Review

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Women's Health

Links between alcohol consumption and breast cancer: a look at the evidence

Alcohol consumption by adult women is consistently associated with risk of breast cancer. Several questions regarding alcohol and breast cancer need to be addressed. Menarche to first pregnancy represents a window of time when breast tissue is particularly susceptible to carcinogens. Youth alcohol consumption is common in the USA, largely in the form of binge drinking and heavy drinking. Whether alcohol intake acts early in the process of breast tumorigenesis is unclear. This review aims to focus on the influences of timing and patterns of alcohol consumption and the effect of alcohol on intermediate risk markers. We also review possible mechanisms underlying the alcohol-breast cancer association.

Keywords: alcohol • benign breast disease • breast cancer • mammographic density • mechanism • risk factor

Ying Liu[1], Nhi Nguyen[1] & Graham A Colditz*[,1,2]

[1]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, 660 S Euclid Ave, St Louis, MO 63110, USA

[2]Alvin J Siteman Cancer Center at Barnes-Jewish Hospital & Washington University School of Medicine, 660 S Euclid Ave, St Louis, MO 63110, USA

*Author for correspondence: Tel.: +1 314 454 7939 colditzg@wustl.edu

Alcohol is considered by the International Agency for Research on Cancer to be causally related to breast cancer risk [1], with a 7–10% increase in risk for each 10 g (~1 drink) alcohol consumed daily by adult women [2–4]. This association is observed in both premenopausal and postmenopausal women. Compared with other organs, breast appears to be more susceptible to carcinogenic effects of alcohol. The risk of breast cancer is significantly increased by 4–15% for light alcohol consumption (≤1 drink/day or ≤12.5 g/day) [2,5,6] which does not significantly increase cancer risk in other organs of women [7]. This raises a clinical and public health concern because nearly half of women of child-bearing age drink alcohol and 15% of drinkers at this age have four or more drinks at a time [8]. Approximately 4–10% of breast cancers in the USA are attributable to alcohol consumption [2,5,6], accounting for 9000–23,000 new invasive breast cancer cases each year. Therefore, better understanding of how alcohol consumption increases breast cancer risk is crucial for developing breast cancer prevention strategies. As previous meta-analyses and systemic reviews comprehensively summarized the association between adult alcohol consumption and breast cancer risk [3,5,9,10], here we reviewed the recent epidemiologic evidence, with special emphasis on timing and patterns of alcohol consumption and the effect of alcohol on intermediate markers. In addition, we discussed up-to-date mechanisms that have been proposed to explain the association and provide guidance for clinicians on preventive messages.

Epidemiology

Importance of early-life exposures to breast cancer development

It is increasingly recognized that early-life exposures can affect a woman's lifetime risk of breast cancer [11–14]. Late onset of menstruation, early pregnancy and early onset of menopause are each associated with decreased risk for breast cancer. Breast cancer risk accumulates across a woman's life course; however, the most rapid accumulation occurs from menarche to first pregnancy [15–18]. Both human and animal model data have demonstrated that environmental exposures during adolescence and early adulthood are more important in breast cancer development than

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exposures later in life [6,12,14,19–22]. There may be a critical period of increased biologic vulnerability between menarche, when breast tissue undergoes rapid proliferation, until the completion of the first pregnancy, when multiple biologic changes in the breast occur and make epithelial cells resistant to becoming transformed into cancer cells [22–26]. This is well exemplified by the observed higher risk of breast cancer among female survivors from the atomic bomb explosions in Hiroshima and Nagasaki who were less than 20 years old at the time of bombing [12]. Experimental animal models do also support a critical role of early life exposures on breast cancer development. Compared with rats that have undergone full-term pregnancy, young virgin rats with undifferentiated mammary glands are more likely to develop breast tumors when exposed to chemical carcinogens [27].

Alcohol consumption during adolescence & early adulthood & risk of breast cancer

Given the increased susceptibility of nulliparous breast tissue to neoplastic transformation, alcohol, a breast carcinogen, consumed in adolescent and early adult years may be relevant to breast cancer development. Several epidemiologic studies have evaluated alcohol consumption across the life course in relation to breast cancer risk. The majority of these studies reported that recent drinking, but not drinking in early adult life, was significantly associated with breast cancer risk [28–33]. However, case–control studies showed a significant increase in breast cancer risk associated with early age at which women started to drink (<25 years) [34] and with alcohol consumption before age 30 years [35,36]. Alcohol consumption before age 30 years was dose dependently associated with premenopausal breast cancer risk, with a 34% increase in risk for every 13 g/day (~1 drink/day) of intake, but not with postmenopausal breast cancer risk [32]. In a prospective analysis of more than 105,000 predominantly postmenopausal women in the Nurses' Health Study (NHS), Chen *et al.* [2] compared alcohol consumed before and after age 40 with regard to breast cancer risk and reported statistically significant associations of similar magnitude (relative risk [RR]: 1.07–1.08 per 10 g/day consumption) for drinking in age ranges. However, these studies did not distinguish between drinking before first pregnancy and drinking after first pregnancy, but evaluated alcohol consumption at specific chronological age ranges.

Animal studies have demonstrated that dietary exposure to ethanol during puberty stimulates morphologic changes in mouse mammary glands, including increases in ductal branching (targets for malignant transformation) and epithelial proliferation and density [37,38]. However, ethanol exposure of parous mice at the beginning of natural postlactational involution has no effect on mammary gland structure and did not influence the regression of the lactating mammary gland to a resting state [39].

We recently refined the risk assessment approach to specifically address how alcohol intake between menarche and first pregnancy influences subsequent breast cancer risk among women in the Nurses' Health Study II (NHS II), a separate large cohort of US female nurses born from 1946 to 1964 [6]. Parous women ages 24–44 years with no prior history of breast cancer in 1989 (n = 91,005) were included in the analysis. During up to 20 years of follow-up, 1609 women were diagnosed with breast cancer. Compared with women who abstained from alcohol before first pregnancy, the risk of breast cancer was significantly increased by 34% (RR: 1.34; 95% CI: 1.00–1.80) for those with intake of ≥15 g per day (~1.5 drinks/day). Alcohol intake before first pregnancy was dose dependently associated with breast cancer risk, with the relative risk of 1.11 (95% CI: 1.00–1.23; p = 0.05) for each additional 10 g/day intake. In our study, alcohol consumed after first pregnancy was moderately, but nonsignificantly, associated with breast cancer risk, with the relative risk of 1.09 (95% CI: 0.96–1.23) for every 10 g/day consumption. The observed association with alcohol consumption before first pregnancy was independent of drinking after first pregnancy. Moreover, the increase in risk of breast cancer for alcohol intake before first pregnancy was stronger when the time interval between menarche and first pregnancy was longer. For every 10 g/day alcohol consumed before first pregnancy, the risk of breast cancer was increased by 14% among women with an interval of 10–14 years between menarche and first pregnancy and by 25% among women with an interval of 15 or more years.

These epidemiologic results, along with animal data, suggest that alcohol exposure before first pregnancy can lead to morphologic changes in the breast, which may predispose to breast cancer development. In addition, a longer exposure to alcohol during this susceptible period may confer excessive breast cancer risk. Early-life alcohol consumption appears to contribute to both pre- and post-menopausal breast cancer.

Alcohol intake & intermediate markers of breast cancer risk

Proliferative benign breast disease (BBD) and mammographic density are well-confirmed intermediate end points of breast cancer risk that are generally evaluated in epidemiologic studies of the breast cancer etiology. A recent analysis of women participating in the breast cancer surveillance consortium showed

that proliferative BBD and high breast density independently predict the risk of subsequent breast cancer [40]. As expected, women with both atypical hyperplasia and high breast density were at the greatest risk of breast cancer, with the relative risk of 5.34 (95% CI: 3.52–8.09). While alcohol consumption is consistently associated with increased risk of breast cancer, studies examining the associations of alcohol intake with proliferative BBD and mammographic density reported inconsistent results.

Benign breast disease

BBD encompasses a highly heterogeneous group of lesions that differ in their histopathologic features and clinical prognosis. Based on the criteria of Dupont and Page [41], BBD is generally divided into nonproliferative lesions, proliferative lesions without atypia and atypical hyperplasias. The presence of proliferative benign lesions influences subsequent breast cancer risk; the risk is increased by 30–90% among women with proliferative BBD and no atypia, and by fourfold to fivefold among those with atypical hyperplasia [42–45]. Women with proliferative BBD have higher risk of breast cancer in the same breast as well as in the contralateral breast.

Few epidemiologic studies have assessed the association between alcohol consumption and risk for BBD. Two case–control studies of risk factors for BBD did not find an association between alcohol consumption in the year preceding diagnosis and BBD risk [46,47], which is consistent with a prospective analysis of postmenopausal women participating in the Women's Health Initiative (WHI) randomized clinical trials [48]. One potential explanation for the lack of association is that these studies did not focus on an etiologically relevant exposure period. We recently examined the contributions of alcohol consumption before and after first pregnancy to risk of proliferative BBD [6]. We limited the analysis to cases confirmed by centralized pathologic review and diagnosed among parous women in the NHS II cohort. Compared with nondrinkers before first pregnancy, the risk for proliferative BBD was increased by 26% for those with daily intake of 5.0–14.9 g (~0.5–1.5 drinks) before first pregnancy and by 39% for those with daily intake of ≥15 g before first pregnancy. Overall, the risk for proliferative BBD was increased by 16% (95% CI: 2–32%) for each additional 10 g of alcohol consumed daily before first pregnancy. The increase in risk for proliferative BBD was more pronounced among women with a longer time interval between menarche and first pregnancy, although the difference was not statistically significant. In contrast, alcohol consumption after first pregnancy was not related to risk of proliferative BBD. The increased risk of proliferative BBD was also observed among women in the NHS II who reported moderate alcohol consumption between ages 18 and 22 years (RR: 1.15; 95% CI: 1.03–1.28 for each additional 10 g/day intake) [49]. Among daughters of women in the NHS II, daily intake of one drink of alcohol between ages 16 and 22 years was associated with a 50% (95% CI: 19–90%) increased risk of biopsy confirmed BBD [50]. Taken together, these results suggest that alcohol consumption during adolescence and early adulthood may have a greater adverse effect on risk of proliferative BBD as compared with alcohol intake in late adult years.

Mammographic density

Mammographic density is defined as the proportion of radiologically dense fibroglandular tissue in the breast. Mammographic density, assessed by either a qualitative approach or a quantitative measure of the radiodense area of the breast, is consistently associated with increased risk of breast cancer [51]. A meta-analysis of 14,000 cases and 226,000 noncases revealed that women with more than 75% mammographic density have almost five-times the risk of breast cancer compared with women with less than 5% mammographic density [52].

The relationship between alcohol consumption and mammographic density is inconsistent. Some studies observed a weak, but significant, positive trend of increasing mammographic density with increased adult alcohol consumption in both pre- and postmenopausal women [53–57]. But, there was no significant association between alcohol consumption and mammographic density in other studies [58–63]. In general, a positive association was reported in the studies where the overall alcohol consumption level was higher, such as more than 15% of participants consuming more than 10 g of alcohol per day. Women in the Minnesota Breast Cancer Family cohort, comprising breast cancer patients' first- and second-degree female relatives and spouse of male relatives, were asked about their age at initiation of alcohol intake and alcohol consumption before age 18 during the follow-up [59]. Women who reported ever drinking alcohol before age 18 had a higher mammographic density than women who never drank during adolescence. However, this difference was nullified by adjustment for breast cancer risk factors. There was a suggestive trend toward higher mammographic density for women who reported heavier and frequent drinking before age 18 and those who drank regularly in adolescence and continued throughout adulthood. In a New York birth cohort (born 1959–1963), alcohol consumption was asked separately for each decade of

life (prior to age 21, 21–29, 30–39 and 40 or older) and mammograms were obtained during the follow-up of female participants ages of 38–42 [64]. In that study, recent alcohol intake was more strongly associated with mammographic density than average lifetime alcohol intake. Compared with nondrinkers, those who reported seven or more drinks per week in the past year had 12.3% (95% CI: 4.3–20.4%) higher density. Mammographic density was inversely associated with alcohol consumption before age 21 but positively associated with alcohol consumption in other periods of life.

Drinking patterns & breast cancer risk

Alcohol consumption is common in adolescents and young adults in the USA, although the minimum legal drinking age is 21 years. More than one of four people aged 12–20 reported alcohol use during the past 30 days [65]. Nearly 70% of youth alcohol consumption is in the form of binge drinking [66], defined as consuming four or more alcoholic drinks on one occasion. Having multiple drinks in the same sitting results in higher alcohol levels in blood than having a single drink at one time, which can trigger different metabolic pathways [9]. Hence, women who report seven drinks on the weekend but no alcohol consumption on the weekdays may have higher risk of breast cancer as compared with those who consistently have one drink every day. Breast cancer risk is generally assessed in epidemiologic studies for an average amount of alcohol intake in a specified time/age period, which does not account for the effect of a large amount of alcohol consumed at any one time.

We identified three epidemiologic studies examining the relationship between binge drinking and breast cancer risk. Binge drinking is related to increased risk of breast cancer in two prospective studies among nurses. In the Danish Nurse Cohort study, binge drinking was evaluated on the last weekday and on weekends [67]. Women reporting binge drinking on weekends had a relative risk of 1.49 for 10–15 drinks and a relative risk of 2.51 for 16–21 drinks as compared with women reporting 1–3 drinks, while a lower risk was observed for a small number of women who drank more. Regarding binge drinking on the last weekday, the highest relative risk of 1.55 was observed for four to five drinks compared with intake of one drink. In the NHS, breast cancer risk was increased by 21% in adult binge drinkers compared with nondrinkers, after controlling for cumulative alcohol consumption [2]. A case–control study reported a nonsignificant increase in the risk for binge drinking with an odds ratio of 1.50, which was restricted to women who consumed 91 g/week (~7 drinks/week) or more of alcohol [68].

Type of alcoholic beverage & breast cancer

The influence of alcohol on breast cancer development might vary across types of alcohol. Red wine is thought to have a protective effect on cancer development and cardiovascular systems due to polyphenolic compounds from grape skin. Antioxidant, anti-inflammatory and anticancer functions of polyphenols in red wine occur through various molecular and biochemical processes. The compounds resveratrol, quercetin and catechin are three polyphenolic compounds, accounting for 70% of the red wine polyphenols [69]. They are structurally similar to estrogen and act as both antagonists and agonists on estrogen receptor (ER) [70–72]. Data from *in vitro* studies and a small randomized clinical trial suggest that resveratrol suppresses estrogen production from androgens by controlling the activity of aromatases [73,74]. Exposures to resveratrol in breast cancer cells inhibit DNA methyltransferases, the enzymes catalyzing DNA methylation and prevent epigenetic silencing of the *BRCA1* tumor suppressor protein [75].

Beer is a complex mixture of bioactive compounds. The most-studied constituents of beer are phenolic compounds, which are derived from malt (70–80%) and hop (20–30%). Some hop-derived compounds, such as xanthohumol and hop bitter acids, are considered as potential cancer chemopreventive agents that are able to interfere with the initiation, promotion and progression of carcinogenesis [76]. They have impacts on signaling pathways that control carcinogen metabolism, inflammatory reaction, angiogenesis and invasion, and induce apoptosis and cell differentiation [77]. Similar to resveratrol, xanthohumol has mixed estrogenic/antiestrogenic properties and inhibits aromatase activities *in vitro* [76]. However, these compounds are present in beer at very low levels and their absorption in the body is limited.

Relatively few epidemiologic studies evaluated the associations of types of alcohol with breast cancer and its intermediate risk markers. Although ethanol contents vary across different types of alcoholic beverages, similar breast cancer risk is reported for all types of alcoholic beverages [2,78,79]. Few studies examined types of alcohol in relation to intermediate breast cancer risk markers. In premenopausal women from the New York Women's Birth cohort, mammographic density was inversely associated with red wine consumption during adolescence and early adulthood and positively associated with beer and white wine intake in early adult years [64]. A significant positive association between recent white wine intake and mammographic density was also observed among postmenopausal women [64], which was consistent with data from the Minnesota breast cancer family cohort showing a positive association for white wine and an inverse

association for red wine in postmenopausal women [60]. A study among Mediterranean women reported a positive association between increasing levels of wine intake and mammographic density; it did not examine the associations by types of wine [80]. However, there was no association between adult alcohol intake and risk of proliferative BBD by types of beverages in postmenopausal women [48]. Overall, the epidemiologic evidence supports alcohol content – not type of beverage – that drives breast cancer risk. Available data regarding mammographic density by types of alcohol are limited.

Possible mechanisms

Despite a consistent association between alcohol consumption and breast cancer risk, the underlying mechanisms remain unclear. The most commonly investigated pathways include the effect of alcohol on circulating estrogen levels and ER in mammary epithelial cells and the carcinogenic role of ethanol metabolites. Recent *in vivo* and *in vitro* studies suggest other mechanisms through which alcohol may play a role in breast tumorigenesis, such as the effect of alcohol on epithelial–mesenchymal transition (EMT), epithelium–stroma interaction and epigenetic regulation of gene expression in the breast.

Estrogen & ER

Prolonged exposure to estrogens has been related to the elevated incidence of breast cancer in humans. Epidemiologic studies in postmenopausal women receiving hormonal therapy (HT) have revealed that high levels of estrogen in blood are associated with elevated risk of subsequent breast cancer [81,82]. Mammary adenocarcinomas were induced in rats continuously exposed to supraphysiological doses of estrogens, while fibroadenomas were found in rats dosed with low estrogen levels over long periods of time [83,84]. The proliferative effect of estrogens on breast epithelial cells is considered to be mediated by its nuclear receptor, ER-α [85]. However, estrogen may induce breast cancer through a genotoxic, ER-α-independent pathway [86–89].

In premenopausal adult women, alcohol intake has been associated with higher circulating levels of estradiol and estrone [90–92]. A controlled diet study reported that consumption of 30 g ethanol (~2.5 drinks) per day for three menstrual cycles was associated with a 28% increase in plasma estradiol and a 21% increase in plasma estrone among women ages 21–40 years [93]. The alcohol-related increase in plasma estradiol was restricted to women using oral contraceptives in another controlled diet study [94]. In addition, a shorter menstrual cycle was reported by premenopausal women with moderate alcohol consumption as compared with nondrinkers, suggesting an increased exposure to endogenous estrogens [95]. For adolescent girls, the impact of alcohol consumption on sex hormone levels remains unclear. Martin *et al.* [96] reported that alcohol use was positively related to blood estradiol and testosterone levels among girls in high schools. In contrast, Block *et al.* [97] reported that moderate alcohol consumption lowered estrogen levels in adolescent girls ages 12–18 years.

A recent meta-analysis of eight prospective studies among postmenopausal women showed that alcohol intake is positively associated with all the sex hormones, with the strongest association for dehydroepiandrosterone sulfate (DHEAS), but inversely associated with sex hormone-binding globulin [98]. DHEAS is an androgen and can be metabolized to estrogen in the breast by aromatase. In a randomized trial including 51 postmenopausal women without HT, serum estrone sulfate and DHEAS were increased by 8 and 5% in women consuming 15 g of alcohol per day, respectively [99]. There is an even more pronounced effect of moderate alcohol intake on blood estradiol levels in postmenopausal women who were taking HT. Compared with nondrinkers using estrogen, postmenopausal women who consumed 15.6 g of alcohol per week and took 1 mg of estradiol daily had a 3.3-fold increase in serum estradiol levels [100]. Alcohol may increase circulating sex hormone levels through an increase in the hepatic redox state and inhibition of the activity of sulfotransferase and 2-hydroxylase, resulting in a decrease in steroid degradation [94,101]. Another explanation is the increased aromatase activity following chronic alcohol consumption, which leads to an enhanced conversion of testosterone to estrogens [102,103].

The elevated levels of intracellular estrogens resulting from alcohol intake may act through the ER to promote breast tumor growth. Ethanol stimulates proliferation of ER^+ but not ER^- breast cancer cells, causing a 10- to 15-fold increase in transcriptional activity of ER [104,105]. Ethanol increases ER-α expression through the JNK1 pathway [106]. Several epidemiologic studies have shown that alcohol was more strongly associated with hormone-receptor-positive breast tumors than with other types of breast cancer [10], consistent with an underlying hormonal basis for the association between alcohol intake and breast cancer. The risks for ER^+/PR^+ and ER^+/PR^- breast cancer increased by 8% (95% CI: 2–15%) and 12% (95% CI: 0–25%), respectively, per drink consumed per day among postmenopausal women [107], which is comparable to the 12% (95% CI: 8–15%) increase in risk of ER^+ tumors per 10 g/day of alcohol consumption reported in a meta-analysis of four prospective studies and 16 case–control studies [10]. In the NHS cohort,

alcohol consumption appeared to be more strongly associated with risk of ER^+/PR^+, ER^+/PR^- than with risk of ER^-/PR^-, but the difference was not significant [2]. Among parous women in the NHS II, we found that alcohol consumption before first pregnancy tended to be more strongly related to risks of ER^+/PR^+ tumors (RR: 1.18; 95% CI: 1.03–1.34; $p_{heterogeneity} = 0.06$) compared with the risks for ER^+/PR^- tumors (RR: 0.86; 95% CI: 0.60–1.22) and ER^-/PR^- tumors (RR: 0.84; 95% CI: 0.60–1.16) [6]. Together, these results support the hypothesis that alcohol may enhance breast tissue's sensitivity to estrogens and predominantly increase the risk of breast cancer expressing the hormone receptors.

Alcohol metabolism

The ethanol-induced increase in sex hormones is thought to promote proliferation of already initiated mammary epithelial cells but not cause neoplastic transformation of normal epithelial cells [108]. Another possibility is related to carcinogenic products of alcohol metabolism. In the human body, alcohol is converted to acetaldehyde primarily by alcohol dehydrogenase and further to acetate by acetaldehyde dehydrogenase and xanthine oxidoreductase [5,109]. Acetaldehyde rapidly binds to DNA and proteins and produces DNA adducts, which results in DNA point mutations, DNA crosslinks and chromosomal aberrations [110–112]. In addition, acetaldehyde inhibits the repair of oxidative DNA damages induced by alkylating agents [113].

Although liver is a primary site where acetaldehyde and free radicals are produced in the process of alcohol metabolism, normal human breast tissue has the capacity to metabolize ethanol at low concentrations and alcohol dehydrogenase is expressed in the human breast epithelial cells [9,114]. In rats, acetaldehyde accumulates in mammary tissue for prolonged periods of time after a single oral dose of ethanol and finally reaches a level considerably higher than in blood [115,116]. This is primarily due to increasing production of acetaldehyde in mammary tissue, the limited ability to detoxify acetaldehyde in mammary tissue and acetaldehyde produced elsewhere and delivered to mammary tissue via blood [108].

In addition to acetaldehyde, reactive oxygen species (ROS) are derived from alcohol metabolism and have been implicated in alcohol-associated breast carcinogenesis. Similar to acetaldehyde, ROS can damage DNA by causing mutation and strand breaks [109]. ROS are involved in both the initiation and progression of cancer [117]. Xanthine oxidoreductase and aldehyde oxidase, two enzymes involved in acetaldehyde metabolism, can generate ROS and are also present in mammary tissue [109]. Thus, exposure to alcohol may increase oxidative DNA damage in breast tissue. This hypothesis is supported by a recent *in vitro* study in which alcohol-derived salsolinol significantly enhanced 8-oxo-dG formation, an indicator of oxidative damage, in normal mammary epithelial cells [118]. Elevated levels of 8-oxo-dG adducts in DNA play a fundamental role in breast cancer [119].

Other mechanisms

The EMT is essential for the normal development and also emerging as an important mechanism for cancer progression [120]. During the EMT, epithelial cells lose their polarity and tight cell–cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. The EMT phenotype is characterized by upregulation of matrix metalloprotease (MMP) and vimentin but downregulation of E-cadherin. Forsyth *et al.* [121] reported that alcohol triggers EMT in breast cancer cells through EGFR–Snail signaling. The ethanol concentrations used in this study were equivalent to those that could be generated by moderate alcohol intake in humans.

Carcinogenic effects of ethanol may not only target breast epithelial cells. Ethanol may also affect stromal cells and interfere with the tumor–stroma interaction. MMPs enhance tumor invasion and metastasis by degrading the extracellular matrix and promoting cell migration [122]. High levels of MMP-2 and MMP-9 expression in tumor tissue have been correlated with enhanced metastasis and poor prognosis in breast cancer patients [123–125]. In most cases, these two MMPs are not produced by malignant epithelial cells, but by surrounding tumor stroma, particularly stromal fibroblasts [126,127]. Ethanol activates MMP-2 production by fibroblasts in a dose-dependent manner and culture medium collected from ethanol-exposed fibroblasts significantly alters the invasive behavior of breast cancer cells and mammary epithelial cells [128]. In addition, a recent *in vitro* study showed that ethanol promotes the adhesion of breast cancer cells to fibronectin, an important component of the extracellular matrix through suppression of the Nm23 metastatic suppressor gene and subsequent enhancement of fibronectin receptor ITGA5 expression [129]. Ethanol stimulates migration and invasion of breast cancer cells [129], particularly those overexpressing HER2 [130]. Breast tumors overexpressing HER2 account for about 20–30% of breast cancer cases and generally have poor prognosis [131–135].

Emerging evidence suggests the impact of alcohol on epigenetic regulation of gene expression [136]. Epigenetic dysregulation is a key mechanism for tumor initiation and progression. Abnormal DNA methylation is the best understood epigenetic cause of disease. Global hypomethylation can result in chromosome instability,

and region-specific hypermethylation has been linked with the silencing of tumor suppressor genes. Chronic alcohol intake has been linked to lower leukocyte DNA global methylation in humans [137]. In a study of the methylation profiles of breast tumors, Christensen *et al.* [138] showed a trend toward decreased methylation with increasing alcohol intake, and a trend toward increased methylation with increasing dietary folate. There were no individual CpG loci showing statistically significant alcohol-related changes in methylation in that study. However, other studies reported that alcohol consumption was related to altered methylation patterns for several genes, including hypermethylation of ER-α [139] and tumor suppressor gene E-cadherin and hypomethylation of p16 [140].

Several mechanisms may mediate the effect of alcohol on DNA methylation, including reduced folate bioavailability and inhibition of key enzymes in one-carbon metabolism that leads to reduced production of the major methyl donor S-adenosylmethionine [5,136]. In addition, alcohol and acetaldehyde affect methylation patterns by suppression of activity and expression of enzymes involved in DNA methylation. Alcohol can adversely affect folate metabolism by inhibiting the intestinal absorption, reducing the hepatic storage and increasing the renal excretion [141]. Folate, as a methyl donor in one-carbon metabolism, is essential for DNA synthesis and methylation. Several prospective studies showed that the adverse effect of alcohol consumption on breast cancer risk was restricted to women with low folate intake, and a protective effect of high folate intake (generally ≥600 μg/day) on breast cancer risk was observed primarily among women with high alcohol consumption [142–146]. However, our previous work showed that folate intake during adolescence did not reduce the risk of proliferative BBD associated with alcohol intake during adolescence and early adulthood among women in the NHS II [49]. This null finding could be due to the average level of folate intake (310 μg/day) in our sample that was too low to detect a modifying effect of folate on the risk for alcohol-associated proliferative BBD.

Conclusion & clinical implications

Moderate alcohol consumption is consistently associated with increased risk of breast cancer, particularly hormone receptor-positive subtypes. Given the increased susceptibility of breast tissue to tumorigenesis between menarche and first pregnancy and the high prevalence of alcohol use in adolescent girls and young women, understanding how alcohol intake before first pregnancy influences breast cancer development is important for breast cancer prevention. Women should understand the accumulation of risk across the life course and the lifelong increase in risk of breast cancer from moderate and heavy consumptions in early adult years. We have reported that the risks of breast cancer and proliferative BBD are increased by 11 and 16% for one drink/day alcohol intake before first pregnancy when breast tissue is likely at its most vulnerable stage. This translates into 4% of breast cancer cases and 11% of proliferative BBD cases attributable to alcohol consumed before first pregnancy, the estimates controlled for alcohol consumed after first pregnancy. Breast cancer prevention efforts should not only target midlife and older women, but also include adolescent girls and young women. Healthcare providers should be aware of the adverse effect of youth drinking on a woman's lifelong risk of breast cancer and provide behavioral counsels to their patients.

The US Preventive Services Task Force recommends that clinicians screen adults aged 18 years or older for alcohol misuse and provide persons engaged in risky or hazardous drinking with brief behavioral counseling interventions [147]. This general recommendation, such as the WHO recommending limited intake, misses the critical importance of adolescent and early adult alcohol intake among women. The available evidence is insufficient to assess screening and behavioral counseling interventions in primary care settings to reduce alcohol misuse in adolescents. However, greater attention by primary care physicians and other health professionals to identifying and preventing underage drinking remains a priority, as adolescents today will experience breast cancer over the coming 50 years.

Alcohol may be involved in breast tumor initiation and promotion through increasing sex hormone levels, enhancing breast epithelial cell responsiveness to sex hormones, producing genotoxic metabolite acetaldehyde and oxidative stress. Recent *in vitro* studies suggest that alcohol stimulates migration and invasion of breast cancer cells through interfering with the epithelium–stroma interaction and enhancing EMT. Aberrant patterns of DNA methylation also could be part of the pathogenic mechanisms that lead to alcohol-induced cancer development.

Despite a dose-dependent association between alcohol and breast cancer risk, it remains unclear about a threshold level of alcohol consumption above which the increased risk of breast cancer becomes clinically significant. Moderate alcohol consumption appears to reduce the risk of developing or dying from heart disease, the leading cause of death in the USA. This cardiovascular benefit is observed primarily in middle-aged and older people. There is paucity of data regarding the association between alcohol intake and cardiovascular health in young adults. A pooled analysis of eight prospective studies including more than 190,000

women showed a lower risk of coronary heart disease for low-to-moderate alcohol intake in young women (age <50 years) [148]. The beneficial effect in young women might be negligible due to their low absolute risk for coronary heart disease. Healthcare providers should discuss with their patients about drinking habits as well as weighing the risk and benefit of low-to-moderate alcohol intake. The 2010 US Dietary Guidelines for Americans recommends that adult women who consume alcohol should limit their exposure to no more than one drink per day. Given a small, but significant, association between light and moderate alcohol intake and breast cancer risk, the recommended amount of alcohol may be inappropriate for some women in terms of potential breast cancer risk. Improved understanding of breast cancer risk accumulation and provider guidance on risk of breast cancer can lead to reductions in alcohol intake during the critical period of breast cancer risk accumulation and lower lifelong risk for breast cancer.

Executive summary

Background

- Alcohol is classified as a breast carcinogen by the International Agency for Research on Cancer.
- This review focuses on timing and patterns of alcohol consumption, the effect of alcohol on intermediate breast cancer risk markers and possible mechanisms.

Timing of alcohol consumption & breast cancer

- Exposures between menarche and first pregnancy, a stage when breast tissue is most susceptible to neoplastic transformation, can affect a woman's lifetime risk of breast cancer.
- Dietary exposure to ethanol during puberty – but not ethanol exposures after lactation – induces morphologic changes in mouse mammary glands that predispose to breast cancer development.
- Alcohol consumption before first pregnancy is dose dependently associated with a significant increase in risk of breast cancer, which is independent of alcohol consumption after first pregnancy.
- A prolonged alcohol consumption before first pregnancy confers excess risk of breast cancer.

Alcohol intake & intermediate markers of breast cancer risk

- Alcohol consumption during adolescence and early adulthood may have a greater adverse effect on risk of proliferative benign breast disease as compared with alcohol drinking in late adult years.
- There is no clear pattern of relationship between alcohol consumption and mammographic density.

Drinking patterns & breast cancer risk

- Most young drinkers binge drink.
- Few studies evaluated binge drinking in relation to breast cancer, with increased risk observed in prospective studies.

Type of alcoholic beverage & breast cancer

- Some constituents of red wine and beer have an anticancer property and are considered as potential chemopreventive agents
- Epidemiologic evidence suggests that alcohol content, rather than types of alcohol, is associated with risk for breast cancer.

Possible mechanisms

- Alcohol intake increases circulating levels of estrogens in both premenopausal and postmenopausal women, which might occur through reduced steroid degradation and increased aromatase activity.
- Alcohol influences breast cancer development potentially through ER-dependent pathways as alcohol substantially enhances transcriptional activity of ER and is more strongly associated with hormone receptor positive breast tumors.
- Carcinogenic products of alcohol may play a critical role in breast carcinogenesis as key alcohol metabolism enzymes are present in normal human breast tissue.
- New biologic mechanisms include the promotion of epithelial–mesenchymal transition, the activation of matrix metalloproteases leading to extracellular matrix degradation and cellular migration, the enhancement of breast cancer cells adhering to fibronectin and epigenetic regulation of gene expression possibly through interfering with folate metabolism.

Conclusion & clinical implications

- Alcohol intake in adolescent and early adult years increases risks of breast cancer and proliferative benign breast disease, suggesting that breast cancer prevention efforts should begin early in life.
- Given the high prevalence of youth alcohol consumption, healthcare providers should educate girls and young women about the lifelong risk of breast cancer from alcohol consumption across the life course.
- Future studies will need to evaluate drinking patterns across the life course in relation to breast cancer risk, identify what components of lifestyles could modify the adverse effect of alcohol and characterize the biologic changes stimulated by alcohol in breast tissue.

Future perspective

It is estimated that 10.8 million young people in the USA between the ages of 12 and 20 years (28.2% of this age group) are current drinkers, of which nearly 7.2 million (18.8%) are binge drinkers, and 2.3 million (6.0%) are heavy drinkers. Hence, future studies are needed to examine drinking patterns over the lifetime in relation to breast cancer risk. In addition, little is known about what components of lifestyles could modify the adverse effect of alcohol consumption on breast cancer development.

A better understanding of the biologic changes stimulated by alcohol leading to cancer in breast tissue would help develop novel markers that could be used in cancer prevention by allowing us to identify a subgroup of the most susceptible women and providing therapy targets. In particular, identification of the molecular processes linking alcohol to breast cancer would provide guidance on safe levels of alcohol drinking and could provide the basis for women's informed decisions regarding frequency and volume of alcohol consumption.

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References

1 IARC working group on the evaluation of carcinogenic risks to humans. *Alcohol Consumption and Ethyl Carbamate. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.* International Agency for Research on Cancer, Lyon, France (2010).

2 Chen WY, Rosner B, Hankinson SE, Colditz GA, Willett WC. Moderate alcohol consumption during adult life, drinking patterns, and breast cancer risk. *JAMA* 306(17), 1884–1890 (2011).

3 Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *JAMA* 286(17), 2143–2151 (2001).

4 Smith-Warner SA, Spiegelman D, Yaun SS *et al.* Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* 279(7), 535–540 (1998).

5 Seitz HK, Pelucchi C, Bagnardi V, La Vecchia C. Epidemiology and pathophysiology of alcohol and breast cancer: update 2012. *Alcohol Alcohol.* 47(3), 204–212 (2012).

6 Liu Y, Colditz GA, Rosner B *et al.* Alcohol intake between menarche and first pregnancy: a prospective study of breast cancer risk. *J. Natl Cancer Inst.* 105(20), 1571–1578 (2013).

7 Bagnardi V, Rota M, Botteri E *et al.* Light alcohol drinking and cancer: a meta-analysis. *Ann. Oncol.* 24(2), 301–308 (2013).

8 Centers for Disease Control and Prevention. Alcohol use and binge drinking among women of childbearing age-United States, 2006–2010. *MMWR* 61(28), 534–538 (2012).

9 Brooks PJ, Zakhari S. Moderate alcohol consumption and breast cancer in women: from epidemiology to mechanisms and interventions. *Alcohol. Clin. Exp. Res.* 37(1), 23–30 (2013).

10 Suzuki R, Orsini N, Mignone L, Saji S, Wolk A. Alcohol intake and risk of breast cancer defined by estrogen and progesterone receptor status – a meta-analysis of epidemiological studies. *Int. J. Cancer* 122(8), 1832–1841 (2008).

11 Colditz GA, Frazier AL. Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. *Cancer Epidemiol. Biomarkers Prev.* 4(5), 567–571 (1995).

12 Land CE, Tokunaga M, Koyama K *et al.* Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1990. *Radiat. Res.* 160(6), 707–717 (2003).

13 Trichopoulos D, Adami HO, Ekbom A, Hsieh CC, Lagiou P. Early life events and conditions and breast cancer risk: from epidemiology to etiology. *Int. J. Cancer* 122(3), 481–485 (2008).

14 Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC. Adolescent and adult soy intake and risk of breast cancer in Asian–Americans. *Carcinogenesis* 23(9), 1491–1496 (2002).

15 Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: data from the Nurses' Health Study. *Am. J. Epidemiol.* 152(10), 950–964 (2000).

16 Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG. 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. *Nature* 303(5920), 767–770 (1983).

17 Rosner B, Colditz GA. Nurses' health study: log-incidence mathematical model of breast cancer incidence. *J. Natl Cancer Inst.* 88(6), 359–364 (1996).

18 Rosner B, Colditz GA, Willett WC. Reproductive risk factors in a prospective study of breast cancer: the Nurses' Health Study. *Am. J. Epidemiol.* 139(8), 819–835 (1994).

19 Anderson LN, Cotterchio M, Boucher BA, Kreiger N. Phytoestrogen intake from foods, during adolescence and adulthood, and risk of breast cancer by estrogen and progesterone receptor tumor subgroup among Ontario women. *Int. J. Cancer* 132(7), 1683–1692 (2013).

20 Lee SA, Shu XO, Li H *et al.* Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *Am. J. Clin. Nutr.* 89(6), 1920–1926 (2009).

21 Maruti SS, Willett WC, Feskanich D, Rosner B, Colditz GA. A prospective study of age-specific physical activity and premenopausal breast cancer. *J. Natl Cancer Inst.* 100(10), 728–737 (2008).

22 Russo IH, Russo J. Pregnancy-induced changes in breast cancer risk. *J. Mammary Gland Biol. Neoplasia* 16(3), 221–233 (2011).

23 Meier-Abt F, Milani E, Roloff T *et al.* Parity induces differentiation and reduces Wnt/Notch signaling ratio and proliferation potential of basal stem/progenitor cells isolated from mouse mammary epithelium. *Breast Cancer Res.* 15(2), R36 (2013).

24 Medina D. Pregnancy protection of breast cancer: new insights reveal unanswered questions. *Breast Cancer Res.* 15(3), 103 (2013).

25 Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JR. Long-term effect of a first pregnancy on the secretion of prolactin. *N. Engl. J. Med.* 316(5), 229–234 (1987).

26 Bernstein L, Pike MC, Ross RK, Judd HL, Brown JB, Henderson BE. Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. *J. Natl Cancer Inst.* 74(4), 741–745 (1985).

27 Russo J, Russo IH. Toward a physiological approach to breast cancer prevention. *Cancer Epidemiol. Biomarkers Prev.* 3(4), 353–364 (1994).

28 Swanson CA, Coates RJ, Malone KE *et al.* Alcohol consumption and breast cancer risk among women under age 45 years. *Epidemiology* 8(3), 231–237 (1997).

29 Tjonneland A, Christensen J, Thomsen BL *et al.* Lifetime alcohol consumption and postmenopausal breast cancer rate in Denmark: a prospective cohort study. *J. Nutr.* 134(1), 173–178 (2004).

30 Horn-Ross PL, Canchola AJ, West DW *et al.* Patterns of alcohol consumption and breast cancer risk in the California Teachers Study cohort. *Cancer Epidemiol. Biomarkers Prev.* 13(3), 405–411 (2004).

31 Holmberg L, Baron JA, Byers T *et al.* Alcohol intake and breast cancer risk: effect of exposure from 15 years of age. *Cancer Epidemiol. Biomarkers Prev.* 4(8), 843–847 (1995).

32 Longnecker MP, Newcomb PA, Mittendorf R *et al.* Risk of breast cancer in relation to lifetime alcohol consumption. *J. Natl Cancer Inst.* 87(12), 923–929 (1995).

33 Berstad P, Ma H, Bernstein L, Ursin G. Alcohol intake and breast cancer risk among young women. *Breast Cancer Res. Treat.* 108(1), 113–120 (2008).

34 van't Veer P, Kok FJ, Hermus RJ, Sturmans F. Alcohol dose, frequency and age at first exposure in relation to the risk of breast cancer. *Int. J. Epidemiol.* 18(3), 511–517 (1989).

35 Harvey EB, Schairer C, Brinton LA, Hoover RN, Fraumeni JF Jr. Alcohol consumption and breast cancer. *J. Natl Cancer Inst.* 78(4), 657–661 (1987).

36 Young TB. A case–control study of breast cancer and alcohol consumption habits. *Cancer* 64(2), 552–558 (1989).

37 Masso-Welch PA, Tobias ME, Vasantha Kumar SC *et al.* Folate exacerbates the effects of ethanol on peripubertal mouse mammary gland development. *Alcohol* 46(3), 285–292 (2012).

38 Singletary K. Ethanol and experimental breast cancer: a review. *Alcohol. Clin. Exp. Res.* 21(2), 334–339 (1997).

39 Garofalo JM, Bowers DM, Browne RW *et al.* Mouse mammary gland is refractory to the effects of ethanol after natural lactation. *Comp. Med.* 63(1), 38–47 (2013).

40 Tice JA, O'Meara ES, Weaver DL, Vachon C, Ballard-Barbash R, Kerlikowske K. Benign breast disease, mammographic breast density, and the risk of breast cancer. *J. Natl Cancer Inst.* 105(14), 1043–1049 (2013).

41 Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. *N. Engl. J. Med.* 312(3), 146–151 (1985).

42 Dupont WD, Parl FF, Hartmann WH *et al.* Breast cancer risk associated with proliferative breast disease and atypical hyperplasia. *Cancer* 71(4), 1258–1265 (1993).

43 London SJ, Connolly JL, Schnitt SJ, Colditz GA. A prospective study of benign breast disease and the risk of breast cancer. *JAMA* 267(7), 941–944 (1992).

44 Marshall LM, Hunter DJ, Connolly JL *et al.* Risk of breast cancer associated with atypical hyperplasia of lobular and ductal types. *Cancer Epidemiol. Biomarkers Prev.* 6(5), 297–301 (1997).

45 Page DL, Dupont WD, Rogers LW, Rados MS. Atypical hyperplastic lesions of the female breast: a long-term follow-up study. *Cancer* 55(11), 2698–2708 (1985).

46 Rohan TE, Cook MG. Alcohol consumption and risk of benign proliferative epithelial disorders of the breast in women. *Int. J. Cancer* 43(4), 631–636 (1989).

47 Friedenreich C, Bryant H, Alexander F, Hugh J, Danyluk J, Page D. Risk factors for benign proliferative breast disease. *Int. J. Epidemiol.* 29(4), 637–644 (2000).

48 Cui Y, Page DL, Chlebowski RT *et al.* Alcohol and folate consumption and risk of benign proliferative epithelial disorders of the breast. *Int. J. Cancer* 121(6), 1346–1351 (2007).

49 Liu Y, Tamimi RM, Berkey CS *et al.* Intakes of alcohol and folate during adolescence and risk of proliferative benign breast disease. *Pediatrics* 129(5), e1192–e1198 (2012).

50 Berkey CS, Willett WC, Frazier AL *et al.* Prospective study of adolescent alcohol consumption and risk of benign breast disease in young women. *Pediatrics* 125(5), e1081–e1087 (2010).

51 Boyd NF, Martin LJ, Yaffe MJ, Minkin S. Mammographic density and breast cancer risk: current understanding and future prospects. *Breast Cancer Res.* 13(6), 223 (2011).

52 McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* 15(6), 1159–1169 (2006).

53 Boyd NF, Connelly P, Byng J *et al.* Plasma lipids, lipoproteins, and mammographic densities. *Cancer Epidemiol. Biomarkers Prev.* 4(7), 727–733 (1995).

54 Masala G, Assedi M, Bendinelli B *et al.* Glycemic index, glycemic load and mammographic breast density: the EPIC Florence longitudinal study. *PLoS ONE* 8(8), e70943 (2013).

55 Cabanes A, Pastor-Barriuso R, Garcia-Lopez M *et al.* Alcohol, tobacco, and mammographic density: a population-based study. *Breast Cancer Res. Treat.* 129(1), 135–147 (2011).

56 Vachon CM, Kuni CC, Anderson K, Anderson VE, Sellers TA. Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States). *Cancer Causes Control* 11(7), 653–662 (2000).

57 Berube S, Diorio C, Verhoek-Oftedahl W, Brisson J. Vitamin D, calcium, and mammographic breast densities. *Cancer Epidemiol. Biomarkers Prev.* 13(9), 1466–1472 (2004).

58 Qureshi SA, Couto E, Hofvind S, Wu AH, Ursin G. Alcohol intake and mammographic density in postmenopausal Norwegian women. *Breast Cancer Res. Treat.* 131(3), 993–1002 (2012).

59 Vachon CM, Sellers TA, Janney CA *et al.* Alcohol intake in adolescence and mammographic density. *Int. J. Cancer* 117(5), 837–841 (2005).

60 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.* 9(2), 151–160 (2000).

61 Maskarinec G, Takata Y, Pagano I, Lurie G, Wilkens LR, Kolonel LN. Alcohol consumption and mammographic density in a multiethnic population. *Int. J. Cancer* 118(10), 2579–2583 (2006).

62 Herrinton LJ, Saftlas AF, Stanford JL, Brinton LA, Wolfe JN. Do alcohol intake and mammographic densities interact in regard to the risk of breast cancer? *Cancer* 71(10), 3029–3035 (1993).

63 Gapstur SM, Lopez P, Colangelo LA, Wolfman J, Van Horn L, Hendrick RE. Associations of breast cancer risk factors with breast density in Hispanic women. *Cancer Epidemiol. Biomarkers Prev.* 12(10), 1074–1080 (2003).

64 Flom JD, Ferris JS, Tehranifar P, Terry MB. Alcohol intake over the life course and mammographic density. *Breast Cancer Res. Treat.* 117(3), 643–651 (2009).

65 *Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings*. Substance Abuse and Mental Health Services Administration, MD, USA (2012).

66 Eaton DK, Kann L, Kinchen SA. Youth risk behavior surveillance-United States, 2011. *CDC Morb. Mort. Surveil. Summ.* 61, 1–162 (2012).

67 Morch LS, Johansen D, Thygesen LC *et al.* Alcohol drinking, consumption patterns and breast cancer among Danish nurses: a cohort study. *Eur. J. Public Health* 17(6), 624–629 (2007).

68 Kinney AY, Millikan RC, Lin YH, Moorman PG, Newman B. Alcohol consumption and breast cancer among black and white women in North Carolina (United States). *Cancer Causes Control* 11(4), 345–357 (2000).

69 Damianaki A, Bakogeorgou E, Kampa M *et al.* Potent inhibitory action of red wine polyphenols on human breast cancer cells. *J. Cell. Biochem.* 78(3), 429–441 (2000).

70 Ratna WN, Simonelli JA. The action of dietary phytochemicals quercetin, catechin, resveratrol and naringenin on estrogen-mediated gene expression. *Life Sci.* 70(13), 1577–1589 (2002).

71 Harris DM, Besselink E, Henning SM, Go VL, Heber D. Phytoestrogens induce differential estrogen receptor alpha- or Beta-mediated responses in transfected breast cancer cells. *Exp. Biol. Med.* 230(8), 558–568 (2005).

72 Chakraborty S, Levenson AS, Biswas PK. Structural insights into Resveratrol's antagonist and partial agonist actions on estrogen receptor alpha. *BMC Struct. Biol.* 13, 27 (2013).

73 Shufelt C, Merz CN, Yang Y *et al.* Red versus white wine as a nutritional aromatase inhibitor in premenopausal women: a pilot study. *J. Womens Health (2002).* 21(3), 281–284 (2012).

74 He S, Sun C, Pan Y. Red wine polyphenols for cancer prevention. *Int. J. Mol. Sci.* 9(5), 842–853 (2008).

75 Hardy TM, Tollefsbol TO. Epigenetic diet: impact on the epigenome and cancer. *Epigenomics* 3(4), 503–518 (2011).

76 Gerhauser C. Beer constituents as potential cancer chemopreventive agents. *Eur. J. Cancer* 41(13), 1941–1954 (2005).

77 Monteiro R, Calhau C, Silva AO *et al.* Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J. Cell. Biochem.* 104(5), 1699–1707 (2008).

78 Petri AL, Tjonneland A, Gamborg M *et al.* Alcohol intake, type of beverage, and risk of breast cancer in pre- and postmenopausal women. *Alcohol. Clin. Exp. Res.* 28(7), 1084–1090 (2004).

79 Ellison RC, Zhang Y, McLennan CE, Rothman KJ. Exploring the relation of alcohol consumption to risk of breast cancer. *Am. J. Epidemiol.* 154(8), 740–747 (2001).

80 Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, Palli D. Dietary and lifestyle determinants of mammographic breast density: a longitudinal study in a Mediterranean population. *Int. J. Cancer* 118(7), 1782–1789 (2006).

81 Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J. Natl Cancer Inst.* 90(11), 814–823 (1998).

82 Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J. Natl Cancer Inst.* 94(8), 606–616 (2002).

83 Russo J, Russo IH. Experimentally induced mammary tumors in rats. *Breast Cancer Res. Treat.* 39(1), 7–20 (1996).

84 Russo IH, Russo J. Mammary gland neoplasia in long-term rodent studies. *Environ. Health Persp.* 104(9), 938–967 (1996).

85 Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J. Biol. Chem.* 276(40), 36869–36872 (2001).

86 Cavalieri E, Rogan E, Chakravarti D. The role of endogenous catechol quinones in the initiation of cancer and neurodegenerative diseases. *Methods Enzymol.* 382, (293–319 (2004).

87 Chakravarti D, Mailander PC, Li KM *et al.* Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene* 20(55), 7945–7953 (2001).

88 Saeed M, Rogan E, Fernandez SV, Sheriff F, Russo J, Cavalieri E. Formation of depurinating N3Adenine and N7Guanine adducts by MCF-10F cells cultured in the presence of 4-hydroxyestradiol. *Int. J. Cancer* 120(8), 1821–1824 (2007).

89 Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med.* 354(3), 270–282 (2006).

90 Mendelson JH, Lukas SE, Mello NK, Amass L, Ellingboe J, Skupny A. Acute alcohol effects on plasma estradiol levels in women. *Psychopharmacology* 94(4), 464–467 (1988).

91 Muti P, Trevisan M, Micheli A *et al.* Alcohol consumption and total estradiol in premenopausal women. *Cancer Epidemiol. Biomarkers Prev.* 7(3), 189–193 (1998).

92 Dorgan JF, Reichman ME, Judd JT *et al.* The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control* 5(1), 53–60 (1994).

93 Reichman ME, Judd JT, Longcope C *et al.* Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. *J. Natl Cancer Inst.* 85(9), 722–727 (1993).

94 Sarkola T, Makisalo H, Fukunaga T, Eriksson CJ. Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol. Clin. Exp. Res.* 23(6), 976–982 (1999).

95 Cooper GS, Sandler DP, Whelan EA, Smith KR. Association of physical and behavioral characteristics with menstrual cycle patterns in women age 29–31 years. *Epidemiology* 7(6), 624–628 (1996).

96 Martin CA, Mainous AG 3rd, Curry T, Martin D. Alcohol use in adolescent females: correlates with estradiol and testosterone. *Am. J. Addict.* 8(1), 9–14 (1999).

97 Block GD, Yamamoto ME, Mallick E, Styche A. Effects on pubertal hormones by ethanol abuse in adolescents. *Alcoholism* 17, 505 (1993).

98 Key TJ, Appleby PN, Reeves GK *et al.* Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br. J. Cancer* 105(5), 709–722 (2011).

99 Dorgan JF, Baer DJ, Albert PS *et al.* Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J. Natl Cancer Inst.* 93(9), 710–715 (2001).

100 Ginsburg ES, Mello NK, Mendelson JH *et al.* Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA* 276(21), 1747–1751 (1996).

101 Eagon PK. Alcoholic liver injury: influence of gender and hormones. *World J. Gastroenterol.* 16(11), 1377–1384 (2010).

102 Gavaler JS, Van Thiel DH. The association between moderate alcoholic beverage consumption and serum estradiol and testosterone levels in normal postmenopausal women: relationship to the literature. *Alcohol. Clin. Exp. Res.* 16(1), 87–92 (1992).

103 Sarkola T, Fukunaga T, Makisalo H, Peter Eriksson CJ. Acute effect of alcohol on androgens in premenopausal women. *Alcohol Alcohol.* 35(1), 84–90 (2000).

104 Fan S, Meng Q, Gao B *et al.* Alcohol stimulates estrogen receptor signaling in human breast cancer cell lines. *Cancer Res.* 60(20), 5635–5639 (2000).

105 Singletary KW, Frey RS, Yan W. Effect of ethanol on proliferation and estrogen receptor-alpha expression in human breast cancer cells. *Cancer Lett.* 165(2), 131–137 (2001).

106 Zhang Q, Jin J, Zhong Q, Yu X, Levy D, Zhong S. ERalpha mediates alcohol-induced deregulation of Pol III genes in breast cancer cells. *Carcinogenesis* 34(1), 28–37 (2013).

107 Li CI, Chlebowski RT, Freiberg M *et al.* Alcohol consumption and risk of postmenopausal breast cancer by subtype: the women's health initiative observational study. *J. Natl Cancer Inst.* 102(18), 1422–1431 (2010).

108 Fanelli SL, Maciel ME, Diaz Gomez MI *et al.* Further studies on the potential contribution of acetaldehyde accumulation and oxidative stress in rat mammary tissue in the alcohol drinking promotion of breast cancer. *J. Appl. Toxicol.* 31(1), 11–19 (2011).

109 Wright RM, McManaman JL, Repine JE. Alcohol-induced breast cancer: a proposed mechanism. *Free Radic. Biol. Med.* 26(3–4), 348–354 (1999).

110 Dumitrescu RG, Shields PG. The etiology of alcohol-induced breast cancer. *Alcohol* 35(3), 213–225 (2005).

111 Terry MB, Gammon MD, Zhang FF *et al.* ADH3 genotype, alcohol intake and breast cancer risk. *Carcinogenesis* 27(4), 840–847 (2006).

112 Lorenti Garcia C, Mechilli M, Proietti De Santis L, Schinoppi A, Kobos K, Palitti F. Relationship between DNA lesions, DNA repair and chromosomal damage induced by acetaldehyde. *Mutat. Res.* 662(1–2), 3–9 (2009).

113 Seitz HK, Maurer B. The relationship between alcohol metabolism, estrogen levels, and breast cancer risk. *Alcohol Res. Health* 30(1), 42–43 (2007).

114 Triano EA, Slusher LB, Atkins TA *et al.* Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. *Cancer Res.* 63(12), 3092–3100 (2003).

115 Castro GD, de Castro CR, Maciel ME *et al.* Ethanol-induced oxidative stress and acetaldehyde formation in rat mammary tissue: potential factors involved in alcohol drinking promotion of breast cancer. *Toxicology* 219(1–3), 208–219 (2006).

116 Castro GD, Delgado de Layno AM, Fanelli SL, Maciel ME, Diaz Gomez MI, Castro JA. Acetaldehyde accumulation in rat mammary tissue after an acute treatment with alcohol. *J. Appl. Toxicol.* 28(3), 315–321 (2008).

117 Moller P, Wallin H. Adduct formation, mutagenesis and nucleotide excision repair of DNA damage produced by reactive oxygen species and lipid peroxidation product. *Mutat. Res.* 410(3), 271–290 (1998).

118 Murata M, Midorikawa K, Kawanishi S. Oxidative DNA damage and mammary cell proliferation by alcohol-derived salsolinol. *Chem. Res. Toxicol* 26(10), 1455–1463 (2013).

119 Malins DC, Haimanot R. Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Res.* 51(19), 5430–5432 (1991).

120 Kalluri R, Weinberg RA. The basics of epithelial–mesenchymal transition. *J. Clin. Invest.* 119(6), 1420–1428 (2009).

121 Forsyth CB, Tang Y, Shaikh M, Zhang L, Keshavarzian A. Alcohol stimulates activation of Snail, epidermal growth factor receptor signaling, and biomarkers of epithelial–mesenchymal transition in colon and breast cancer cells. *Alcohol. Clin. Exp. Res.* 34(1), 19–31 (2010).

122 Duffy MJ, Maguire TM, Hill A, McDermott E, O'Higgins N. Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast Cancer Res.* 2(4), 252–257 (2000).

123 Nanda DP, Sil H, Moulik S, Biswas J, Mandal SS, Chatterjee A. Matrix metalloproteinase-9 as a potential tumor marker in breast cancer. *J. Environ. Pathol. Toxicol. Oncol.* 32(2), 115–129 (2013).

124 Ranogajec I, Jakic-Razumovic J, Puzovic V, Gabrilovac J. Prognostic value of matrix metalloproteinase-2 (MMP-2), *Matrix Med. Oncol.* 29(2), 561–569 (2012).

125 Talvensaari-Mattila A, Paakko P, Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 (MMP-2) is associated with survival in breast carcinoma. *Br J. Cancer* 89(7), 1270–1275 (2003).

126 Singer CF, Kronsteiner N, Marton E *et al.* MMP-2 and MMP-9 expression in breast cancer-derived human fibroblasts is differentially regulated by stromal-epithelial interactions. *Breast Cancer Res. Treat.* 72(1), 69–77 (2002).

127 Crawford HC, Matrisian LM. Tumor and stromal expression of matrix metalloproteinases and their role in tumor progression. *Invasion Metastasis* 14(1–6), 234–245 (1994).

128 Aye MM, Ma C, Lin H, Bower KA, Wiggins RC, Luo J. Ethanol-induced *in vitro* invasion of breast cancer cells: the contribution of MMP-2 by fibroblasts. *Int. J. Cancer* 112(5), 738–746 (2004).

129 Wong AW, Paulson QX, Hong J *et al.* Alcohol promotes breast cancer cell invasion by regulating the Nm23-ITGA5 pathway. *J. Exp. Clin. Cancer Res.* 30, 75 (2011).

130 Xu M, Bower KA, Chen G *et al.* Ethanol enhances the interaction of breast cancer cells over-expressing ErbB2 with fibronectin. *Alcohol. Clin. Exp. Res.* 34(5), 751–760 (2010).

131 Dawood S, Hu R, Homes MD *et al.* Defining breast cancer prognosis based on molecular phenotypes: results from a large cohort study. *Breast Cancer Res. Treat.* 126(1), 185–192 (2011).

132 Onitilo AA, Engel JM, Greenlee RT, Mukesh BN. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. *Clin. Med. Res.* 7(1–2), 4–13 (2009).

133 Kennecke H, Yerushalmi R, Woods R *et al.* Metastatic behavior of breast cancer subtypes. *J. Clin. Oncol.* 28(20), 3271–3277 (2010).

134 Carey LA, Perou CM, Livasy CA *et al.* Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 295(21), 2492–2502 (2006).

135 Tao L, Y., Tamimi RM, Collins LC *et al.* The association between vascular endothelial growth factor expression in invasive breast cancer and survival varies with intrinsic subtypes and use of adjuvant systemic therapy: results from the Nurses' Health Study. *Breast Cancer Res. Treat.* 129(1), 175–184 (2011).

136 Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. *Alcohol Res.* 35(1), 25–35 (2013).

137 Choi JY, James SR, Link PA *et al.* Association between global DNA hypomethylation in leukocytes and risk of breast cancer. *Carcinogenesis* 30(11), 1889–1897 (2009).

138 Christensen BC, Kelsey KT, Zheng S *et al.* Breast cancer DNA methylation profiles are associated with tumor size and alcohol and folate intake. *PLoS Genet.* 6(7), e1001043 (2010).

139 Zhu K, Davidson NE, Hunter S *et al.* Methyl-group dietary intake and risk of breast cancer among African-American women: a case–control study by methylation status of the estrogen receptor alpha genes. *Cancer Causes Control* 14(9), 827–836 (2003).

140 Tao MH, Marian C, Shields PG *et al.* Alcohol consumption in relation to aberrant DNA methylation in breast tumors. *Alcohol* 45(7), 689–699 (2011).

141 Pufulete M, Al-Ghnaniem R, Leather AJ *et al.* Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. *Gastroenterology* 124(5), 1240–1248 (2003).

142 Baglietto L, English DR, Gertig DM, Hopper JL, Giles GG. Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study. *BMJ* 331(7520), 807 (2005).

143 Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF *et al.* Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am. J. Clin. Nutr.* 83(4), 895–904 (2006).

144 Tjonneland A, Christensen J, Olsen A *et al.* Folate intake, alcohol and risk of breast cancer among postmenopausal women in Denmark. *Eur. J. Clin. Nutr.* 60(2), 280–286 (2006).

145 Sellers TA, Kushi LH, Cerhan JR *et al.* Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology* 12(4), 420–428 (2001).

146 Zhang S, Hunter DJ, Hankinson SE *et al.* A prospective study of folate intake and the risk of breast cancer. *JAMA* 281(17), 1632–1637 (1999).

147 Whitlock EP, Polen MR, Green CA, Orleans T, Klein J. Behavioral counseling interventions in primary care to reduce risky/harmful alcohol use by adults: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 140(7), 557–568 (2004).

148 Hvidtfeldt UA, Tolstrup JS, Jakobsen MU *et al.* Alcohol intake and risk of coronary heart disease in younger, middle-aged, and older adults. *Circulation* 121(14), 1589–1597 (2010).