MINIREVIEW

Caffeine and the Development of the Normal and Neoplastic Mammary Gland

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Abstract. **There has been much interest in the role of dietary factors in the etiology and progression of breast disease. Due to its wide consumption and the many biochemical and physiological effects it exerts, caffeine has been extensively examined in both clinical and experimental animal studies. To date, clinical studies investigating a possible relationship between caffeine consumption and breast disease in humans have yielded inconsistent and inconclusive results. In experimental animal studies utilizing laboratory mice and rats, caffeine has been shown to stimulate mammary gland lobulo-alveolar development and secretion. The development of mammary gland tumors can be either stimulated or suppressed depending upon the animal species and strain, and the stage of tumorigenesis (initiation/promotion) at which caffeine is administered. Many laboratories have proposed that antagonism of the adenosine receptor is the most plausible mechanism to account for the many biological activities of caffeine. However, other mechanisms by which caffeine act cannot be discounted. The significant modifying role of caffeine on normal and neoplastic mammary gland development in experimental animals provides a biological foundation from which to implicate caffeine as a potential modulator of developmental growth of normal, benign, and carcinomatous human breast tissues.** [P.S.E.B.M. 1994, Vol 207]

Caffeine (1,3,7,trimethylxanthine) is a naturally occurring plant alkaloid found in coffee, tea, and cocoa, and used as an additive in many soft drinks and medications. Caffeine belongs to a group of purine-based compounds collectively referred to as methylxanthines (MX), which also includes, theophylline and theobromine. Caffeine has been shown to have a wide variety of biochemical and physiological effects and is the most widely consumed drug today in many parts of the world. In humans, caffeine has been reported to be associated with an increased risk of developing cancer in a number of different organ sites (1–3). However, an equal number of reports have shown no association between caffeine consumption and cancer risk (4–6). Since 1979, when Minton *et al.* (7, 8) reported an association between caffeine consumption and fibrocystic breast disease (FBD), there has been much interest in the possible relationship between caffeine consumption and breast disease. This review will serve three purposes: (i) to summarize the results of clinical studies examining the possible role of caffeine in the development of benign and malignant human breast disease; (ii) to summarize the results of experimental animal studies examining the effects of caffeine on normal and neoplastic rodent mammary gland development; and (iii) to consider the

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various mechanisms by which caffeine may affect the development of breast disease.

Caffeine and Benign Breast Disease (BBD): Studies Which Report an Association

Minton *et al.* (7, 8) were the first to suggest that caffeine consumption was associated with the symptoms of FBD. When caffeine and other MX-containing beverages were eliminated from the diet of women with clinically diagnosed FBD, a majority of women (13 of 20) experienced complete disappearance of palpable breast nodules and other symptoms within 1–6 months after completely abstaining from all MX consumption. Only 1 of 27 women who continued MX consumption experienced resolution of her disease. Cyclic AMP and cyclic GMP levels were found to be higher in fibrocystic breast tissue when compared with normal breast tissue.

Brooks *et al.* (9) attempted to document the results of Minton *et al.* (7, 8) and found that among women who abstained from MX consumption for 6 months, 91% had improvements in palpable breast findings, and 88% had improvements in self-reported breast symptoms. In addition, 85% of patients exhibited improvements in graphic thermal patterns using graphic stress telethermometry.

Minton *et al.* (10) presented a third report supporting the hypothesis that MX consumption is associated with the symptoms of FBD. In women who completely abstained from MX consumption, 83% experienced complete resolution of their disease and 15% experienced a significant improvement. Symptoms resolved in 25% and improved in 50% of women who reduced their MX consumption by more than half. Among women who did not alter their MX consumption, only 17% experienced a resolution of their symptoms, and 8% experienced an improvement.

Using discordant twins in a case control study, Odenheimer *et al.* (11) showed a significantly positive association between FBD and coffee consumption. Two groups were studied: 90 pairs of twins in which one twin had a history of biopsy-proven FBD and her twin did not (44 monozygotic pairs and 46 dizygotic pairs) and 48 pairs of twins in which one twin had clinically diagnosed FBD and her twin was free of disease at examination and reported no history of breast disease (25 monozygotic pairs and 23 dizygotic pairs). In the cases with a history of biopsy-proven FBD, the odds ratio (OR) for moderate coffee consumption (1–4 cups/day) versus none, or moderate consumption versus heavy consumption (at least 5 cups/day) was 1.6. In the cases with clinically diagnosed FBD, women with FBD were more likely to consume coffee than their twins (OR:4.2, $P < 0.05$). In both groups, the association was stronger for the monozygotic than the dizygotic twins.

Boyle *et al.* (12) reported that the occurrence of FBD was positively associated with average daily consumption of caffeine. The OR ranged from 1.5 (31–150 mg caffeine/day) to 2.3 (greater than 500 mg caffeine/day). The OR was approximately the same when acute or chronic disease controls were considered separately. Women who had FBD along with concomitant fibroadenoma also showed a positive association with caffeine consumption. The OR were 1.9 (31–250 mg caffeine/day), 2.7 (251–500 mg caffeine/day), and 2.5 (greater than 500 mg caffeine/day). When mean ductal atypia scores were considered, a positive trend was observed among both the low and high atypia groups. Strong associations were found for atypical lobular hyperplasia and sclerosing adenosis with papillomatosis or papillary hyperplasia. For atypical lobular hyperplasia, a 5-fold increase was observed in women consuming over 500 mg caffeine/day (OR: 5.0) when compared with those consuming up to 250 mg caffeine/day (OR: 1.0). For sclerosing adenosis with papillomatosis or papillary hyperplasia, the OR ranged from 3.6 (31–250 mg caffeine/day) to 6.2 (greater than 500 mg caffeine/day). Among controls only, an increase in breast symptoms was observed with caffeine use. For breast pain, the OR was 3.2 when comparing controls who consumed greater than 500 mg caffeine/day with those consuming 30 mg or less/day. The corresponding OR was 1.9 for the formation of breast lumps. No relationship was found between caffeine consumption and the presence of fibroadenomas.

Similar to Boyle *et al.* (12), La Vecchia *et al.* (13) also showed a definite caffeine dose response relationship and an association specific for FBD. The relative risk (RR) estimates of FBD for women who consumed 1–2, or 3 or more, cups of coffee/day were 4.1 and 6.4, respectively, when hospitalized controls were the comparison group, and 2.0 and 3.7, respectively, when outpatient controls were the comparison group. The RR estimates were higher, but not significantly changed, when total MX consumption (coffee plus tea) was considered. The relationship between total MX consumption and FBD was increased with increasing duration of use. No association was found between MX consumption and the presence of fibroadenomas.

The effects of theophylline therapy of FBD in asthmatic women were studied by Hindi-Alexander *et al.* (14). Theophylline was significant only when adjusted for age, pregnancy, and menopause ($P < 0.03$) and was borderline significant without adjustments ($P < 0.07$). Caffeine was only significant with no adjustment ($P < 0.04$). Total MX consumption was found to be a contributing factor in FBD severity with or without adjustments.

Caffeine and BBD: Studies Which Report No Association

Heyden (15) in an editorial review of the original work of Minton *et al.* (7, 8) concluded that there was no reason to associate caffeine and FBD.

Lawson *et al.* (16) reported a modest positive association between caffeine and FBD. The RR were: 1.4 (1–3 cups of coffee/day), 1.5 (4–6 cups of coffee/day), and 1.3 (7 or more cups of coffee/day). However, the authors concluded that the data gave little support for a role of MX in the development of FBD.

Ernster *et al.* (17) found a statistically significant ($P < 0.001$) reduction in clinically palpable breast findings in women abstaining from MX consumption when compared with controls. However, the absolute reduction was slight (a mean of one-half point per quadrant), and before-after mammograms for a small subset of women abstaining from MX consumption were unchanged.

Marshall *et al.* (18) noted no difference in coffee and tea consumption patterns between controls and women with BBD. Higher levels of coffee consumption were not associated with an elevated risk of BBD. When high coffee consumption was adjusted for high tea consumption, no increase in risk was observed. Higher levels of tea consumption were associated with a decreased risk of BBD even when adjusted for coffee consumption.

The clinical course of FBD over a 6-month period was followed by Heyden and Muhlbaier (19), while recording the usual MX consumption of the study subjects. All nodules disappeared from 21 (15% of all) breasts, and 125 (87% of all) breasts showed some change in position or number of nodules during the 6-month study period. The MX consumption remained constant throughout the study (4.2 mg/day decrease from the start to the finish of the study).

Lubin *et al.* (20) reported that the histological type of BBD and degree of ductal atypia showed no association with coffee or MX consumption. No dose response effect was observed.

Schairer *et al.* (21) found no evidence for an association between MX consumption and BBD (trend test $P = 0.47$). When cases with FBD were examined separately according to pathological subtypes (atypia, hyperplasia, sclerosing adenosis, and cysts) no association was also found. No relationship was observed between menstrual breast tenderness and MX consumption among women with FBD.

A 5-year retrospective study and a 6-month prospective study of women with FBD was done by Heyden and Foder (22). No association between MX consumption and FBD was reported.

A critical review of the literature on the association between caffeine and FBD was written by Levinson and Dunn (23) which concluded that there was little evidence to support the association.

Allen and Frobery (24) reported that decreased caffeine consumption did not result in any significant reduction in palpable breast nodules or in a lessening of breast pain and tenderness. However, the authors found discrepancies between the actual study results and the follow-up survey results. The follow-up survey revealed that 42% of the study subjects who had reduced their caffeine consumption during the study reported a decrease in nodularity and breast pain and tenderness during the study. The actual study results did not support the follow-up survey results.

Summary

Approximately 50%–90% of women have FBD (25). Many of these women experience symptoms of cyclic breast pain and tenderness (26) and eliminating or reducing MX consumption has been proposed as a safe and easy way to reduce these symptoms. Unfortunately, no *consistent* beneficial effect on FBD symptoms has been shown when MX consumption is eliminated or reduced. Similarly, studies have shown no *consistent* role of MX in the etiology of FBD. The inconsistencies of these studies may be due, at least in part, to a lack of careful and precise methodologies. For example, in some studies (i) objective diagnostic techniques were not utilized; (ii) the examiner was aware of the patients' level of caffeine consumption; and (iii) no reliable measure of compliance was used to document the level of caffeine consumption. In other studies, (i) no consideration was given to assess normal fluctuations in breast symptoms as a function of the menstrual cycle; (ii) the study period was too short for a meaningful analysis; and (iii) the criteria for case/control selection and/or the number of case/controls were insufficient. Thus, due to the inconsistency of the studies to date (there are an equal number of studies describing a positive and a negative association), a clear and decisive positive relationship between MX consumption and the genesis and/or progression of FBD cannot be provided at this time. Considering the widespread occurrence of FBD in the United States and throughout the world, increased efforts toward evaluating the role of MX in the etiology and/or maintenance of this disease would appear to be justifiable.

Caffeine and Breast Cancer (BC)

Stocks (27) published a comparison study between the mortality rates from cancers of different sites and the annual consumption of coffee and tea in 20 countries. A strong positive association with tea ($P = 0.009$) and an insignificant positive association with coffee consumption was found for breast cancer (BC).

Armstrong and Doll (28) correlated various dietary and environmental variables with cancer incidence and mortality rates. A nonsignificant positive association between coffee consumption and BC was observed ($r = 0.42$ and 0.37, for incidence and mortality, respectively).

Lawson *et al.* (16) found a modest positive association between hot beverage (coffee and tea) consumption and BC. The estimated RR for women who drank 1–3, 4–6, and 7 or more cups of coffee/day were 1.3, 1.5, and 1.1, respectively, when compared with those who consumed no coffee or tea. No dose response effect was observed.

Lubin *et al.* (29) in a case control study of diet and BC observed a nonsignificant increase in risk (RR: 1.2) in women who drank more than five cups of coffee or tea/day when compared with women drinking five or less cups/day. When adjusted for beef/pork consumption, this association remained positive, but reduced.

Mansel *et al.* (30) reported that coffee drinkers (greater than 6 cups/day) had an elevated risk of BC when compared with nondrinkers (RR: 1.26, $P < 0.05$). When coffee drinkers of 1–5 cups/day were compared with those consuming greater than 6 cups/day, no increase in the RR was found. Also, no difference was found when comparing those who drank 1–5 cups/day with abstainers.

Among Seventh-Day Adventists, Phillips and Snowdon (31) found a minimum reduction in mortality for BC and no relationship between coffee and BC. In a subsequent study (32) by the same authors, no association between coffee consumption and mortality from BC was found among Seventh-Day Adventists.

Lubin *et al.* (33) found that BC patients tend to consume less coffee and total MX than control groups, but no dose response effect was noted. A nonsignificant negative association was found between cups of coffee consumed and BC when comparing women who drank greater than or equal to 4 cups of coffee/day and those who drank less than or equal to 1 cup of coffee/week. All three ethnic subgroups in this study showed the above association. This pattern was stronger among high fat consumers after adjustments for confounding hormonal factors (linear trend P values = 0.06 and 0.05, respectively, for surgical and neighborhood controls). When the consumption of MX among BC patients was compared with that of BBD patients, a diminished risk was found.

No association between recent coffee and tea consumption and BC was found by Rosenberg *et al.* (34). After adjusting for confounding variables, the RR estimates for consuming at least 5 cups of coffee/day were 1.2 compared with the noncancer controls and 1.1 compared with the cancer controls. Among women with FBD, coffee consumption was not associated with an increase in risk of BC. Tea or decaffeinated coffee consumption was not associated with an increased risk.

Le (35) reported that the risk of BC was inversely associated with coffee consumption. The OR for BC were 0.8 for less than 3 cups of coffee/day and 0.6 for 3 or more cups of coffee/day (P value for linear trend = 0.003).

Pozner *et al.* (36) reported that women with moderately to well differentiated BC had a higher caffeine and coffee consumption than women with poorly differentiated BC. The authors concluded that there was a significant relationship between BC differentiation and caffeine and coffee consumption.

Jacobsen *et al.* (37) found no association between coffee consumption and BC (RR: 0.81 for greater than or equal to 7 cups of coffee/day versus less than or equal to 2 cups of coffee/day, P value for linear trend = 0.73).

La Vecchia *et al.* (38) found that coffee consumption does not increase the risk of BC. The RR estimates for women who consumed less than 2, 2–3, and 4 or more cups of coffee/day were 1.5, 1.3, 1.0, respectively, when compared with women who never consumed coffee. No association was apparent with duration of coffee use or use of other MX containing beverages.

MX consumption was reported not to be associated with an increased risk of BC by Schairer *et al.* (39). Evidence for a negative association was apparent with consumers of more than 125 mg of MX/day. The test for trend was significant ($P = 0.03$) although no consistent dose response effect was observed. Analysis by age showed that women under the age of 50 had no consistent pattern of BC risk with MX consumption, whereas, in women 50 years old or greater, there was evidence of a negative association.

Phelps and Phelps (40) found that the partial correlation of BC and caffeine consumption after accounting for the effects of fat intake is negative and significant ($P < 0.05$). This negative partial correlation disappeared when the data were weighted by population in each country.

Rohan and McMichael (41) reported that there was relatively little variation in BC risk in association with total caffeine or total MX consumption among the total study population of postmenopausal women. However, among premenopausal women, there was an increased risk at higher levels of consumption which was not dose-dependent. No evidence for an effect of MX at high levels of fat intake was found.

In case control studies of diet and BC, both Iscovich *et al.* (42) and Katsouyanni *et al.* (43) found no positive association between caffeine-containing beverage consumption and BC. However, a borderline

significant negative trend was observed in the case-neighborhood control analysis done by Iscovich *et al.* (42).

Vatten *et al.* (44) observed an overall weak non-significant negative association between daily coffee consumption and BC. However, this association depended on the body mass index of the women. In women with a Quetelet index less than 24, there was a significant (trend = 5.1, $P = 0.02$) inverse relationship between coffee consumption and BC risk. In women with a Quetelet index greater than or equal to 24, there was a nonsignificant (trend = 2.3, $P = 0.13$) positive association between coffee consumption and BC risk. The interaction effect between coffee consumption and body mass index was significant (trend = 10.2, $P = 0.02$).

Summary

The results of studies examining a possible role for MX in the etiology of BC have been inconsistent. The study results range from a weak positive association, to no association, to a weak negative association. Thus, compelling *consistent* epidemiological evidence for a role of caffeine or MX in the etiology and/or progression of BC has to date not been provided. Considering the vase array of confounding variables that influence the genesis and/or progression of this disease, it is extremely difficult to identify potential disease-modifying factors that possess less than striking positive or negative regulatory activities. Caffeine and/or MX may indeed modify this neoplastic process; further research efforts will be required to resolve this relationship.

Caffeine and Rodent Mammary Gland Tumorigenesis

Wurzner *et al.* (45) performed a 2-year feeding study of instant coffees in Sprague-Dawley rats. The average daily coffee intake was 3.5 g/kg for female rats. Mammary gland fibroadenomas were observed in nine controls and in from two to nine females of each treatment group. Only one mammary adenocarcinoma was reported in this study; this tumor was observed in the decaffeinated coffee-treated group.

A long-term study (104 week) on the effects of caffeine in female Wistar rats was done by Takayama and Kuwabara (46). Caffeine was administered via the drinking water at levels of 0.1% and 0.2%. In the control group, 16 (32%) rats were found to have mammary tumors. Among the caffeine-treated groups, 23 (48%) rats had mammary tumors in the 0.1% treatment group, and 17 (34%) rats had mammary tumors in the 0.2% treatment group. The number of adenocarcinomas were the following: control, 1 of 16; 0.1% caffeine, 3 of 23; and 0.2% caffeine, 8 of 17.

Mohr *et al.* (47) administered caffeine via the drinking water for 2 years to female Sprague-Dawley rats. Treatment levels used were the following: 0 (two control groups), 200, 430, 930, and 2000 mg caffeine/liter. Mammary gland fibroadenoma incidence was 27, 26, 24, 20, 20, and 13, respectively, while mammary adenocarcinoma incidence was 0, 1, 4, 3, 4, and 1, respectively. There were 50 rats in each group.

A 2-year toxicity/carcinogenicity study of fresh brewed coffee in rats that were initially exposed *in utero* to coffee was done by Palm *et al.* (48). Fresh brewed coffee was administered via the drinking water at concentrations of 25%, 50%, and 100% to female Sprague-Dawley rats derived from females treated *in utero* with coffee. Focal mastitis and cystic alveolar dilation of the mammary glands were significantly increased ($P < 0.05$) in females of the 100% coffee group sacrificed at 1 year, but was not significantly increased in animals examined at 2 years. Among female animals sacrificed and those dying during the study, the incidence of mammary gland fibroadenoma was significantly decreased ($P < 0.05$) in females of the 50% and 100% coffee groups.

Minton *et al.* (49) reported that caffeine administered to female Sprague-Dawley rats via the drinking water had an effect on the latency period of 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumor appearance. Caffeine increased tumor latency in rats fed standard laboratory chow and decreased tumor latency significantly in rats fed a high-fat (20% vegetable) diet. Caffeine was found to increase the number of mammary tumors per tumor-bearing rat regardless of the fat content of the diet.

Welsch *et al.* (50) found that caffeine administration via the drinking water (250 and 500 mg/liter) to female Sprague-Dawley rats temperately enhanced the promotion phase of DMBA-induced mammary tumorigenesis. Caffeine increased mammary carcinoma incidence when treatment was initiated 3 days after DMBA treatment and continued for 21 weeks. This increase in mammary tumor incidence was also observed when caffeine treatment was initiated 20 weeks after DMBA treatment and continued for 6 weeks in both rats with or without mammary tumors at the onset of caffeine treatment. A dose response increase was seen in mammary carcinoma incidence.

Caffeine (1 or 2 mg/ml drinking water) was shown by Petrek *et al.* (51) to have an inhibitory effect on diethylstilbestrol (DES)-induced mammary carcinomas in ACI female rats. In rats without DES, mammary carcinomas did not develop in any of the animals receiving caffeine. In rats with DES treatment, increased caffeine dosage increased the time to first tumor appearance, decreased the number of rats that developed tumors, and decreased the number of tumors overall.

Nagasawa and Konishi (52) studied the relationship between caffeine consumption and the incidence of mammary hyperplastic alveolar nodules (HAN) in four strains of female mice (SHN, SLN, GR/A, and C3H). HAN developed in caffeine-treated (500 mg/liter of drinking water) mice at 60, 120, 60 and 90 days of age in SHN, SLN, GR/A, and C3H mice, respectively. No such changes were observed in the controls at the same respective ages. The total number of HAN were increased in the caffeine-treated groups compared with controls in each species, but was statistically significant in C3H mice only. The same authors (53) found that caffeine significantly ($P < 0.01$) stimulated spontaneous mammary tumorigenesis in both breeder and virgin C3H mice. The stimulation was found to be more marked in virgins than breeders. Caffeine-treated mice also had a higher number of HAN in both breeders ($P < 0.01$) and virgins ($P < 0.05$).

The influence of caffeine and/or coffee consumption on the initiation and promotion phases of DMBA-induced mammary tumorigenesis in female Sprague-Dawley rats was studied by Welsch *et al.* (54). In initiation, either moderate (100–400 mg/liter) or high (860 mg/liter) doses of caffeine or moderate to high dose levels of caffeinated coffee significantly ($P < 0.05$) reduced mammary carcinoma multiplicity (number of mammary tumors/rat), while decaffeinated coffee had no significant effect on mammary tumor multiplicity at either high or moderate dose levels. When caffeine was added to a moderate dose level of decaffeinated coffee, a significant ($P < 0.05$) reduction in mammary tumor multiplicity was observed. In promotion, a prolonged consumption of either moderate levels of caffeine or moderate or high levels of caffeinated coffee had no significant effect on mammary tumor multiplicity. However, in the early stage of promotion, caffeine did exert a significant ($P < 0.05$) stimulatory effect on mammary tumor multiplicity. This effect was temperate and transitory. Caffeine and/or coffee consumption did not significantly effect the percent of rats with mammary carcinomas or the mean latency period of mammary tumor appearance. In a sequential study (55), the influence of caffeine consumption on DMBA-induced mammary tumorigenesis was studied in female Sprague-Dawley rats fed a chemically defined diet containing a standard (5%) or high (20%) level of unsaturated fat. In initiation, caffeine consumption significantly ($P < 0.05$) reduced mammary tumor multiplicity in animals fed either the standard or high-fat diet. In promotion, prolonged caffeine consumption did not significantly effect mammary tumor multiplicity in either diet group. An early promoting effect of caffeine on mammary tumor multiplicity was observed, but did not reach the 5% level of statistical probability. When caffeine was administered during both the initiation and promotion phases, no significant effect on mammary tumor multiplicity was observed. Caffeinated coffee mimicked the effects of caffeine in both initiation and promotion while decaffeinated coffee had no effect on either phase. Caffeine and/or coffee consumption again did not effect the percent of rats with mammary tumors or the mean latency period of mammary tumor appearance.

Welsch *et al.* (56) also studied the influence of caffeine consumption via the drinking water on the development of DMBA-induced mammary tumors in BD2F1 female mice and spontaneous mammary tumorigenesis in C3H mice. In BD2F1 mice treated with either 250 or 500 mg/liter caffeine, mammary tumor multiplicity was increased by 20% and 40%, respectively. In C3H mice treated with either 250 or 500 mg/liter caffeine, mammary tumor multiplicity was increased by 13% and 117%, respectively. The higher dose of caffeine resulted in a significant ($P < 0.05$) increase in the number of mammary carcinomas/mouse in both BD2F1 and C3H mice. The percentage of mice with mammary carcinomas or mean latency period was not effected by caffeine consumption.

VanderPloeg and Welsch (57) examined the effect of caffeine on the development of ovarian hormone-dependent mammary tumors in GR mice. Virgin female GR mice were treated daily for 24 weeks with 17β-estradiol and progesterone, commencing at 8 to 10 weeks of age. One week after the onset of hormone treatment, caffeine (500 mg/liter drinking water) was administered daily until experiment termination to one-half of the hormone treated mice. Hormone treatment induced mammary tumors in 95%–100% of the mice. Caffeine treatment significantly ($P < 0.05$) reduced the mean number of mammary tumors per mouse and significantly ($P < 0.05$) increased the mean latency period of mammary tumor appearance.

VanderPloeg *et al.* (58) studied the influence of caffeine (500 mg/liter drinking water) on development of benign and carcinomatous mammary gland tumors in female rats treated with the carcinogens DMBA and N-methyl-N-nitrosourea (MNU). Eighty-nine percent of the mammary tumors induced by DMBA were benign (adenomas, fibroadenomas, often with cystic secretory activity), and 11% were carcinomas (intraductal and invasive); virtually all of the MNU-induced mammary tumors were carcinomas (99%). Caffeine consumption during the initiation stage in the DMBA-treated rats resulted in a significant decrease in the mean number of mammary carcinomas per rat (50% reduction, $P < 0.01$) and mean number of benign mammary tumors per rat (28% reduction, $P < 0.05$); caffeine consumption during the promotion stage significantly decreased the mean number of benign mammary tumors per rat (57% reduction, $P < 0.001$) while not significantly influencing mammary carcinoma

number. Caffeine consumption did, however, increase the percentage of benign mammary tumors with cystic secretory changes ($P < 0.05$). In contrast, caffeine consumption during either the initiation or promotion stages of MNU-treated rats did not significantly influence this tumorigenic process. That caffeine significantly suppressed the initiation stage of DMBA-induced rat mammary gland tumorigenesis (DMBA requires metabolic activation), while not influencing this stage when MNU was used as a carcinogen (MNU does not require metabolic activation), suggests that caffeine acted via an alteration in carcinogen (DMBA) activation. How caffeine inhibited the development of benign mammary tumors, when administered during the promotion stage, was not determined.

The effect of caffeine administered during the promotion stage (500 mg/liter drinking water) on the development of benign mammary tumors in DMBA-treated female rats was examined further by Wolfrom *et al.* (59). In these studies, DMBA was administered to four different animal models: (i) 55-day-old virgin rats; (ii) 53-day-old ovariectomized, 17β-estradiol-treated virgin rats; (iii) 135-day-old virgin rats; and (iv) 135-day-old parous rats. A high incidence of benign mammary fibroadenomas was observed in each of the four animal models. In addition, in the estrogen-treated ovariectomized animals, a high incidence of secretory mammary gland cysts was observed. Caffeine treatment significantly ($P < 0.05$ to $P < 0.001$) reduced the incidence of benign mammary fibroadenomas in the 55-day-old virgin rat model ($P < 0.01$), in the 53-day-old estrogen-treated ovariectomized virgin rat model ($P < 0.05$ to $P < 0.001$), and in the 135-day-old virgin rat model ($P < 0.05$). The number of benign mammary fibroadenomas was reduced by caffeine in the 135-day-old parous rat model but this reduction was not significant ($P < 0.10$). In addition, in the estrogen-treated ovariectomized virgin rat model, caffeine significantly ($P < 0.05$ to $P < 0.001$) reduced the incidence of mammary gland cysts.

Caffeine and Normal Rodent Mammary Gland Growth

Chronic caffeine administration via the drinking water (500 mg/liter) had no significant effect on mammary gland growth in female C3H mice (60). However, mammary glands from this strain are difficult to evaluate for growth processes as such glands spontaneously develop a large number of hyperplasias and/or dysplasias (e.g., HAN). In contrast, mammary glands obtained from female BALB/c mice are much easier to analyze for growth processes as such glands are relatively free of these pathologies.

Welsch *et al.* (56) studied the effects of caffeine consumption via the drinking water (500 mg/liter) on mammary gland development in female BALB/c mice both *in vivo* and *in vitro*. Mammary gland developmental growth (ductal proliferation and lobulo-alveolar development) was significantly ($P < 0.05$) increased in mice treated with caffeine. This effect was greater when the mice were treated with mammotropic hormones (estrogen and progesterone). *In vitro* (organ culture) studies revealed that mammary glands derived from caffeine-treated mice were more responsive to a mammotropic hormonal (prolactin, growth hormone, aldosterone, estrogen, and progesterone) development growth (proliferation and differentiation) stimulus ($P < 0.05$).

The stimulatory effect of caffeine (500 mg/liter drinking water) on mammary lobulo-alveolar development in female BALB/c mice treated with mammotropic hormones (estrogen and progesterone) as reported by Welsch *et al.* (56) was subsequently confirmed by VanderPloeg *et al.* (61). In an effort to determine whether or not this observed stimulatory effect of caffeine was directly on the mammary gland, small slow-release Elvax-40P pellets containing caffeine were implanted directly into the mammary glands of female BALB/c mice concurrently treated with mammotropic hormones (estrogen and progesterone). No significant stimulatory effect of caffeine was observed (61). Furthermore, the addition of caffeine (100 μM) to the culture media of whole mouse mammary gland (organ cultures) did not enhance lobulo-alveolar development induced by mammotropic hormones. Thus, while a consistent significant stimulatory effect of caffeine on mammary lobulo-alveolar development was observed when caffeine was consumed orally (drinking water), no direct effect of caffeine, when placed directly into the mammary gland *in situ* (or in culture media), on mammae development was observed. These data provide evidence that the stimulatory effect of caffeine on mammary gland lobulo-alveolar development is not manifested via a direct action of caffeine on the mammary gland, but suggests that caffeine is stimulatory via a systemic activity.

The effect of caffeine on modulation of those systemic factors that potentially could enhance mammary gland lobulo-alveolar development in female BALB/c mice was examined by VanderPloeg and Welsch.[2] Estrogen- and progesterone-treated ovariectomized BALB/c mice, given a dose level of caffeine (500 mg/liter drinking water) that enhances mammary lobulo-alveolar development, were sacrificed and whole blood was obtained by decapitation. Blood levels of free fatty acids, glucose, insulin, IGF-1, prolactin, and corticosterone were determined. While each of these

[2] Welsch CW, VanderPloeg LC. Enhancement by caffeine of mammary gland lobulo-alveolar development in mice and rats: A function of increased corticosterone secretion. (J Environ Pathol Toxicol Oncol **13:** (in press), 1994.)

blood components have been reported to enhance mammary gland development in rodents, only one of these (i.e., corticosterone) was consistently and significantly ($P < 0.01$) increased in the blood of the caffeine-treated mice. Glucocorticoids, in particular, corticosterone, have been reported by many groups to enhance mammary gland lobulo-alveolar development and secretion in mice and rats.

Hart and Grimble (62, 63) added caffeine to the drinking water (50 mg/kg body wt) of Wistar rats commencing at the onset of pregnancy and continuing until the 14th day of lactation. Litter weights were significantly ($P < 0.01$) increased in the caffeine-treated rats compared with control rats. Since litter weight (pup number adjusted to be equal among groups) is a good quantitative indicator of lactational performance, such data provide evidence that caffeine can enhance the secretory activity of the rodent mammary gland. In a similar study, Sheffield (64) added caffeine to the drinking water (500 mg/liter) of ND/4 mice during pregnancy. On Day 18 of pregnancy, mammary gland DNA was significantly ($P < 0.05$) increased in the caffeine-treated mice compared with the control mice, providing further evidence that caffeine can enhance mammary gland lobulo-alveolar development in mice. At Day 15 of lactation (caffeine was only administered during pregnancy), litter weight was significantly ($P < 0.05$) increased in the caffeine-treated mice compared with control mice. That caffeine enhanced lactational performance when administered only during pregnancy suggests that the increased lactational performance by caffeine described by Hart and Grimble (62, 63) was due to a caffeine-induced increase in the number of mammary secretory units (alveoli) in lieu of an increase in the amount of secretion per secretory unit.

Summary

It is clear that under controlled experimental conditions, an array of dose levels of caffeine administered orally to laboratory mice or rats can affect normal, hyperplastic, and carcinomatous mammary gland development. The normal mammary gland of mice consuming caffeine appears to have a heightened mammotropic hormone-induced enhancement of mammary gland lobulo-alveolar development. Increased mammary gland lobulo-alveolar development by caffeine can enhance the secretory potential of this gland. Caffeine does not appear to act via a direct action on the mammary gland but appears to act via a systemic factor. This systemic phenomenon may be increased secretion of corticosterone. The development of mammary carcinomas in rodents, as a function of caffeine consumption, can be either stimulated or suppressed, a phenomenon dependent upon animal species and strain and the tumorigenic phase (initiation/promotion) at which time caffeine is administered. Although it is often difficult to extrapolate data derived from experimental animal studies to human populations, a sufficient number of experimental animal studies have been performed which provide, at the very least, a biological basis from which to implicate caffeine as a potential modulator of developmental growth of normal, benign, and carcinomatous human breast tissues.

Mechanisms

Phosphodiesterase (PDE) Inhibition. Minton *et al.* (7, 8) hypothesized that MX, by inhibiting PDE, would cause an increase in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) and thereby lead to FBD symptoms. While it is true that MX have been shown to inhibit PDE (65) activity and that cAMP can influence mammary tumorigenesis (66), this particular mechanism may be unlikely due to the following: (i) MX, in relevant therapeutic concentrations, minimally affects PDE activity (67); and (ii) there appears to be a number of PDE isozymes which can be isolated from various tissues, and MX may selectively antagonize only some forms of these enzymes (68). However, it should be noted that caffeine may increase cAMP/cGMP indirectly via some of the other mechanisms discussed below.

Hormonal. Caffeine has been reported to modulate catecholamine, dopamine, and serotonin secretion and/or metabolism (69–71). These neurohormones have been reported to influence mammary tumorigenesis (72).

Caffeine has been reported to influence anterior pituitary and thyroid function leading to an increase in serum corticosterone, a decrease in serum thyroid-stimulating hormone (TSH) and inhibiting pulsatile secretion of growth hormone (GH) (73, 74). Altering the secretion of such hormones can affect both the normal and neoplastic rodent mammary gland (72). Prolactin (PRL) secretion does not appear to be affected by caffeine (50, 51, 73). Prolactin is an important hormone in rodent mammary gland tumorigenesis (74).

Insulin (I) secretion has been reported to be increased by MX (76) as has blood glucose (77). Insulin and/or glucose have been shown to enhance DMBA-induced mammary tumor growth processes (78).

Free Fatty Acids (FFA). Caffeine has been reported to increase FFA levels in serum (79). Certain serum FFA stimulate the growth and proliferation of both normal and neoplastic rodent mammary epithelial cells (80–82). An increase in serum FFA levels may also lead to alterations in cell membrane fluidity, mammotropic hormone secretion, intercellular communication, and/or hormone/growth factor responsiveness (83).

Calcium (Ca). Caffeine has been reported to increase Ca levels in mammalian tissue (84); Ca levels have been reported to be higher in tumor cells than in normal cells (85). An increase in cellular Ca has been reported to inhibit proliferation of a number of epithelial cell types, including mammary epithelial cells, and to cause differentiation of these cells (86–88). The responsiveness of the rat mammary gland to a carcinogenic insult has been shown to be dependent upon the state of differentiation of the mammary gland (89, 90). It is conceivable that caffeine, by increasing cellular Ca levels, causes a differentiation of the mammary gland and thereby alters its suspectibility to a carcinogenic insult. This phenomenon is a possible explanation of the inhibitory effect of caffeine during the initiation phase of DMBA-induced rat mammary gland tumorigenesis as reported by Welsch *et al.* (54) and VanderPloeg *et al.* (58).

DNA. Caffeine and theophylline have been shown to inhibit the binding of DMBA to murine epidermal cell DNA (91). Caffeine has been reported to inhibit lung tumorigenesis (92), possibly by inhibiting carcinogen binding to DNA. The mechanism by which caffeine might inhibit carcinogen binding to DNA is not certain. It has been reported that caffeine can scavenge hydroxyl radicals (93). Since hydroxyl radicals enhance the binding of carcinogens such as DMBA to DNA, caffeine may inhibit DMBA binding to DNA via a hydroxyl radical scavenging activity. Alternatively, caffeine may inhibit carcinogen binding to DNA by sequestration of the carcinogen (94); such would lower the amount of the carcinogen available to cellular DNA. Inhibition by caffeine of the initiation phase of DMBA-induced rat mammary gland tumorigenesis (54, 58) may be a function of impaired DMBA binding to mammary cell DNA via these processes. Caffeine, however, has been reported to enhance the cytotoxicity and mutagenicity of a number of DNA damaging agents, including carcinogenic chemicals. The mechanism by which caffeine enhances cytotoxicity of these chemicals is believed to be via inhibition of DNA repair (95). Those studies in which caffeine has been reported to enhance carcinogen-induced mammary gland tumorigenesis, e.g., when caffeine and the carcinogen are administered concurrently and over a period of several weeks (e.g., 56), may be via this process.

Metabolic Enzymes. Caffeine is metabolized by the hepatic mixed function monooxygenase enzymes including both the P450 and the polycyclic hydrocarbon inducible P448 systems (96–98). Caffeine has been reported to enhance, inhibit, or have no effect (90–101) on hepatic microsomal enzymes in the rat, depending on the dose level administered and the length of treatment. Caffeine alteration of carcinogen metabolism, via modification of microsomal enzyme activities, is a possible explanation for the inhibitory effect of caffeine during the initiation phase of DMBA-induced mammary tumorigenesis (54, 58).

It has been reported that the metabolism of polycyclic aromatic hydrocarbons (PAH) by cultured mouse keratinocytes is altered by extracellular Ca concentrations (102). The expression and inducibility of enzymes involved in the metabolism of PAH by cultured mouse keratinocytes may be regulated by extracellular Ca concentration and/or a Ca-induced differentiation of the mouse keratinocytes. It is possible that caffeine may effect the enzymes involved in the metabolism of PAH via the ability of the drug to increase cellular Ca levels.

Adenosine Receptors. Adenosine is an endogenous bioactive substance that functions as a regulatory signal in many different tissues and cell types, including heart, blood vessels, smooth muscle, platelets, mast cells, kidney, neurons, and adipocytes. The biological effects of adenosine are generally opposite to those produced by MX and are antagonized by MX in therapeutic concentrations (67).

There are at least two types of adenosine receptors: (i) the A_1 receptor, which has an inhibitory effect on adenylate cyclase (AC); and (ii) the A_2 receptor, which has a stimulatory effect on AC (103). The main criterion for distinguishing these two receptors is through the use of various adenosine analogues (104, 105). Caffeine is equipotent at both receptors (106) and has been shown to antagonize the stimulatory effect of adenosine on cellular cAMP levels in human mammary carcinoma cells (107). A third adenosine receptor (A_3) has been proposed (108) which is not coupled to AC and possibly linked to Ca. Caffeine's ability to alter cellular Ca levels might be due to its interactions with this proposed A_3 adenosine receptor.

Summary

Caffeine and other MX have been shown to evoke a wide variety of biochemical and physiological responses in many different tissues and cell types. However, the relevance of many of these responses in humans is uncertain, given that relatively high levels of caffeine and other MX are required to achieve many of these effects. Dose levels achievable through consumption of MX-containing foods and/or beverages are known to be able to antagonize adenosine receptors while having little or no direct effect on other mechanisms discussed. Given the wide distribution of adenosine and its receptors among many different tissues and cell types, and that the biological effects of MX are opposite to those produced by adenosine, it has been proposed that antagonism of adenosine receptors is the most plausible mechanism to account for the many biological actions of MX. However, other mechanisms by which MX act cannot be discounted. Further research is needed to illuminate the mecha-

nism(s) by which MX act, especially in regard to the developmental growth of the normal, benign, and carcinomatous mammary gland.

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