Effects of wheat bran and energy restriction on onset of puberty, cell proliferation and development of mammary tissue in female rats

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Delayed onset of puberty and mammary development is assumed to reduce the risk of mammary cancer. An animal experiment was performed to investigate the influence of dietary fiber, which is known to affect hormonal balance, on these characteristics. Forty-five immature female rats were randomized into three groups, which were fed ad libitum a low-fiber diet (<0.5% dietary fiber based on white wheat flour), a high-fiber diet (9.2% dietary fiber based on wheat bran), or an energy-restricted low-fiber diet providing 90% of the energy of the ad libitum low-fiber diet. Energy intake in the second and third groups was similar. Wheat bran slightly delayed onset of puberty, whereas restricted energy intake delayed onset of puberty by about six days. At 48–58 days of age, 14 rats of the low-fiber group, 10 of the high-fiber group and 7 of the restricted group were initiated. Development of mammary tissue was rudimentary in rats of the energy-restricted low-fiber group, stronger in the high-fiber group and strongest in the ad libitum low-fiber group. Cell proliferation in mammary tissue was similar for both groups fed ad libitum, but significantly lower in the restricted group. Peroxidase activity, a biomarker for estrogenicity, was lower in the high-fiber group than in the two low-fiber groups. It is concluded that wheat bran and, even more effectively, an imposed restricted energy intake delays onset of puberty and mammary development. This shortens the time for mammary cells to be initiated to tumor cells and hence reduces the risk of mammary cancer development. It seems that wheat bran acts via a reduced energy intake. However, that wheat bran plays a role in the delay of mammary development due to a reduced exposure to estrogen cannot be excluded.

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Epidemiological and experimental (animal) studies have indicated that various parameters affect the risk for mammary cancer development: a delayed menopause (1) and dietary factors such as an increased fiber intake (2) and restricted energy intake (3).

In rats fed a high-fiber (HF) diet based on wheat bran, mean weight of NMU-induced mammary tumors was lower than in rats fed a low-fiber (LF) diet based on white wheat flour (4). Estrogen acts on mammary cells as an inducer of cell proliferation at physiological levels (5). It was postulated that cancer risk is proportional to the number of proliferating cells, which in turn depends on both the number of cells and the rate of cell division in the tissue (6). To decrease cell proliferation of mammary tissue, exposure to estrogen has to be minimized. One way to decrease estrogen exposure might be adding estrogen-binding compounds, i.e. particular fibers, to the diet (7), which results in an enhanced fecal and a lowered urinary estrogen excretion (4). Despite increased fecal estrogen excretion, no lowered plasma estrogen levels were observed in rats fed the HF diet (4). A more sensitive test for the estrogen-mediated effect of fiber on estrogen-sensitive tissue could be found in studying the maturation of rats. The addition of wheat bran to a diet also results in a lowered energy intake and the introduction of lignan precursors. The precursors can be converted by intestinal microflora to lignans, absorbed by the intestinal wall and behave as anti-estrogenic compounds (8, 9). All of these factors affect the parameters studied.

To investigate specifically the effects of both wheat bran and reduced energy intake, an experiment was designed in which three groups of immature rats were fed a HF diet, a LF diet or an energy-restricted LF diet. The parameters studied were onset of puberty, mammary development and cell proliferation of mammary tissue. Exposure of estrogen-sensitive tissue to estrogen was studied by measuring peroxidase activity as a biomarker for estrogenicity (10, 11).

Materials and methods

Animals

Immature female Fisher rats (F-344), 24–25 days of age,
were obtained from Charles River Wiga GmbH, Sulzfeld, Germany. On the second day after arrival the rats were divided into three groups of 15 animals each after computerized randomization to match for initial body weight. The rats were housed individually in suspended hanging-type stainless steel wire-mesh-bottom cages in an air-controlled room (23 ± 1°C) with a relative humidity of 50 ± 10% and a light/dark cycle of 12 h. The animals were weighed weekly.

**Diets**

One group of rats was fed an HF diet based on wheat bran (A-HF group) and another on an LF diet based on white wheat flour (A-LF group), both given ad libitum. The rats of the third group (R-LF group) were fed a diet accounting for 90% of the amount of food consumed by the animals of the A-LF group on the previous day. The LF diet was composed such that the R-LF animals received approximately the same amount of energy as the animals of the A-HF group (12). Water was supplied ad libitum. Food consumption of the A-LF and R-LF groups was recorded daily and food supply of the R-LF group was adjusted accordingly. Food consumption of the A-HF groups was recorded weekly. The diets provided all animals with equal amounts of vitamins, minerals, protein and fat. The lower energy intake of the R-LF group was at the cost of a lower carbohydrate intake relative to the A-LF group. The composition of the diets is summarized in Table 1. Nutrient composition of the diets was analyzed as reported recently (4).

**Onset of puberty**

Vaginal membrane rupture, which indicates the onset of puberty, was controlled twice daily from 31 days of age.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A-HF group</th>
<th>A-LF group</th>
<th>R-LF group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>15.69</td>
<td>22.53</td>
<td>25.70</td>
</tr>
<tr>
<td>White wheat flour</td>
<td>42.00</td>
<td>54.72</td>
<td>49.03</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>23.75</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>3.63</td>
<td>4.24</td>
<td>4.74</td>
</tr>
<tr>
<td>Vitamin ADEK prep.</td>
<td>0.31</td>
<td>0.36</td>
<td>0.40</td>
</tr>
<tr>
<td>Vitamin B mix.</td>
<td>0.20</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>Lard</td>
<td>7.21</td>
<td>8.96</td>
<td>9.95</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>7.21</td>
<td>8.96</td>
<td>9.95</td>
</tr>
<tr>
<td>Protein</td>
<td>22.3</td>
<td>25.6</td>
<td>28.1</td>
</tr>
<tr>
<td>Fat</td>
<td>16.1</td>
<td>18.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>39.0</td>
<td>42.8</td>
<td>39.2</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>9.2</td>
<td>&lt;0.3</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.9</td>
<td>9.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Ash</td>
<td>4.5</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Energy (MJ/100 g)</td>
<td>16.4</td>
<td>18.4</td>
<td>19.0*</td>
</tr>
</tbody>
</table>

*With a food restriction of 10%, energy intake by animals of the R-LF group will be similar to that of the A-HF group.

The estrous cycle was determined by vaginal smears, which were taken at 09.00 and 18.00 from 48 days of age. Smears were fixed in methanol for 5 min and subsequently stained according to Papanicolaou (13). Phases of the cycle were recorded as proestrus, estrus, metestrus or diestrus (14).

**Cell proliferation**

Nine animals of the A-HF group, nine of the A-LF group and seven of the R-LF group, while in metestrus, were injected ip with methyl-3H-thymidine (2 μCi/g body weight, spec. act. 40–60 Ci/mmol. Amersham, ‘s-Hertogenbosch, The Netherlands). The animals were killed 4 h after injection by either anesthesia and aorta bleeding. Both right and left cervical, thoracic, abdominal and inguinal mammary glands, attached to the overlying skin, were dissected. The left part of the mammary tissue was used for estimating peroxidase activity and the right part for measuring cell proliferation (thymidine labeling index, TLI).

The right mammary tissue was fixed in 4% aqueous phosphate-buffered formaldehyde solution (pH 7.0) and used for autoradiography. The tissues were dehydrated and embedded in Technovit 7100 plastic (Kulzer, Wehrheim, Germany). The blocks were sectioned semiserially at a thickness of 5 μm. For each block three preparations and per preparation three sections were obtained. Between sections 15 μm was omitted and for each preparation 50 μm was omitted. The sections were covered with liquid photographic emulsion (Kodak NTB-2, Eastman Kodak, New York) diluted 1:1 with a 2% aqueous glycerol solution. Autoradiograms were exposed in dry, light-tight boxes for four weeks at −28°C, developed in Kodak D19, stained with 0.01% Toluidine Blue and embedded in DePeX mounting medium (BDH, Poole, England). TLI was determined by counting the number of labeled nuclei (indicated by arrow in Figs 1 and 2) and the total number of epithelial cells of the terminal end buds (TEB, Fig. 1) and terminal ducts (TD) or alveolar buds (AB, Fig. 2) Nine sections per mammary tissue and per animal were counted. TLI was expressed as the number of labeled nuclei per 500 epithelial cells. The values obtained for each group were pooled and mean and standard deviation were calculated for each group.

**Mammary development**

The right part of the mammary tissue from rats of the A-HF group (N = 14), the A-LF group (N = 14) and the R-LF group (N = 12) was also used for establishing mammary development. The right glands from rats not used for measuring cell proliferation were dissected from the skin and fixed in acetone. The fixed tissues were further processed according to the AMEX method (15) and embedded in paraffin. Three 5 μm slides, obtained as described above, of each of the embedded tissues were
stained with hematoxylin and eosin. These slides, as well as three slides of tissue of each of the rats used for measuring cell proliferation, were used for classifying mammary tissues into two groups according to development of mammary tissue. Based on histological criteria the first group included poorly developed mammary tissue, i.e. individual transverse structures of collecting ducts and TEB. The second group showed moderately and well-developed tissue, i.e. a fairly large number of collecting ducts and TEB lying close together. The slides were screened blindly two times by two different investigators to obtain reliable scores.

Peroxidase activity in mammary and endometrial cells

The peroxidase (E.C. 1.11.1.7.) activity meant is called "uterine peroxidase", which is soluble in 0.5 mol/l CaCl₂ and is a biomarker for estrogenicity (16). For the sake of clarity, this enzyme will be called peroxidase.

The left part of the mammary tissues, adipose tissue included, from the animals used for establishing mammary development was frozen in liquid nitrogen and stored at –80°C. The left part of mammary tissues from animals injected with labeled thymidine was washed in saline (until the saline was free of radioactivity) and stored at –80°C. Tissues were weighed, minced with scissors and suspended in 10 mmol/l TRIS buffer (final volume 10 ml). The suspension was homogenized with a Potter homogenizer set at 1000 rpm in ice. Five milliliters of the homogenate was centrifuged for 45 min at 35 000 x g and 4°C. The sediment was rehomogenized with 2.5 ml of the peroxidase extraction buffer (10 mmol/l TRIS, 0.5 mol/l CaCl₂) and centrifuged again (35 000 x g, 20 min, 4°C). The supernatant between pellet and fat layer was used for measuring peroxidase activity.

The entire uterus of the sacrificed rats was quickly dissected. Any adhering mesenteric and adipose tissues were removed and the uterus was chilled in 0.9% NaCl. Each uterine cornu from the oviduct to the cervix was opened longitudinally. Endometrial cells were collected by scraping with bent tweezers over the inner surface of the uterus (17). The cells were washed by centrifugation and the pellet was suspended in 1 ml of 10 mmol/l TRIS.
buffer pH 7.2 and used for the peroxidase assay (600 µl) and the DNA assay (see below). To 600 µl of the suspension 3 ml of peroxidase extraction buffer (final CaCl₂ concentration 0.5 mol/l) was added and peroxidase activity was measured according to Lyttle and Desombre (10) with some modifications. 3,3',5,5'-tetramethyl-benzidine (TMB, Sigma, St Louis, MO) was used as substrate and horseradish peroxidase (HRP, Serva, Heidelberg/New York) as standard. Tubes with samples and standards were incubated until the lowest standard points colored light blue. The reaction was stopped with 500 µl of 2 mol/l HCl. To remove denatured proteins, tubes were centrifuged (15 min. 35,000 x g, 4°C). In the supernatants the intensity of the color was measured at 452 nm. Peroxidase activity was expressed per µg DNA as units of “apparent HRP” equaling an absorption unit of color formation per minute.

**DNA analysis**

DNA was measured according to Labarca and Paigen (18) based on the enhancement of fluorescence seen when bisbenz-imidazole (Hoechst 33258) binds to DNA.

Endometrial cells suspended in 10 mmol/l TRIS buffer or an aliquot of the mammary tissue homogenate in TRIS buffer were diluted in 0.05 mol/l sodium phosphate pH 7.4 containing 2.0 mol/l NaCl. Cells were homogenized in a Potter homogenizer. To 500 µl of the homogenate 1 ml buffer containing 1.5 µg Hoechst 33258 was added. After vortexing, fluorescence was measured with a fluorometer (Perkin Elmer 204-A fluorescence spectrophotometer) with the excitation and emission wavelengths adjusted to 356 nm and 458 nm, respectively. The emission units were read on a standard curve for DNA calf thymus (type I, art. D1501; Sigma, St Louis, MO) with a range of 20–2500 ng/tube.

**Statistics**

Differences in mean energy intake, body weight, onset of puberty, cell proliferation and peroxidase activity were tested using Student’s t-test. Differences in development of mammary tissue and estrous cycle were estimated by Pearson’s chi square test with the BMDP program 4F (19). A difference at p < 0.05 was considered statistically significant.
Table 2. Mean food (g·day⁻¹·rat⁻¹) and energy (kJ·day⁻¹·rat⁻¹) intake by female rats (N = 15 per group) fed an HF or an LF diet ad libitum or an energy-restricted LF diet at 27 to 48 days of age.

<table>
<thead>
<tr>
<th></th>
<th>Food</th>
<th>Energy</th>
<th></th>
<th>Food</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>27–34 (Week 1)</td>
<td>A-LF 8.2±0.9</td>
<td>151±15.9b</td>
<td>A-HF 6.7±1.1</td>
<td>109±17.5a</td>
<td>9.6±1.0</td>
</tr>
<tr>
<td></td>
<td>R-LF 5.8±0.2</td>
<td>110±3.8a</td>
<td></td>
<td>R-LF 5.8±0.2</td>
<td>110±3.8a</td>
</tr>
<tr>
<td>34–41 (Week 2)</td>
<td>A-LF 9.6±1.0</td>
<td>176±18.8b</td>
<td>A-HF 9.6±0.6</td>
<td>157±9.4a</td>
<td>10.2±1.2</td>
</tr>
<tr>
<td></td>
<td>R-LF 8.4±0.1</td>
<td>160±13.3a</td>
<td></td>
<td>R-LF 8.4±0.3</td>
<td>159±5.8a</td>
</tr>
<tr>
<td>41–48 (Week 3)</td>
<td>A-LF 6.7±1.1</td>
<td>109±17.5a</td>
<td>A-HF 9.6±0.6</td>
<td>157±9.4a</td>
<td>10.2±1.2</td>
</tr>
<tr>
<td></td>
<td>R-LF 5.8±0.2</td>
<td>110±3.8a</td>
<td></td>
<td>R-LF 5.8±0.2</td>
<td>110±3.8a</td>
</tr>
</tbody>
</table>

Mean ± SD indicated with different letters in one column indicate a significant difference (p<0.05).

Results

Energy intake and body weights

Initial mean weight of the rats in each of the groups was about 44 g. From the start of the experiment until the age of 48 days, energy intake in the A-HF and R-LF groups was similar, but significantly lower than in the A-LF group (Table 2). Although energy intake was similar, mean body weight of the R-LF group was slightly, but significantly, lower than in the A-HF group after the first week (Fig. 3). The higher energy intake by rats of the A-LF group resulted in a significantly higher mean body weight after the first week (compared with the R-LF group) and after the second week of the experiment (compared with both other groups).

Onset of puberty

The A-HF diet slightly delayed and the R-LF diet delayed vaginal opening significantly relative to the A-LF diet (Table 2).

Vaginal smears taken to assess the phase of the cycle indicated that, at 48–58 day of age, 14 rats out of 15 in the A-LF group had an estrous cycle, whereas just 10 animals of the A-HF group and 7 animals of the R-LF group were in cycle (Table 3).

Table 3. Age (mean ± SD in days) at onset of puberty and of being in estrous cycle of groups of rats (N = 15 per group) fed an A-HF, an A-LF or a R-LF diet.

<table>
<thead>
<tr>
<th></th>
<th>Mean age (days) of vaginal membrane rupture</th>
<th>Estrous cycle of animals at 48–58 days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle</td>
<td>No cycle</td>
</tr>
<tr>
<td>A-LF</td>
<td>34.2±2.7</td>
<td>14 1</td>
</tr>
<tr>
<td>A-HF</td>
<td>37.0±4.7 NS</td>
<td>10 5 NS</td>
</tr>
<tr>
<td>R-LF</td>
<td>40.7±6.0**</td>
<td>7 8*</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.001; NS = not significant, relative to the A-LF group.

Cell proliferation

With the procedure described, in only two out of seven rats of the R-LF group had mammary tissue sufficiently developed to establish cell proliferation in TEB cells, while ducts could not be observed in one out of seven slides prepared from the mammary tissue of these rats. In the A-HF group, four out of nine tissues had sufficiently developed to show TEB and in seven out of nine to show ducts. In the A-LF group, in eight out of nine slides TEB and ducts could be observed. TLI (% of labeled cells in a total of 500 cells) in the TEB and ducts in mammary

![Figure 3](https://via-free-access.bioscientifica.com/11/12/2018/08/38/17AM)
Peroxidase activity in endometrial cells was found to be 100-fold higher than in mammary tissue. Differences in activity in endometrial cells were not observed among the groups (rats in metestrus). In a pilot experiment, using female rats fed the LF diet ad libitum from 27 days to about 50 days of age, peroxidase activity was found to be two to three fold higher in endometrial cells from rats in proestrus (about 1400 mU/µg DNA) than in rats in metestrus (about 500 mU/µg DNA).

Discussion

Our experiments show clearly that both dietary fiber and energy restriction affect hormonal processes which find expression in a delayed vaginal membrane rupture, i.e. a delayed puberty, a delayed onset of the estrous cycle and poor mammary development. An important consequence of ad libitum dietary fiber intake seems to be a reduced energy intake. An imposed lowered energy intake seems to have a still stronger effect, energy intake being similar for the A-HF and R-LF groups. A "stress factor" evoked by the absence of sufficient food might play a crucial role in hormonal processes. However, it cannot be excluded that lignans introduced in the body by wheat bran (8) have a low estrogenic activity which compensates for the effects of lower energy intake (9, 20).

In the A-HF and R-LF groups energy intake was similar from the start of the experiment until the age of 48 days. However, a restricted diet has a direct effect on body weight; already after the first week mean body weight in the R-LF group was significantly lower than in the A-LF group. The A-HF diet has a delayed effect on body weight; mean body weight of the A-HF was similar to the A-LF group after the first week but lower after the second week (Fig. 3).

An A-HF diet and, even more effectively, a R-LF diet delays vaginal membrane rupture, i.e. onset of puberty. Eckstein et al. (21), using descendants of Wistar or Charles River rats, showed vaginal opening to occur at 35.6 days which is in good agreement with our findings. We started measuring the estrous cycle when rats were 48 days old, at which time almost all animals of the A-LF group were in cycle. In the A-HF group, and in particular in the R-LF group, more animals were in an irregular or non-cycle state, indicating that wheat bran and, even more effectively, an imposed reduced energy intake affect those hormonal processes which regulate the estrous cycle. The amenorrheal state or delayed menarche has also been noticed in girls who sport intensively (22) or have a high intake of dietary fiber (23–25).

A clear effect of dietary fiber and restricted energy intake is the delay in mammary development (Table 5). This finding corroborates the result of human studies in which dietary intake based on cereal fiber was related to breast development in girls aged 9 to 13 (24).

Cell proliferation of the epithelial cells in mammary
Mammary tissue development in rats

Fig. 4. A 5 μm section of a poorly developed mammary gland (staining by toluidine blue, bar represents 20 μm).

Table 6. Uterine peroxidase activity (mU/μg DNA) in mammary tissue and endometrial cells from rats fed the A-LF, the A-HF or the R-LF diet. Rats were in metestrus at the time of section.

<table>
<thead>
<tr>
<th></th>
<th>Mammary tissue</th>
<th>Endometrial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-LF</td>
<td>10</td>
<td>4.72 ± 3.06a</td>
</tr>
<tr>
<td>A-HF</td>
<td>9</td>
<td>2.62 ± 1.60b</td>
</tr>
<tr>
<td>R-LF</td>
<td>7</td>
<td>3.96 ± 1.28b</td>
</tr>
</tbody>
</table>

*Mean ± s.e.m indicated with different superscripts in one column indicate a significant difference (p<0.05).

tissue was not found to differ between the two groups fed ad libitum. This is in agreement with our previous finding that the incidence of NMU-induced mammary tumours was similar in two groups fed a HF or LF diet (4). It has been suggested that mammary tumor induction by a carcinogen depends principally on the frequency of mammary cell division at the time a carcinogen acts on the gland (26). In the energy-restricted rats cell proliferation was significantly lower. Lok et al. (27), too, found a strong effect of energy restriction on

Fig. 5. Schematic presentation of the hypothesized pathway for the effects of dietary fiber and/or energy on mammary cancer risk. The numbers in parentheses refer to the References. The (+) or (-) effects of lignans depend on the presence of endogenous estrogens. An explanation of this figure is given in the text. GnRH is gonadotropin-releasing hormone.
proliferation of mammary cells of female mice, but this effect became only manifest when restriction was as large as 20%. Peroxidase activity has been suggested to be a measure of estrogen exposure (10). In mammary tissue of the A-HF group a significantly lower peroxidase activity was measured than in tissue of the two low-fiber groups. This suggests that estrogen exposure in mammary tissue of the A-HF animals (when in metestrus) is lower, which is in agreement with earlier studies in which we found a three to fourfold higher fecal estrogen excretion and a lower urinary estrogen excretion in rats fed the HF diet (4). However, a lowered estrogen exposure should result in a lowered epithelial cell proliferation as well, which was not observed (Table 5).

As mammary development is under specific control of oestradiol-17β (28), the poor mammary development in the A-HF and R-LF groups relative to the A-LF group can thus be explained by the lowered estrogen exposure. In the A-HF group this can be explained by the increased fecal estrogen excretion (4), which is still not compensated by a higher estrogen production or, as in the R-LF group, by a lower energy intake. A lower energy intake might inhibit gonadotropin (LH and FSH) secretion and thus decrease estrogen production (29).

The reduced risk for breast cancer in vegetarian women (30) or in women on a fiber-rich diet (2) might be explained as presented schematically in Fig. 5. According to the unifying hypothesis of De Waard and Trichopoulos (31), breast cancer is induced during puberty and adolescence. Only undifferentiated epithelial mammary cells (TEB cells) are believed to be susceptible to carcinogenic compounds (32). Wheat bran, but also a restricted energy intake, shortens the time between the onset of mammary development and fully differentiated mammary tissue, i.e. time after pregnancy. Thus the period during which mammary cells can be initiated to tumor cells is shortened. The presence of about 24% wheat bran (ca. 9.5% dietary fiber) in the diet results in a reduced energy intake of about 10% (4). An energy restriction of 10% affects tumor promotion (reduction of tumor multiplicity and tumor burden) as reviewed previously (33). Furthermore, it is known that wheat bran affects estrogen excretion (4, 34) and hence lowers exposure of mammary tissue to estrogen (Table 6). In the phase of mammary development at an age of about 50 days, the lowered estrogen exposure appears not to affect cell proliferation (Table 4). However, it cannot be excluded that mammary tumors are more sensitive to lower estrogen exposure resulting in a reduced tumor weight as found previously (4, 33). A restricted energy intake, rather than an iso-energetic wheat bran diet, affects the parameters studied, i.e. onset of puberty, estrous cycle and mammary development. This might be explained by a stress factor, as mentioned before, or by the fact that wheat bran contains certain precursors, which can be converted into lignans (9). In the absence of endogenous estrogens in the prepubertal period these lignans might exert estrogenic activity which compensates for the reduced energy intake (20). However, when endogenous estrogens are present, as in the case of puberty and adolescence, the lignans will have antiestrogenic activity (9), which might reduce the (effective) biologic activity of the estrogens, as discussed previously (4). Another explanation is that a reduced energy intake inhibits gonadotropin secretion (29). A lowered LH secretion might affect the estrous cycle and, due to a lowered estrogen production, delay mammary development.

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