs, which would eventually reduce breast cancer incidence.

Supplementary material
Supplementary Tables 1–5 and Figures 1–3 can be found at http://carcin.oxfordjournals.org/

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