

Effects of Maternal Exposure to Cow's Milk High or Low in Isoflavones on Carcinogen-Induced Mammary Tumorigenesis among Rat Offspring

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Abstract

We investigated whether maternal exposure during pregnancy to cow's milk containing endogenous estrogens and insulin-like growth factor 1 (IGF-1) and either high or low levels of isoflavones from dietary legumes (HIM and LIM, respectively) affected carcinogen-induced mammary carcinogenesis in female rat offspring. Pregnant Sprague-Dawley rats were given HIM, LIM, or tap water (control) from gestational day (GD) 11 until birth; hereafter all rats received tap water. Mammary tumorigenesis was induced by administering 7,12-dimethylbenz[a]anthracene (DMBA) on postnatal day 50. No differences in maternal serum estradiol ($P = 0.19$) and IGF-1 levels ($P = 0.15$) at GD 19 or birth weight among the milk and water groups were seen, but estradiol, and IGF-1 levels and birth weight were numerically higher in the LIM group than in the HIM group. Puberty onset occurred earlier in the LIM offspring than in controls ($P = 0.03$). Although the high isoflavone content seemed to prevent the effect on circulating estradiol and IGF-1 levels and advanced puberty onset seen in the LIM group, HIM increased DMBA-DNA adducts in the mammary gland and tended to increase mammary tumorigenesis. In contrast, offspring exposed to LIM *in utero*, did not exhibit increased breast cancer risk, despite having higher estradiol and IGF-1 environment and consequently earlier puberty onset. These results indicate that the phytochemical content in the cow's milk, consumed by a pregnant dam, determines how milk affects the offspring. *Cancer Prev Res*; 4(5): 694–701. ©2011 AACR.

Introduction

Dietary phytoestrogens, particularly isoflavones and lignans, have received a great deal of attention due to their potential to affect hormone-dependent cancers, especially breast cancer. Soy products contain high levels of isoflavones (0.2–6.5 mg/mL; ref. 1), but isoflavones are also present in cow's milk (2, 3), although not in levels comparable to soy products. Equol is usually the far most abundant isoflavone in cow's milk, and the levels vary from 0.045 to 1 µg/mL, depending on the amounts and type of legumes fed to cows. The content of equol is on average 5 to 7 times higher in organic milk than in conventionally produced milk (2), due to the more intensive use of legumes in the feed. Equol is produced from the precursors formononetin

and daidzein by ruminal and intestinal bacteria (4, 5). All cows produce equol, but approximately one third of the Western population is estimated to be able to produce this isoflavone, most likely due to interindividual variability in the intestinal bacterial flora (5). Cow's milk is therefore a potential source of equol for non-equol producers. It is not clear whether equol exposure affects breast cancer risk. In humans, urinary equol excretion correlates with a high intake of soy products and a reduced risk of breast cancer (6), but equol also shows proliferative effects on human breast cancer cells (7), and in rodent studies dietary equol exhibits estrogenic effects (8).

Results from animal studies suggest that maternal dietary or hormonal exposures during pregnancy may have long-lasting effects on mammary tumorigenesis among the female offspring (9–12). For example, maternal exposure to a high-fat diet during pregnancy increases a female offspring's susceptibility to develop carcinogen-induced mammary tumors in rats (9, 13), whereas whole wheat flour, containing lignans, and fish oil reduces the risk (10, 12). The effects of maternal exposure to the isoflavone genistein during pregnancy on offspring's mammary tumorigenesis have also been intensively studied but results are conflicting (14). Neonatal and prepubertal exposure to equol alters the morphology of the mammary gland epithelial tree in a manner that can be considered to be protective, but it has no effect on mammary tumorigenesis (15).

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Some animal (16, 17) and human studies (18) suggest that milk intake increases breast cancer risk. However, most studies have reported either a reduced risk or no change in risk being associated with milk consumption (19, 20). Findings in humans suggest that childhood milk intake reduces breast cancer risk (21, 22). No animal studies have addressed the effect of maternal milk intake during pregnancy on mammary tumorigenesis among the offspring. Human epidemiologic data suggest that high milk consumption during pregnancy is associated with increased birth weight (23), and a high birth weight has been linked to elevated maternal estrogen levels (24) and increased risk of later development of breast cancer (25). Consistent with the human data, mammary cancer risk is increased in rats which had a high birth weight (13).

This study was designed to investigate whether *in utero* exposure to cow's milk high or low in isoflavones affects carcinogen-induced mammary tumorigenesis among the female offspring, compared with a tap water control group.

Materials and Methods

Animals

Twenty-seven female Sprague-Dawley rats were obtained from Charles River Laboratories on at gestational day (GD) 7 and weighed at arrival. The animals were housed individually in standard rat plexiglas cages at a constant temperature and humidity under a 12-hour light–dark cycles. Animals were fed the American Institute of Nutrition (AIN-93G) phytoestrogen-free, semipurified diet *ad libitum* throughout the study period. The study was conducted in accordance with the appropriate institutional and federal regulations.

Maternal liquid exposures

Pregnant dams were randomly assigned to the following 4 treatments ($n = 9$ per group) from GD 11 until they gave birth: low isoflavone milk (LIM), high isoflavone milk (HIM), or tap water (control). As we have previously shown that maternal exposure to 17β -estradiol (E2), either at a very low concentration (0.1 $\mu\text{g/g}$) or at a higher concentration (50 $\mu\text{g/g}$), significantly increases offspring's mammary tumorigenesis, an E2 control group was not included to this study. Fresh milk was provided daily, and the drinking bottles were weighed before and after milk was added to calculate the daily intake. The average daily feed intake was calculated on the basis of the difference in weight of the food provided and the remaining feed. The daily calorie intake (kcal/day) for dams during pregnancy was calculated from the energy content of the AIN93G diet provided in the information leaflet and the kcal content in skim milk (0.35 kcal/g milk).

When pups were born the litter was weighed and all dams were switched to tap water and continued on the AIN93G diet. To avoid litter effect, female pups were cross-fostered 1 to 2 days after the dams gave birth; female pups from 3 to 4 dams were pooled and housed with a nursing

dam which had received the same treatment as the pup's mother during pregnancy. Male pups were sacrificed. All female pups were weaned on postnatal day (PND) 23.

Milk and milk analyses

The 2 milk types were obtained directly from 2 privately owned commercial Danish dairy farms, that were selected on the basis of a screening of bulk milk phytoestrogen content on 20 Danish farms (organic and conventional) representing various different feed ration compositions especially regarding varying types and amounts of legumes. Cows producing HIM were fed a ration consisting of 24% whole crop barley/pea silage, 71% clover grass silage including red clover, and 5% grains (triticale, oats, barley, peas). LIM was obtained from a herd where cows were fed 24% horsebeans, 66% clover grass silage (white clover), and 10% grains (oats). Unprocessed bulk milk from one day's milking was obtained from both herds and stored at -20°C until analyzed or fed to rats as skim milk in the experiment. Milk was centrifuged for 10 minutes at 4,000 rpm, 4°C , and fat discarded to obtain skim milk. All subsequent analysis were carried out on skim milk. The insulin-like growth factor 1 (IGF-1) concentration was determined as described previously (26), and the content of phytoestrogens was measured according to the method described elsewhere (27). Unfortunately this method was not able to detect genistein in milk although it is known typically to be present in the amounts of 0.9 to 3 ng/mL (28, 29) and might have been higher in HIM than LIM.

Pregnancy E2, IGF-1, and isoflavone levels

Blood from all pregnant dams was collected by tail puncture on GD 19. Serum was separated and kept at -80°C until use. A commercial EIA kit from Cayman Chemicals was used to measure serum E2. Serum was purified by diethyl ether extraction prior to E2 analysis according to the manufacturer's instructions. Insulin-like growth factor 1 (IGF-1) was measured by an EIA kit from R&D Systems, Inc., according to manufacturer's instructions. The contents of genistein, daidzein, and equol were measured in serum by using a validated isotope dilution LC-ES/MS (liquid chromatography electrospray mass spectrometry) method (30).

Puberty onset—vaginal opening

Starting on PND 25, female offspring were clinically examined daily to evaluate vaginal opening (VO). Rats were recorded positive for VO when the vagina exhibited complete canalization and patency. VO typically occurs in the Sprague-Dawley rat at approximately 32 days of age under the influence of ovarian and adipose-derived estrogens and other hormones, and represents the initial stages of attaining sexual maturity.

Offspring mammary tumorigenesis

Mammary tumors were induced by oral administration of 10 mg 7,12-dimethylbenz[a]anthracene (DMBA) to

50-day-old female rats exposed to the different liquids *in utero*. The histopathology, estrogen dependence, estrogen and progesterone receptor expression, and antiestrogen responsiveness of DMBA-induced tumors show similarities to estrogen-sensitive human breast cancer (9, 10, 13). The animals were examined for mammary tumors by palpation once per week starting from week 8 post-DMBA treatment. The endpoints for data analysis were (i) number of animals with tumors (tumor incidence), (ii) number of tumors per animal (tumor multiplicity), and (iii) the total tumor volume per animal (volume). The animals were sacrificed when the detectable tumor burden approximated 5% of the total body weight, as required by our institution. All surviving animals, including those that did not develop mammary tumors, were sacrificed 18 weeks after carcinogen administration.

DMBA–DNA adduct measurement

DNA from mammary tissue at 18 weeks after DMBA administration was isolated by standard phenol extraction procedures and DMBA–DNA adducts were subsequently measured by ^{32}P -postlabeling (31). In short, 10 μg DNA was digested by using micrococcal endonuclease (0.06 U/ μL ; Sigma) and spleen phosphodiesterase (0.5 $\mu\text{g}/\mu\text{L}$; Sigma) for 4 hours at 37°C. Subsequently, samples were treated with nuclease P1 (0.26 $\mu\text{g}/\mu\text{L}$; MP Biomedicals) for 30 minutes at 37°C in a total volume of 12.5 μL . The largest fraction of the DNA digest (10.5 μL) was labeled with [γ - ^{32}P]-ATP (50 $\mu\text{Ci}/\text{sample}$; MP Biomedicals) by incubation with T4-polynucleotide kinase (0.68 U/ μL ; Fermentas) for 30 minutes at 37°C. Radiolabeled adduct nucleotide biphosphates were separated by thin-layer chromatography on polyethyleneimine cellulose sheets (Macherey-Nagel). Finally, DMBA–DNA adduct spots were quantified by Phosphor-Imaging technology (Fujifilm FLA-3000). A fraction of the digest (2 μL) was

used to determine the exact amount of DNA in the assay by HPLC-UV.

Statistical analysis

Data obtained on pregnancy-related parameters (birth weight, numbers of pups per litter, and pregnancy hormone levels), tumor volume/animal, and tumor multiplicity at sacrifice, together with DNA adduct formation, were analyzed by 1-way ANOVA followed by between-group comparisons by Tukey's test. Kaplan–Meier curves were used to compare differences in VO and tumor incidence, followed by the log-rank test. All tests were done by the SPSS SigmaStat software and differences were considered significant if the *P* value was less than 0.05.

Results

Milk composition

There were no macronutrient differences between the 2 types of milk (data not shown). The total content of phytoestrogens was approximately 4 times higher in HIM (429 ng/mL) than LIM (101 ng/mL; *P* < 0.001; Table 1), with equol being the most abundant phytoestrogen in HIM (366 ng/mL). However, the levels of enterodiol and enterolactone were significantly higher in LIM (0.21 and 42 ng/mL, respectively) than HIM (0.09 and 19.8 ng/mL, respectively; *P* = 0.04 and *P* = 0.01, respectively; Table 1).

Pregnancy outcome and serum estradiol, IGF-1, and isoflavones

Pregnant dams were started on cow's milk on GD 11 and continued until they gave birth. During pregnancy, the intake of liquid was higher in the 2 milk groups (*P* < 0.001), but milk-treated dams also consumed significantly less feed than the tap water group (*P* < 0.001),

Table 1. Content of IGF-1 and phytoestrogens in LIM and HIM

	LIM	HIM	<i>P</i> ^a
IGF-1, ng/mL	2.9 ± 0.06	1.2 ± 0.1	<0.001
Phytoestrogens ^b (ng/mL)			
Formononetin	6.0 ± 0.6	7.9 ± 0.3	0.054
Biochanin A	5.2 ± 0.6	12.0 ± 5.5	0.17
Daidzein	1.7 ± 0.6	5.8 ± 0.3	0.01
Prunetin	–	7.5 ± 2.4	–
Equol	37 ± 2.0	366 ± 4.9	<0.001
Matairesinol	–	0.63 ± 0.5	–
Secoisolariciresinol	9.0 ± 0.05	9.1 ± 0.4	0.43
Enterodiol	0.21 ± 0.03	0.09 ± 0.02	0.04
Enterolactone	42.4 ± 3.4	19.8 ± 0.1	0.01
Coumestrol	–	–	–
Total	101 ± 3.3	429 ± 11.9	<0.001

NOTE: All values are mean ± SEM.

^aProbability for no difference between the 2 milk types based on an unpaired *t* test.

^bMilk samples from 2 different days of the study period from GD 11 until birth were obtained and analyzed.

Table 2. Feed and liquid intake in dams during pregnancy ($n = 7-9$ per group) exposed to LIM, HIM, or tap water (control) from GD 11 until they gave birth

	Maternal treatment			P
	LIM	HIM	Control	
Food intake, g/day	15.2 ± 0.3 ^a	16.9 ± 0.8 ^a	19.2 ± 0.8 ^b	<0.001
Liquid intake, g/day	48.4 ± 3.0 ^a	52.4 ± 2.0 ^a	30.2 ± 2.3 ^b	<0.001
Energy intake, kcal/day	75.9 ± 1.8	84.1 ± 3.3	74.9 ± 3.5	0.07
Daily phytoestrogen exposure, µg/kg body weight	16.9 ± 1.1	75.1 ± 2.2	0	<0.001
Female birth weight, g/pup	7.4 ± 0.3	6.8 ± 0.2	7.0 ± 0.1	0.14
Male birth weight, g/pup	7.9 ± 0.4	7.1 ± 0.1	7.6 ± 0.1	0.09
Female/male ratio	1.3 ± 0.2	1.0 ± 0.3	0.9 ± 0.3	0.54

NOTE: All values are mean ± SEM. Birth weight of pups and female/male ratio in the litters are given here.

^{a,b}Different superscripts indicate statistical difference at the $P < 0.05$ level.

and therefore intake of energy (kcal/day) was not significantly different among the 3 groups ($P = 0.07$; Table 2). The total daily phytoestrogen exposure was determined between GD 19 and birth, and it was 16.9 ± 1.1 body weight for LIM dams and 75.1 ± 2.2 µg/kg body weight for HIM dams ($P < 0.001$; Table 2). Neither milk group differed from tap water control offspring in female ($P = 0.14$) and male ($P = 0.09$) birth weight, and the female/male offspring ratio was similar among the 3 groups (Table 2). However, both female and male offspring of HIM dams tended to be lighter than the offspring of LIM dams, although maternal energy intake was highest in the HIM group.

There was no difference in serum levels of E2 at GD 19 ($P = 0.19$), but when HIM and LIM were compared with each other, the E2 concentration was significantly lower in the HIM than the LIM group (419 pg/mL vs. 482 pg/mL, $P = 0.03$); control dams had E2 concentrations in between

the 2 values (468 pg/mL; Table 3). Similarly, HIM-exposed dams exhibited significantly lower IGF-1 level at GD 19 (782 ng/mL) compared with LIM (904 ng/mL; $P = 0.03$) and control dams (824 pg/mL), but the difference among the 3 groups did not reach statistical significance ($P = 0.15$; Table 3). Serum levels of isoflavones were low in all dams and neither LIM nor HIM altered the circulating levels of genistein or daidzein at GD 19, compared with water controls (Table 3). Equol levels were undetectable in most dams (in 23 of 27 dams). As isoflavones cross the placenta and the aglycone (biologically active) form accumulates in the fetus (32), it is possible that had we been able to measure isoflavones in the fetus, differences would have been seen.

Puberty onset

Maternal exposure to LIM during pregnancy caused an earlier puberty onset in the female offspring, determined by

Table 3. Serum 17β-estradiol (E2), IGF-1 and isoflavones in dams at GD 19 ($n = 5-9$ per group) exposed to LIM, HIM, or tap water (control) from GD 11 until they gave birth

	Maternal treatment			P
	LIM	HIM	Control	
E2, pg/mL	482 ± 10.2	419 ± 17.8 ^a	468 ± 26.1	0.19
IGF-1, ng/mL	904 ± 32.7	782 ± 37.4 ^a	824 ± 52.1	0.15
Equol ^b , µmol/L	Below detection	Below detection	Below detection	
Genistein ^b , µmol/L	0.23 ± 0.07	0.23 ± 0.08	0.29 ± 0.03	0.76
Daidzein ^c , µmol/L	0.33 ± 0.10	0.26 ± 0.10	0.37 ± 0.02	0.63

NOTE: All values are mean ± SEM.

^a $P < 0.05$, compared with the LIM group.

^bLimit of detection (LOD): 0.05 µmol/L; limit of quantification (LOQ): 0.15 µmol/L. Equol could be detected in 4 of 27 samples and therefore considered below detection. Genistein could be detected in 22 of 27 samples and a surrogate value 0.05 µmol/L was inserted for samples below the LOD.

^cLOD: 0.03 µmol/L; LOQ: 0.08 µmol/L. Daidzein could be detected in 24 of 27 samples and a surrogate value 0.03 µmol/L was inserted for samples below the LOD.

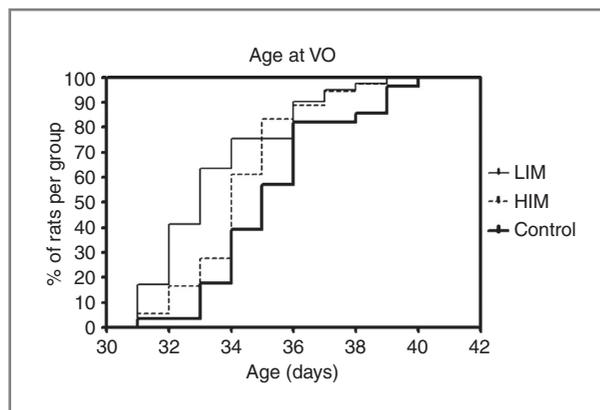


Figure 1. Effects of maternal exposure to tap water (control), LIM, or HIM from GD 11 until birth on VO among female offspring ($n = 28$, 47, and 36 in the control, LIM, and HIM groups, respectively). Vaginal opening occurred significantly earlier in the LIM group ($P = 0.03$) compared with the control group.

assessing the age of VO, compared with the control group (log rank = 7.169, $P = 0.03$; Fig. 1). No difference was seen between the HIM and control offspring. The age at which 50% of the rats showed VO was 33.6 ± 0.3 , 34.3 ± 0.3 , and 35.1 ± 0.4 for the LIM, HIM, and the control groups, respectively.

DMBA–DNA adducts

DMBA–DNA adducts were determined at the end of the tumor monitoring period (18 weeks) in nontumorous mammary tissue. Although DMBA–DNA adduct levels that were initially formed after the exposure will gradually decline in time due to DNA repair and cell turnover, DMBA–DNA adducts were still detectable in all samples. DMBA-induced DNA adduct levels were 2-fold higher in the HIM than in the control rat mammary glands (23.4 ± 3.1 vs. 11.2 ± 3.4 adducts per 10^8 nucleotides, $P < 0.001$; Fig. 2). DNA adduct levels in mammary glands of rats that received LIM *in utero* did not differ from the levels observed in control rats (16.3 ± 3.0 adducts per 10^8 nucleotides; Fig. 2).

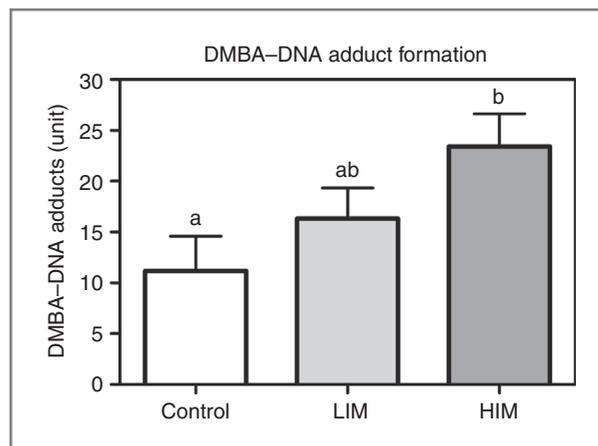


Figure 2. Effects of maternal exposure during pregnancy to tap water (control), LIM, or HIM on DMBA–DNA adduct formation measured in nontumorous mammary tissue at the end of the tumor monitoring period (18 weeks) among the offspring. Values represents mean \pm SEM. Bars marked with different letters are significantly different from each other ($P < 0.05$).

Tumorigenesis

There was no difference between LIM and HIM offspring relative to water offspring with respect to mammary tumor incidence (log rank = 2.544, $P = 0.28$; Fig. 3a). Compared with water and HIM of which 74% and 75% developed mammary tumor, respectively, maternal LIM intake during pregnancy resulted in the lowest incidence (60% mammary tumors). The number of mammary tumors per animal at sacrifice (tumor multiplicity; Fig. 3b) was nonsignificantly higher in the HIM offspring (2.08 ± 0.37) than in the tap water control and LIM offspring (1.42 ± 0.34 and 1.32 ± 0.27 , respectively; $P = 0.19$). The final tumor burden at sacrifice (total tumor volume) was 34% higher in the HIM group ($6,241 \pm 1,161$ vs. $4,815 \pm 1,301$ and $4,797 \pm 990$ mm³ the control and LIM offspring, respectively; Fig. 3c).

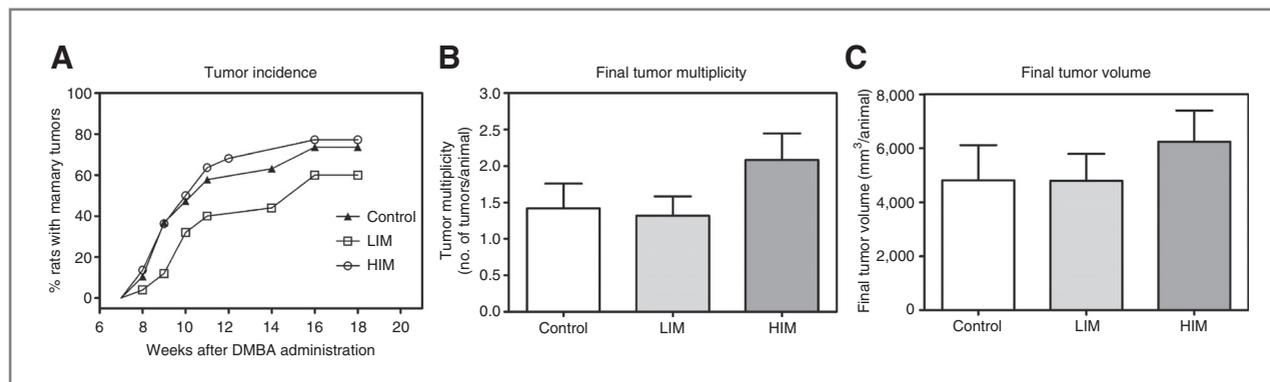


Figure 3. Effects of maternal exposure during pregnancy to tap water (control), LIM, or HIM on offspring tumor incidence (% rats with tumors; A), tumor multiplicity measured as the number of tumors per animal at the time of sacrifice (B), and tumor volume measured as the volume of tumors per animal (C) at the time of sacrifice ($n = 19$, 25, and 24 in the control, LIM, and HIM groups, respectively). Values are expressed as mean \pm SEM.

Discussion

Maternal dietary intake of excess fat or other dietary factors during pregnancy have been shown to affect female offspring's later susceptibility to develop mammary cancer in rats (10–12). This increase in risk is thought to occur due to an increase in fetal estrogenic environment caused by the maternal diet (33, 34). Our study is the first to investigate the effect of maternal milk consumption during pregnancy on the offspring's risk of mammary cancer. Milk contains endogenous estrogens and IGF-1 (26, 35) and various dietary phytoestrogens (2, 3, 27, 29), particularly isoflavones and lignans, with concentrations reflecting the composition of the cow's diet. These phytoestrogens have potential to exert estrogenic effects. Different feeding practices have been reported to result in up to 10-fold difference in milk's phytoestrogen content (118 vs. 1,215 ng/mL; ref. 3), but the 2 milk types in our experiment only reached a 4-fold difference in total phytoestrogen content. Further, milk phytoestrogens occur almost entirely as biologically inactive glucuronide and sulfate conjugates (28, 36) that are hydrolyzed by intestinal glucuronidases and sulfatases on ingestion before free biologically active forms can be absorbed.

We fed pregnant rats skim milk which contained either high or low levels of isoflavones; control dams received tap water. Skim milk was chosen for this study to avoid any confounding effects caused by possible differences in fatty acid composition between the LIM and HIM, and to minimize the effect of unconjugated biologically active endogenous estrogens that are suggested to increase with fat content (37). Phytoestrogens in milk are reported to be independent of milk fat content (28). HIM contained significantly more daidzein and its metabolite equol than LIM. The latter, in turn, contained significantly higher levels of enterodiol and enterolactone, two key lignan metabolites. These findings probably reflect the fact that cows producing HIM consumed a feed ration which included red clover silage containing high levels of isoflavones (29) typically used in organic milk production, whereas LIM cows consumed a feed ration typical for conventional milk production which includes white clover having a relatively high content of lignan precursors (38).

The different content of isoflavones in the two milk types could not be detected as a difference in circulating levels of genistein, daidzein, and equol at GD 19, perhaps reflecting the overall relatively low content of phytoestrogens in these 2 types of cow's milk compared with, for example, soy milk. Lignans were not determined. However, because the serum levels of E2 and IGF-1 at GD 19 were lower in the HIM than the LIM dams, the higher phytoestrogen content of HIM may have affected the dams. Commercial milk contains relatively high levels of both IGF-1 and estrogens (26, 37), and this could explain why these 2 tended to be higher in the LIM group than in the controls. The fact that they were lower in the HIM dams is consistent with previous findings in women showing that red clover-derived isoflavones nonsignificantly reduce circulating IGF-1 levels (39) and

that intake of soy-derived isoflavones is associated in some, but not all studies, with lower serum estrogen levels (40). The tendency of higher circulating levels of IGF-1 and E2 levels in the dams consuming LIM than in the control dams is consistent with milk containing relatively high levels of both (26, 35), and perhaps thereby also increasing them in individuals consuming milk (41), although milk protein consumption is believed to stimulate IGF-1 synthesis over IGF-1 absorption (42).

Offspring of dams consuming HIM during pregnancy exhibited significantly higher levels of DMBA-induced DNA adducts in their mammary glands than the controls; no difference was seen between the control and LIM offspring. Consistent with this, mammary tumor multiplicity and total tumor burden tended to be higher in the HIM group than in the other 2 groups. Animal studies have shown an increase in mammary cancer risk among offspring of dams which were exposed to high levels of isoflavones during pregnancy (10, 14, 43). Maternal exposure during pregnancy in these earlier studies was purified genistein, whereas milk phytoestrogens are a mixture of several different phytoestrogens with isoflavones daidzein and equol being the most abundant in HIM. Equol exists in 2 enantiometric forms: S-(−) and R-(+); S-equol is the naturally occurring metabolite of formononetin and daidzein in the rumen of cows and in the human intestine (4, 5, 44) and most likely the sole form in milk. In a recent study of carcinogen-induced mammary tumorigenesis in rats, S-equol administration from day 35 onwards showed no chemopreventive action, nor was it stimulatory, whereas R-equol reduced mammary tumorigenesis significantly (45). In another study, rats were exposed to S- or R-equol during neonatal life or prepubertally (day 21–35; ref. 15). The exposures did not significantly alter later mammary tumorigenesis. The results obtained in our study suggest that *in utero* exposure to equol in milk increases, rather than protects against, breast cancer. This is consistent with earlier studies which indicate that *in utero* and prepubertal isoflavone exposures are reported to have opposing effects on later breast cancer risk (14). However, the possibility that reduced lignan levels in the HIM, compared with the LIM, led to increased tumorigenesis, rather than high isoflavone levels, cannot be ruled out. Lignans have been linked to reduced mammary cancer risk in animal studies (46).

Offspring of dams consuming LIM milk during pregnancy exhibited several changes which are predictive of increased breast cancer risk. Specifically, their dams had the highest pregnancy IGF-1 and E2 levels, and they tended to have higher birth weight and they exhibited the earliest puberty onset. High pregnancy E2 levels increase offspring's mammary tumorigenesis (9), and it has been proposed that high IGF-1 also increases the risk (47). Human epidemiologic studies have reported that a high milk intake during pregnancy is associated with increased birth weight (23). Further, high birth weight is a risk factor for breast cancer in both humans and rats (25, 48). Early puberty onset is known to be related to increased breast

cancer risk (49), although the effect has become less significant in recent years (50). Thus, regardless of the increased presence of breast cancer biomarkers in the LIM group suggesting that these rats should develop most mammary tumors, they were not at an increased risk, illustrating that the chosen biomarkers are not always predictive of mammary tumorigenesis risk.

In summary, maternal HIM intake during pregnancy, relative to tap water intake, led to increased carcinogen-induced DNA adduct formation in the mammary gland and nonsignificantly increased some key parameters of tumorigenesis among the offspring. In contrast, maternal intake of LIM, which contained more lignans than HIM, had no effect on offspring's mammary cancer risk, although it increased the dam's pregnancy estradiol and IGF-1 levels, induced higher birth weight, and accelerated puberty onset.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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