Dietary energy restriction inhibits estrogen-induced mammary, but not pituitary, tumorigenesis in the ACI rat

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Because of the suggested role of energy consumption and the well-documented role of estrogens in the etiology of breast cancer, we have examined the effect of a 40% restriction of dietary energy consumption on the ability to induce mammary tumorigenesis in female ACI rats. Experiments herein test the hypothesis that at least part of the inhibitory effect of energy restriction on mammary tumorigenesis is exerted downstream of potential effects of dietary manipulation on the production of estrogens by the ovaries. Ovary-intact ACI rats were fed a control or a 40% energy-restricted diet and were either treated continuously with E2 from subcutaneous Silastic tubing implants or received no hormone treatment. Mammary cancers rapidly developed in E2-treated rats fed the control diet; within 216 days of initiation of E2 treatment 100% of the population at risk exhibited palpable mammary tumors. Dietary energy restriction markedly inhibited E2-induced mammary tumorigenesis, as evidenced by significant reductions in tumor burden as well as a significant increase in the latency to the appearance of the first palpable cancer. The inhibitory actions of dietary energy restriction on E2-induced mammary tumorigenesis were associated with an inhibition of E2-stimulated mammary cell proliferation. However, this inhibition was insufficient to block induction of lobuloalveolar hyperplasia or appearance of focal regions of atypical epithelial hyperplasia. These data suggest that dietary energy restriction inhibits E2-induced mammary cancer by attenuating or retarding the progression of atypical hyperplasia to carcinoma. Expression of progesterone receptor (PR) was up-regulated within the focal regions of atypical hyperplasia and the carcinomas induced by E2, regardless of whether the rats were fed the control or energy-restricted diet. However, circulating progesterone was reduced by dietary energy restriction, suggesting a possible mechanism for inhibition of mammary tumorigenesis. Dietary energy restriction did not inhibit the ability of administered E2 to induce prolactin (PRL)-producing pituitary tumors and associated hyperprolactinemia, indicating that the inhibitory effects of dietary energy restriction on mammary tumorigenesis are tissue specific and independent of circulating E2 and PRL.

Introduction

Several dietary factors, including dietary energy consumption, have been implicated as determinants of breast cancer risk in human populations (1). For example, both prospective and case-control studies associate height and/or body mass index with breast cancer risk and provide indirect evidence that energy consumption and/or balance influence the development of breast cancer (2,3). Supporting these epidemiologic data are numerous studies demonstrating that dietary energy restriction markedly inhibits mammary tumorigenesis in a variety of rodent models (4–8). In several laboratory studies, the inhibition of mammary tumorigenesis by energy restriction has been associated with reductions in mammary epithelial cell proliferation or circulating levels of estrogens and/or prolactin (PRL) (8–17). Because these ovarian and pituitary hormones regulate mammary gland growth, differentiation and function, it has been hypothesized that reduction of hormone output by the ovary and/or pituitary is one important mechanism through which the inhibitory effects of energy restriction on mammary tumorigenesis are manifested (16–18).

Abundant data from epidemiologic studies demonstrate that ovarian hormones, particularly estrogens, play an important role in the etiology of breast cancer (19–21). Moreover, prophylactic treatment with the anti-oestrogen tamoxifen reduced by ~50% the incidence of breast cancer in a population of women at high risk of this disease (22). It has been hypothesized that estrogens contribute to breast cancer etiology, at least in part, by increasing the rate of mammary cell proliferation, thereby promoting the accumulation of somatic mutations (23). Cell proliferation in the human breast epithelium is highest during the luteal phase of the menstrual cycle, when circulating progesterone is highest (24). Together, the data suggest that both estrogens and progestins contribute to breast cancer etiology.

Recent studies from our laboratory reveal the female ACI rat to be a unique and physiologically relevant animal model for the study of breast cancer. Ovary-intact ACI rats rapidly develop mammary cancers when the level of 17β-estradiol (E2) in the systemic circulation is maintained in the high physiologic range by release of E2 from subcutaneous implants, whereas mammary cancers very rarely develop in this rat strain in the absence of exogenous E2 (25). Continuous stimulation of the mammary gland by E2 results in induction of lobuloalveolar hyperplasia, subsequent appearance of focal regions of atypical epithelial hyperplasia, and ultimately, development of multiple, independently arising, mammary cancers (25,26). Ovariectomy markedly inhibits development of E2-induced mammary cancers, and the epithelial cells within both the focal regions of atypical hyperplasia and the mammary carcinomas exhibit increased expression of progesterone recep-

Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2-deoxyuridine; COP, Copenhagen; E2, 17β-estradiol; DMBA, dimethylbenz[a]anthracene; MNU, N-methyl-N-nitrosourea; PRL, prolactin; PR, progesterone receptor; SEM, standard error of the mean; SD, standard deviation.

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tor (PR) relative to the surrounding epithelium (25,26). These data suggest that progesterone and its receptor may contribute to the development of E2-induced mammary cancers, and that atypical hyperplasia may be a precursor lesion to carcinoma.

In contrast to the high degree of susceptibility of the ACI rat strain to E2-induced mammary cancers, the genetically related Copenhagen (COP) rat strain is much less susceptible to E2-induced mammary tumorigenesis (27,28). Interestingly, the mammary epithelia of the female ACI rat exhibits a greater proliferative response to E2 than does that of the female COP rat, suggesting a possible mechanism for the observed differences in the susceptibilities of these two strains to E2-induced mammary cancers (26). In a genetic cross between the susceptible ACI rat and the resistant COP rat, susceptibility to E2-induced mammary cancers is inherited as an incompletely dominant phenotype (29). Thus, the ACI rat provides a genetically defined and physiologically relevant model in which to study diet/hormone interactions in mammary cancer development.

Because of the suggested role of energy consumption and the well-documented role of estrogens in the etiology of breast cancer, we have examined the effect of a 40% restriction of dietary energy consumption on E2-induced mammary tumorigenesis in female ACI rats. To our knowledge, this is the first study to examine the action of dietary energy restriction in an animal model where mammary cancer is induced solely through the actions of a naturally occurring hormone. Because exogenous E2 is the inducing agent in this model, this study allowed us to test the hypothesis that at least part of the inhibitory effect of energy restriction on mammary tumorigenesis is downstream of potential effects of energy restriction on output of estrogens by the ovaries. Moreover, because the administered E2 maintains production of pituitary derived PRL at a high level (30,31), this study also allowed us to examine the ability of dietary energy restriction to inhibit mammary tumorigenesis independent of any inhibitory effect on pituitary PRL output.

The data presented herein demonstrate that dietary energy restriction markedly inhibited development of E2-induced mammary cancers in the ACI rat, apparently by attenuating or retarding the progression of pre-neoplastic lesions to carcinoma.

Materials and methods

Care and treatment of animals

All protocols involving live animals were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center. Female ACI rats were obtained from Harlan (Indianapolis, IN) and were housed one animal per cage within a barrier animal facility with controlled temperature, humidity and lighting cycle (12 h light/12 h dark). The rats were initially fed a semi-purified diet that was formulated in accordance with guidelines established by the American Society of Nutritional Science (32). One week later, the rats were randomly assigned to groups fed either this control diet or an energy-restricted diet. The compositions of these diets are illustrated in Table 1, and the methods used in their preparation have been described previously (30,33,34). The rats fed the control diet were allowed to eat ad libitum, and the amount of food consumed by these animals was monitored weekly. The rats fed the energy-restricted diet were fed each day, at the beginning of the dark phase of the lighting cycle, 0.64 g of food per g of food consumed per day by the rats fed the control diet. Consequently, the animals fed the energy-restricted diet consumed 40% less energy, derived from carbohydrate and fat, but equivalent amounts of protein, vitamins, minerals, fiber and other nutrients, relative to that consumed by animals fed the control diet. The rats were allowed continuous access to tap water. Body weights were monitored weekly.

Silastic tubing implants, either empty or containing 27.5 mg of crystalline E2, were prepared as described previously (25,27). When the animals were at a mean of 10.3 g of diet/day throughout the course of the experiment (Figure 1A). Food consumption was increased by 4% to 11.2 g/day (P < 0.05), in rats treated with E2. When fed the control diet, both untreated and E2-treated rats grew rapidly until ~84 days of age (Figure 1B). Thereafter, both the

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Table 1. Formulation of experimental diets

<table>
<thead>
<tr>
<th>Diet component</th>
<th>Control diet</th>
<th>EnRes diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>31.1</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose</td>
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<td>11.4</td>
</tr>
<tr>
<td>Dextrin</td>
<td>49.9</td>
<td>38.1</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.0</td>
<td>7.8</td>
</tr>
<tr>
<td>AIN mineral mix</td>
<td>3.5</td>
<td>5.4</td>
</tr>
<tr>
<td>AIN vitamin mix</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Grams fed per grams consumed by control animals</td>
<td>1.0</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Legend:

*Ingredients were obtained from Harlan-Teklad or Sigma Chemical Co. Grams of each component per 100 g of diet are indicated. AIN, American Institute of Nutrition.*

Results

Effects of estrogen treatment on food consumption and growth

Untreated female ACI rats fed the control diet consumed an average of 10.3 g of diet/day throughout the course of the experiment (Figure 1A). Food consumption was increased by 4%, to 11.2 g/day (P < 0.05), in rats treated with E2. When fed the control diet, both untreated and E2-treated rats grew rapidly until ~84 days of age (Figure 1B). Thereafter, both the
Dietary energy restriction inhibits estrogen-induced mammary tumorigenesis

treatment and diet on food consumption and growth. Female ACI rats were obtained at 42 days of age, allowed unlimited access to the control diet for 7 days, and then assigned at random to either the control diet or the energy-restricted diet. Food consumption by animals fed the control diet was monitored weekly. A 40% restriction of energy consumption was imposed by feeding 0.64 g of the energy-restricted diet per g consumed by rats fed the control diet. Treatment with E2 was initiated when the animals were 59 days of age. (A) Each data point represents the average amount of diet consumed per rat per day ± SD. Symbols: filled circle, control diet, untreated; filled square, control diet, E2 treated; filled triangle, 40% energy restricted, untreated; filled inverted triangle, 40% energy restricted, E2 treated.

untreated and E2-treated rats continued to grow at a reduced rate until ~150 days of age, when growth in the E2-treated rats virtually ceased and the average body weight in the untreated population began to diverge from that of the treated population. At 231 days of age, the untreated animals significantly more (P < 0.05) than the E2-treated animals. Together, the data indicate that E2 exerts an inhibitory effect on growth that is independent of food consumption. A 40% restriction of dietary energy consumption markedly inhibited growth. Untreated animals fed the energy-restricted diet grew at a slow, but steady, rate over the course of the experiment. At completion of the study, the energy-restricted, E2-treated, animals weighed 38% (P < 0.05) less than the E2-treated rats fed the control diet.

Dietary energy restriction markedly inhibits estrogen-induced mammary tumorigenesis

Administration of E2 to female ACI rats fed the control diet resulted in the rapid development of mammary tumors (Figure 2A). In this population, the first palpable mammary tumor was detected 69 days following the initiation of E2 treatment, and 100% of the treated population exhibited one or more palpable mammary tumors within 216 days of treatment. The median and mean latencies to the appearance of the first palpable mammary tumor in E2-treated rats fed the control diet were 118 and 126 days, respectively. A 40% restriction of dietary energy consumption markedly inhibited development of E2-induced mammary tumors. In the energy-restricted E2-treated population, the first palpable mammary tumor was observed.
following 104 days of treatment, and 59% (10/17) of the population at risk had tumors by 207 days. The median and mean latencies in the energy-restricted rats bearing E2-induced mammary tumors were 150 and 154 days, respectively. Both median latency and final mammary tumor incidence differed significantly between the groups of E2-treated animals fed the control diet versus the energy-restricted diet \( (P < 0.001) \). Five of 21 rats in the energy-restricted, E2-treated group exhibited morbidity, apparently due to an E2-induced pituitary tumor, and were killed following 176, 188 (two rats), 195 and 201 days of E2 treatment. Four of these animals were free of palpable mammary tumors at the time of death. Untreated, ovary-intact, ACI rats fed either the control or energy-restricted diet did not develop mammary tumors over the course of this experiment (Figure 2A).

In addition to reducing mammary tumor incidence and increasing latency, consumption of an energy-restricted diet significantly reduced mammary tumor burden. A total of 145 mammary tumors were induced in the population of 21 ACI rats fed the control diet and treated with E2 for 177 ± 26 days, a yield of 6.9 tumors/rat at the time of death (Figure 2B). In contrast, only 18 mammary tumors were induced in the 21 ACI rats fed the energy-restricted diet and treated with E2 for 193 ± 19 days, a yield of 0.9 tumors/rat. The total volume of mammary tumor tissue, defined as the sum of the volumes of all the mammary tumors observed per rat at necropsy, averaged 5584 mm\(^3\) in the population of E2-treated rats fed the control diet, compared with 458 mm\(^3\) in the population of E2-treated rats fed the energy-restricted diet (data not shown). The differences in tumor number and volume observed in the E2-treated animals fed the control or energy-restricted diets were both statistically significant \( (P < 0.01) \).

Dietary energy restriction inhibits estrogen-induced cell proliferation in normal, but not neoplastic, mammary tissues

Cell proliferation within the mammary epithelium was examined following 84 days of E2 treatment, a time preceding the appearance of the vast majority of induced mammary tumors (Figure 3A), and following 180–210 days of E2 treatment, a range of time points when animals were being killed due to the presence of mammary tumors (Figure 3B). A marked stimulatory effect of E2 on mammary epithelial cell proliferation in rats fed the control diet was evident at both time points; the fraction of cells staining positive for BrdU was increased from ~0.5% in untreated rats to 3.5–4.0% in E2-treated rats. The ability of E2 to induce mammary cell proliferation was partially, but significantly \( (P < 0.01) \), attenuated in animals fed the energy-restricted diet. In these animals, the fraction of cells staining positive for BrdU was ~2% at both time points. Dietary energy restriction did not consistently affect mammary cell proliferation in untreated rats. Although dietary energy restriction attenuated the ability of E2 to stimulate cell proliferation within the hyperplastic mammary epithelium, cell proliferation in E2-induced mammary tumors was not inhibited by energy restriction. Approximately 7% of cells in E2-induced mammary tumors incorporated BrdU regardless of whether the rats consumed the control or the energy-restricted diet (Figure 3C). This level of cell proliferation was significantly greater \( (P < 0.01) \) than in the surrounding hyperplastic mammary epithelium.

Effects of dietary energy restriction and estrogen on mammary gland morphogenesis and expression of PR

Examination of mammary gland whole mounts revealed that the glands of untreated female ACI rats fed the control diet were comprised of ducts exhibiting higher order branching as well as numerous lateral and alveolar buds (Figure 4A). In contrast, the mammary glands of rats fed the energy-restricted diet exhibited a lesser degree of ductal branching and bud development (Figure 4B). Representative ducts for untreated rats fed the control and the energy-restricted diet are illustrated (Figure 4C and D). The interlobular stroma of rats fed the control diet (Figure 4C) was comprised of adipocytes that were generally more uniform in size and larger than were the stromal adipocytes from animals fed the energy-restricted diet.
Fig. 4. Effects of E2 and dietary energy restriction on mammary gland development. Mammary tissues were evaluated by microscopic examination of mammary gland whole mounts (A, B, E, F) and thin sections stained with hematoxylin and eosin (C, D, G, H). Relative to mammary glands from untreated female ACI rats fed the control diet (A), glands from untreated rats fed the energy-restricted diet (B) exhibited fewer branches and buds. Interlobular adipocytes were larger and more uniform in size in female rats fed the control diet (C), when compared with the adipocytes of untreated, energy-restricted rats (D). ACI rats fed either the control (E and G) or energy-restricted (F and H) diet and treated continuously with E2 for 84 days exhibited marked lobuloalveolar hyperplasia. Stromal adipocytes were larger in E2-treated rats fed the control diet (G) relative to rats fed the energy-restricted diet (H).

Fig. 5. Histologic features of focal regions of atypical hyperplasia, intraductal carcinoma and invasive carcinoma induced in female ACI rats by continuous treatment with E2. Focal regions of atypical epithelial hyperplasia were observed in female ACI rats fed either the control (A) or the energy-restricted diet (B) and treated with E2 for 84 days. Many of the atypical hyperplastic lesions from rats fed either the control (C) or the energy-restricted (D) diets and treated with E2 beyond the 84 day time point, exhibited dilated lumens. Although intraductal carcinomas of the comedo type comprised the majority of the E2-induced mammary tumors in rats fed either experimental diet (E, control diet; F, energy-restricted diet), invasive carcinomas exhibiting desmoplastic reaction (G, control diet; H energy-restricted diet) were also observed.

(Figure 4D). Examination of mammary gland whole mounts (Figure 4E and F) and stained sections (Figure 4G and H) revealed that treatment with E2 for 84 days induced marked lobuloalveolar hyperplasia in female ACI rats fed either the control or energy-restricted diet. Interlobular stromal adipocytes were generally larger in E2-treated rats fed the control diet relative to E2-treated rats fed the energy-restricted diet (Figure 4G and H). Similar results were observed in animals examined at later time points.

Focal regions of atypical epithelial hyperplasia were observed in the mammary glands of female ACI rats fed either the control or energy-restricted diet and treated with E2 for 84 days (Figure 5A–D). The number of these lesions increased as the duration of E2 treatment was extended beyond this time point (data not shown). These focal regions of atypical hyperplasia were characterized by an expanded acinus comprised of cells exhibiting slightly enlarged nuclei and dense cytoplasmic staining resulting from a decrease in vacuolation (Figure 5A and B). In addition, many of the atypical hyperplastic foci were further characterized by the presence of secretions within a dilated lumen (Figure 5C and D). Lesions of this latter type were common in the mammary glands of E2-treated rats fed either the control or the energy-restricted diet. Histologic examination revealed that 60–80% of the tumors that developed in E2-treated animals fed the control or the energy-restricted diet were carcinomas of the comedo type (Figure 5E and F), whereas 20% were cribriform carcinomas. Papillary carcinomas comprised 20% of the tumors induced in animals fed the control diet, but were not observed among the 18 tumors induced in animals fed the energy-restricted diet. Carcinomas exhibiting invasive features were observed in animals fed either the control or the energy-restricted diet (Figure 5G and H).

Intense PR immunoreactivity was observed within the nuclei of a subset of ductal epithelial cells from untreated female
Effects of E2 and dietary energy restriction on expression of PR in normal, hyperplastic and neoplastic mammary tissues. Cells expressing PR were identified by immunohistochemistry. (A, C, E, G and I) PR expression in rats fed the control diet and (B, D, F, H and J) PR expression in rats fed the energy-restricted diet. PR was expressed by a subset of mammary epithelial cells from untreated ACI rats (age matched to E2-treated animals illustrated in C–J), and immunostaining appeared more robust in tissues from untreated rats fed the control diet (A), relative to untreated rats fed the energy-restricted diet (B). PR was also expressed by a subset of epithelial cells from the hyperplastic mammary tissues of E2-treated rats (C, D), and this expression was not affected by diet. The great majority of cells within the focal regions of atypical hyperplasia (E–H) and intraductal carcinomas (I and J) induced by E2 exhibited increased PR expression, relative to the surrounding epithelium. Dietary energy restriction did not detectably affect PR expression in the atypical hyperplasias or carcinomas.

ACI rats fed the control diet (Figure 6A), whereas the number of cells staining positive for PR appeared to be reduced in the mammary glands of untreated animals fed the energy-restricted diet (Figure 6B). PR expression was also exhibited by a subset of cells in the hyperplastic mammary tissues of E2-treated rats with energy-restricted diet (Figure 6D). Virtually all of the epithelial cells within the focal regions of atypical hyperplasia induced by E2 exhibited strong immunoreactivity to PR, regardless of whether these lesions exhibited secretions (Figure 6E and G), and dietary energy restriction did not affect PR expression in these atypical lesions (Figure 6F and H). Similarly, dietary energy restriction did not affect PR expression in E2-induced mammary carcinomas, in which nearly all of the cells exhibited strong immunoreactivity to PR (Figure 6I and J). The data strongly suggest that the inhibitory actions of dietary energy restriction on E2-induced mammary tumorigenesis are independent of PR expression.

Dietary energy restriction does not inhibit estrogen-induced pituitary tumorigenesis and associated hyperprolactinemia

In female ACI rats fed the control diet, the average pituitary weight was 6.7-fold increased, from 10.4 ± 0.2 mg (mean ± SEM) in untreated rats to 69.6 ± 0.5 mg in rats treated with E2 for 177 ± 26 (SD) days (Figure 7A). Pituitary weights of rats fed the energy-restricted diet and treated with E2 for 193 ± 19 days averaged 97.2 ± 6.6 mg, an increase of 13.5-fold over the 7.2 ± 0.1 mg observed in age-matched untreated rats fed the restricted diet. Treatment with E2 increased circulating PRL 190-fold, from 17 ± 6 ng/ml in untreated rats, to 3224 ± 70 ng/ml in female ACI rats fed the control diet for 177 ± 26 days (Figure 7B). Each data point represents the mean serum PRL level (± SEM, n = 21). The horizontal axis (A and B) represents the average duration of E2 treatment (± SD). Symbols: filled circle, control diet, untreated; filled square, control diet, E2 treated; filled triangle, energy restricted diet, untreated; filled inverted triangle, energy restricted diet, E2 treated. Numerals: 1, indicates a statistically significant difference (P < 0.05) between untreated and E2-treated animals fed the same diet; 2, indicates a statistically significant difference (P < 0.05) between similarly treated animals fed the different diets.
E2 levels did not differ significantly when examined following 84 days of treatment. Circulating mammary tumorigenesis was also assessed in animals from the tumor study killed following varying lengths of E2 treatment. Prolactinemia in ovary-intact ACI rats. In addition, these data clearly indicate that dietary energy restriction does not inhibit the ability of administered E2 to induce development of PRL-producing pituitary tumors and associated hyper-prolactinemia in ovary-intact ACI rats. Therefore, we conclude that dietary energy restriction exerts its marked inhibitory effect on E2-induced mammary tumorigenesis in a unique and physiologically relevant animal model.

In the present study, focal regions of atypical hyperplasia, which may be precursors to carcinomas, were common in the mammary glands of E2-treated ACI rats, regardless of whether the animals consumed the control or the energy-restricted diet. Therefore, we conclude that dietary energy restriction exerts its marked inhibitory effect on E2-induced mammary tumorigenesis in a unique and physiologically relevant animal model.

Data presented herein indicate that a 40% restriction of dietary energy consumption markedly inhibits mammary tumorigenesis in female ACI rats treated continuously with E2. Although dietary energy restriction has been demonstrated to inhibit mammary tumorigenesis in several other rodent models (4–8), this is the first demonstration that dietary energy restriction inhibits tumorigenesis in an animal model in which mammary cancers are induced solely by the administration of an estrogen, either naturally occurring or synthetic, or another hormonal agent. This study provides novel insights into the interactions between dietary and endocrine factors in the etiology of mammary cancer in a unique and physiologically relevant animal model.

Analysis of circulating estradiol and progesterone

When examined following 84 days of treatment, circulating E2 levels did not differ significantly between treated rats fed the control or the energy-restricted diet (Figure 8A). Circulating E2 levels in animals killed following varying lengths of E2 treatment ranging from 103 to 216 days in rats fed the control diet and from 158 to 216 days in rats fed the energy-restricted diet averaged 120 ± 13 and 238 ± 30 pg/ml, respectively. The observed levels of circulating E2 approximate those observed in the rat during pregnancy (36). Circulating E2 levels in untreated rats were not measured. During the rat estrous cycle, circulating E2 levels oscillate between ~20 and 75 pg/ml.

Treatment with E2 for 84 days resulted in an ~2-fold increase in the level of circulating progesterone, in ACI rats fed either the control or energy-restricted diet (Figure 8B). Interestingly, dietary energy restriction reduced circulating progesterone by ~50% in both untreated and E2-treated ACI rats, when examined at the 84 day time point. Circulating progesterone was also assayed in animals from the tumor study killed following varying lengths of E2 treatment. Progesterone levels in untreated and E2-treated rats fed the control diet averaged 13 ± 2 and 21 ± 3 ng/ml, respectively, whereas levels in untreated and E2-treated rats fed the energy-restricted diets averaged 9 ± 1 and 11 ± 2 ng/ml, respectively. Each of these observed levels of circulating progesterone is within the physiologic range. The data indicate that dietary energy restriction reduces circulating progesterone in both untreated and E2-treated ACI rats.

Discussion

Data presented herein indicate that a 40% restriction of dietary energy consumption markedly inhibits mammary tumorigenesis in female ACI rats treated continuously with E2. Although dietary energy restriction has been demonstrated to inhibit mammary tumorigenesis in several other rodent models (4–8), this is the first demonstration that dietary energy restriction inhibits tumorigenesis in an animal model in which mammary cancers are induced solely by the administration of an estrogen, either naturally occurring or synthetic, or another hormonal agent. This study provides novel insights into the interactions between dietary and endocrine factors in the etiology of mammary cancer in a unique and physiologically relevant animal model.

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It has often been hypothesized that dietary energy restriction exerts marked inhibitory effects on tumorigenesis in different cancer models by inhibiting cell proliferation (7,9,11). Lok et al. (10) demonstrated a correlation between the inhibitory effects of differing degrees of dietary energy restriction (10–40%) on mammary tumorigenesis and mammary cell proliferation in female Swiss Webster mice. Sinha et al. (12) demonstrated that the marked inhibitory effect of a 50% restriction of food consumption on DMBA-induced mammary tumorigenesis in female Sprague–Dawley rats was associated with a significant reduction in the [3H]thymidine labeling index in the mammary gland. Thompson et al. (8,38,39) demonstrated that dietary energy restriction reduced cell proliferation and
increased apoptosis in putative precursor lesions induced by MNU in the mammary glands of female Sprague–Dawley rats, and correlated these effects of energy restriction with reduced expression in the mammary epithelium of cyclin D1, an activator of G1 to S cell-cycle progression, and increased expression of p27Kip1, an inhibitor of specific cyclin-dependent kinases. In the present study, a 40% restriction of dietary energy consumption significantly inhibited E2-induced mammary epithelial cell proliferation in the ACI rat, even in the presence of continuously elevated E2 and PRL. However, energy restriction did not block development of E2-induced lobuloalveolar hyperplasia or focal regions of atypical epithelial hyperplasia. Therefore, we consider it unlikely that this partial inhibition of E2-stimulated mammary cell proliferation directly contributed to the marked inhibitory effect of energy restriction on E2-induced mammary tumorigenesis observed in the present study. It is interesting to note that, in contrast to the observed inhibitory effect of dietary energy restriction on mammary cell proliferation in the E2-treated ACI rats, no inhibition of cell proliferation was observed within the E2-induced mammary tumors. The data differ from those of Thompson et al. (8,38) who observed a significant inhibitory effect of dietary energy restriction on cell proliferation within mammary adenocarcinomas induced by MNU in female Sprague–Dawley rats. These differing observations may result from genetic differences in the E2-induced and MNU-induced models or from endocrine related differences in the tumors and/or animals.

It has been suggested that one mechanism through which energy restriction may inhibit mammary tumorigenesis is by reducing output of ovarian estrogens and/or pituitary PRL (16–18). Sylvester et al. (40) demonstrated that administration of estradiol benzoate and/or haloperidol, a dopamine antagonist that stimulates PRL secretion, at least partially abrogates the inhibitory effect of a 50% restriction of food consumption on DMBA-induced mammary tumorigenesis in female Sprague–Dawley rats. Similarly, elevation of circulating PRL, produced by pituitary isografts, eliminates the inhibitory effect of a 30% restriction of energy consumption on mammary tumorigenesis in female C3H mice (15). A novel observation from the present study is that dietary energy restriction is capable of dramatically inhibiting mammary tumorigenesis in the ACI rat even when circulating E2 and PRL are maintained at high levels as a consequence of administration of exogenous E2.

We have demonstrated that ovarietomy markedly inhibits development of E2-induced mammary cancers in ACI rats and have interpreted this observation to suggest that rapid development of mammary cancers in E2-treated ACI rats requires the actions of one or more ovarian factors, in addition to E2 (25). Progesterone is one ovarian hormone that may be required for the rapid development of E2-induced mammary cancers in this animal model. Epidemiologic studies suggest a role for progesterone in the etiology of breast cancer in humans (20,41–44). As discussed above, both the focal regions of atypical epithelial hyperplasia and mammary cancers induced in ACI rats by E2 exhibit increased expression of PR, relative to the surrounding epithelium (26). In the present study, PR expression was not detectably affected by dietary energy restriction. However, energy restriction did significantly reduce the level of circulating progesterone in the E2-treated rats. At this time it is not known to what extent, if any, the observed reduction in circulating progesterone contributed to the inhibition of E2-induced mammary tumorigenesis by dietary energy restriction.

In summary, we have demonstrated that dietary energy restriction significantly inhibits E2-induced mammary tumorigenesis in the ACI rat as evidenced by reduced mammary cancer incidence and burden and increased latency to the appearance of the first palpable mammary cancer. This inhibition was tissue specific, associated with reductions in circulating progesterone and may result from an attenuation or retardation of the progression of atypical hyperplasia to carcinoma. Because of the well-documented role of E2 in the etiology of human breast cancers, we believe that this animal model provides a valuable tool for the study of interactions between dietary, hormonal and genetic factors in mammary tumorigenesis.

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