Diet-Gene Interactions in Estrogen-Induced Mammary Carcinogenesis in the ACI Rat

Djuana M. E. Harvell,*† Tracy E. Strecker,* ** Benjamin Xie,* Linda K. Buckles,* ** Martin Tochacek,* ** Rodney D. McComb† and James D. Shull*†**

Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE.

ABSTRACT It is well accepted that hormonal, dietary and genetic factors each influence breast cancer risk. However, the underlying mechanisms and the extent to which these factors interact are largely unknown. We have demonstrated that the female ACI rat exhibits a unique genetically conferred propensity to develop mammary cancers when treated with physiological levels of 17β-estradiol (E2). More recently, we have mapped to rat chromosome 5 a strong genetic modifier of susceptibility to E2-induced mammary cancers, termed estrogen-induced mammary cancer 1 (Emca1), and have identified potential Emca1 candidate genes. Because estrogens have been inextricably linked to the genesis of breast cancer in humans, the ACI rat model has the potential to reveal novel physiologically relevant insights into how the contributory actions of E2 are modified by specific dietary factors. In the present study, we have examined the ability of a 40% restriction of dietary energy consumption to inhibit E2-induced mammary carcinogenesis. The hypothesis tested was that energy restriction will inhibit mammary carcinogenesis even when circulating E2 remains elevated through administration of exogenous hormone. The data presented herein strongly suggest that energy restriction inhibits E2-induced mammary carcinogenesis in the ACI rat at least partly by retarding progression of atypical hyperplastic foci to carcinoma. J. Nutr. 131: 3087S–3091S, 2001.

KEY WORDS: • ACI rat • estrogen • energy restriction • mammary cancer • pituitary

Numerous studies implicate ovarian hormones as critical factors in the etiology of breast cancer (1–3). For example, oophorectomy before menopause was shown to significantly reduce the risk of breast cancer. Moreover, prophylactic treatment with the antiestrogen tamoxifen was shown in a recent clinical trial to reduce by approximately 50% the incidence of breast cancer in a population of women at high risk for this disease (4). Estrogens have been hypothesized to contribute to breast cancer etiology by increasing the rate of mammary cell proliferation, thereby promoting the accumulation of somatic mutations (5). The exact mechanism through which estrogens contribute to the development of breast cancer is not known.

Diet is another important determinant of breast cancer risk in human populations. Numerous prospective and case-control studies associate height, body mass index or both with breast cancer risk and provide indirect evidence that energy consumption and balance influence breast cancer development (6,7). Supporting these epidemiological data are numerous studies demonstrating that dietary energy restriction without malnutrition markedly inhibits mammary carcinogenesis in both rat and mouse models (8–12). Mechanisms postulated to explain how dietary energy restriction may inhibit mammary carcinogenesis include inhibition of mammary epithelial cell proliferation and reduction of circulating estrogen and prolactin, two hormones known to regulate mammary gland growth, differentiation and function (12–18).

The female ACI rat provides a physiologically relevant and genetically defined animal model for studying diet-hormone interactions in mammary cancer development. Data from our laboratory demonstrate that continuous treatment with the naturally occurring estrogen, 17β-estradiol (E2), rapidly induces mammary cancers in ovary-intact ACI rats whereas mammary cancers rarely develop in the absence of exogenous estrogen in this strain (19; D.M.E. Harvell, T. E. Strecker, B. Xie, K. L. Pennington, R. D. McComb and J. D. Shull, University of Nebraska Medical Center, unpublished data). The ACI rat provides a unique opportunity to study the influence of dietary energy restriction on mammary cancer development in a rodent model that exhibits a high degree of susceptibility to E2-induced mammary carcinogenesis in the absence of obesity.

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2 To whom correspondence should be addressed. E-mail: jshull@unmc.edu

3 **Abbreviations used: ACI, AxC-Irish; E2, 17β-estradiol.
Dietary energy restriction inhibits estrogen-induced mammary carcinogenesis. Administration of E2 rapidly induced mammary cancers in ACI rats fed the control diet. In this population the first palpable mammary tumor was detected 69 d after the initiation of E2 treatment, and 100% of the animals exhibited one or more palpable mammary tumors within 216 d of E2 administration (Table 1). In contrast,  

**TABLE 1**  
*The effect of dietary energy restriction on E2-induced mammary carcinogenesis in the ACI rat*  

<table>
<thead>
<tr>
<th>Tumor incidence</th>
<th>Control group</th>
<th>Energy-restricted group</th>
</tr>
</thead>
<tbody>
<tr>
<td>At risk</td>
<td>100% (21/21)</td>
<td>59% (10/17)*</td>
</tr>
<tr>
<td>Total</td>
<td>100% (21/21)</td>
<td>48% (10/21)*</td>
</tr>
<tr>
<td>Appearance of first tumor (days E2)</td>
<td>69</td>
<td>104</td>
</tr>
<tr>
<td>Median</td>
<td>118</td>
<td>150*</td>
</tr>
<tr>
<td>Mean3</td>
<td>126 ± 29 (n = 21)</td>
<td>154 ± 35 (n = 10)</td>
</tr>
<tr>
<td>Average tumor, n/rat</td>
<td>6.9</td>
<td>0.9*</td>
</tr>
<tr>
<td>Total tumor yield</td>
<td>145</td>
<td>18*</td>
</tr>
<tr>
<td>Mean3 tumor volume, mm³</td>
<td>5,584 ± 772</td>
<td>458 ± 227*</td>
</tr>
<tr>
<td>Mean3 length E2 treatment, d</td>
<td>177 ± 26 (103–216)⁵</td>
<td>193 ± 19 (158–216)⁵</td>
</tr>
</tbody>
</table>

1 Where not indicated, values were assessed among animals in the total population.
2 Four animals exhibited morbidity, apparently because of pituitary tumors. These animals were killed 176, 188, 195 and 210 d after the initiation of E2 treatment and were tumor free at necropsy.
3 Mean ± standard deviation (SD).
4 Mean ± standard error of the mean (SEM).
5 Range given in parentheses.
* Significantly different from E2 treated ACI rats fed the control diet, P < 0.05.

**METHODS**  
The source of experimental animals, reagents and supplies as well as the methods relating to care, feeding and hormonal treatment of animals have been described previously (19,24–28). Ovary-intact ACI rats were obtained at age 42 d and individually housed. One week later, the animals were randomly assigned to experimental groups fed either a control or energy-restricted diet, defined as 40% reduction in total consumption of energy derived from fat and carbohydrates without reduction in consumption of essential macronutrients. At age 59 d, treatment with the naturally occurring estrogen, E2, was administered via silastic tubing implants. Thereafter, the rats were examined twice weekly for the presence of palpable mammary tumors and were killed when the largest mammary tumor reached 1.5–2.0 cm in diameter or if signs of morbidity were observed. At necropsy, the location and size of each tumor were recorded. The diameter for each tumor was determined in two perpendicular dimensions, and the tumor volume was calculated using the formula \( V = \frac{4}{3} \pi r^3 \), where \( r \) is half the average diameter. Mammary cell proliferation was assayed by BrdU immunohistochemistry as described previously (20). The levels of E2 (19), prolactin (20) and progesterone (29) in serum from trunk blood were measured by radioimmunoassay as described previously. Statistical significance was assessed using Student’s t test and two-way ANOVA with Newman-Keul’s post hoc test. Mammary tumor latency and incidence were analyzed by the log rank test for comparison of cumulative incidence curves and the Fischer’s exact test, respectively. Values of \( P < 0.05 \) were considered statistically significant.
among animals fed the energy-restricted diet and treated with E2 the first palpable mammary tumor was observed following 104 d of treatment, and 59% (10/17) of the population at risk had tumors by 207 d. Differences in both median latency and final mammary tumor incidence between the groups of E2 treated animals fed the control diet versus the energy-restricted diet (P < 0.01) were statistically significant. Differences in tumor number and volume observed in the E2-treated animals fed the control or energy-restricted diets were also statistically significant (P < 0.01). Five of 21 rats in the energy restricted, E2-treated group exhibited morbidity, apparently because of an E2-induced pituitary tumor, and were killed after 176, 188 (2 rats), 195 and 201 d of E2 treatment. Four of these animals were free of palpable mammary tumors at the time of death. Circulating E2 levels were equivalent in the E2-treated animals fed either diet. These data indicate that dietary energy restriction can reduce mammary tumor incidence, multiplicity and tumor size as well as increase the latency to the appearance of the first palpable mammary tumor in female ACI rats after continuous E2 treatment. Untreated ovary-intact ACI rats fed either the control or energy-restricted diet did not develop mammary tumors over the course of this experiment.

**Dietary energy restriction inhibits mammary cell proliferation in normal but not in neoplastic mammary tissue.** To address the mechanism through which dietary energy restriction may inhibit E2-induced mammary carcinogenesis in the female ACI rat, we examined the effect of dietary energy restriction on mammary cell proliferation. Cell proliferation within the mammary epithelium was examined after 84 d of E2 treatment, a time preceding the appearance of most mammary tumors, and after 180–210 d of E2 treatment, a range of times when animals were being killed because of the presence of mammary tumors. In ACI rats fed the control diet, a marked stimulatory effect of E2 on mammary epithelial cell proliferation was evident at both the early and later times; the fraction of cells staining positive for BrdU was increased from approximately 0.5% in untreated rats to 3.5–4.0% in E2-treated rats. In the rats fed the energy-restricted diet, the ability of E2 to induce mammary cell proliferation was partially but significantly (P < 0.01) attenuated; the fraction of cells staining positive for BrdU was approximately 2% at both times.

We also examined the effects of dietary energy restriction on cell proliferation in E2-induced mammary tumors of the ACI rat. Approximately 7% of cells in E2-induced mammary tumors incorporated BrdU regardless of whether the rats consumed the control or the energy-restricted diet. This level of cell proliferation was significantly greater than in the surrounding hyperplastic mammary epithelium. Therefore, the inhibitory effect of dietary energy restriction on E2-induced mammary cell proliferation is restricted to normal mammary epithelium.

**Dietary energy restriction appears to retard progression of atypical hyperplasia to carcinoma.** We previously demonstrated the presence of focal regions of atypical epithelial hyperplasia in the mammary tissue of ACI rats treated with E2 for as few as 12 wk (20). These atypical hyperplastic foci were characterized by an expanded acinus composed of cells exhibiting slightly enlarged nuclei and dense cytoplasmic staining. Interestingly, the focal regions of both the atypical epithelial hyperplasia and the mammary cancers induced in ACI rats by E2 exhibit increased expression of progesterone receptor relative to the surrounding epithelium, suggesting a link between these lesions (20). It was, therefore, of interest to determine whether consumption of an energy-restricted diet would inhibit the development of atypical hyperplastic foci or modulate progesterone receptor expression in ACI rats after E2 treatment. 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 12 wk. The number of these lesions increased as the treatment was extended beyond this time, and lesions were at least as common in the mammary glands of treated rats fed the energy-restricted diet as in treated rats fed the control diet. Histological examination revealed that the majority of the tumors that developed in the E2-treated animals were carcinomas of the comedo type regardless of whether the animals were fed the control or the energy-restricted diet. Carcinomas exhibiting invasive features were also observed in E2-treated animals regardless of the diet fed. These data suggest that dietary energy restriction exerts its marked inhibitory effect on E2-induced mammary carcinogenesis at a stage subsequent to the development of atypical mammary epithelial hyperplasia (Fig. 1).

**FIGURE 1** Model of the inhibitory actions of dietary energy restriction on E2-induced mammary carcinogenesis. A–D: the progression of mammary carcinogenesis in ACI rats fed a control diet and treated with estrogen. Consumption of an energy-restricted diet appears to exert its inhibitory effects on E2-induced mammary carcinogenesis at a stage subsequent to the development of atypical mammary epithelial hyperplasia and may act by inhibiting the progression of this lesion to carcinoma.
The data described herein demonstrate that a 40% restriction of dietary energy consumption can inhibit mammary carcinogenesis in ovary-intact ACI rats treated continuously with E2. Although dietary energy restriction has been demonstrated to inhibit mammary carcinogenesis in several rat and mouse models (8–12), our findings are novel in that they demonstrate the ability of energy restriction to inhibit mammary carcinogenesis in an animal model where mammary cancer is induced solely through the actions of a naturally occurring hormone.

We do not know the mechanism through which dietary energy restriction inhibits E2-induced mammary cancer development. The inhibitory actions of energy restriction were associated with a reduction in E2-stimulated mammary cell proliferation. However, this inhibition was insufficient to block induction of lobuloalveolar hyperplasia or focal regions of atypical hyperplasia. These data suggest that dietary energy restriction inhibits E2-induced mammary cancers by attenuating the progression of atypical hyperplasia to carcinoma (Harvell et al. unpublished observations, 2001). This inhibition did not appear to result from a reduction in progesterone receptor expression, either in the atypical hyperplasia or carcinomas, but may result in part from reductions in circulating progesterone levels. Dietary energy restriction did not inhibit the ability of administered E2 to induce prolactin-producing pituitary tumors and associated hyperprolactinemia, indicating that the inhibitory effect of dietary energy restriction on mammary carcinogenesis is tissue specific and independent of circulating prolactin. This absence of an inhibitory effect of energy restriction on E2-induced pituitary tumorigenesis in ovary-intact ACI rats confirms and extends our published observation that dietary energy restriction on E2-induced pituitary tumorigenesis in ovariec- tomized ACI rats (27). In contrast, we (24–26,28) have demonstrated a marked inhibitory effect of dietary energy restriction on estrogen-induced pituitary tumorigenesis in the F344 rat strain. Together, these data indicate that the inhibitory actions of energy restriction on estrogen-induced pituitary tumorigenesis are strain specific and dependent on genetic background.

Because exogenous E2 is the sole inducing agent in this model, our study clearly demonstrates that the inhibitory effect of energy restriction on mammary carcinogenesis is downstream of potential effects of energy restriction on output of estrogens by the ovaries. However, inhibition of ovarian progesterone output by dietary energy restriction may contribute to the reduction in mammary cancers observed in the energy-restricted rats. Moreover, because the administered E2 maintains production of pituitary prolactin at a high level, we conclude that the ability of dietary energy restriction to inhibit mammary carcinogenesis is independent of any potential effect on pituitary prolactin output.

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