Dehydroepiandrosterone intake protects against 7,12-dimethylbenz(a)anthracene-induced mammary tumor development in the obese Zucker rat model

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Abstract. Obesity has been epidemic in the US for over two decades; almost 65% of adults in the US are overweight. Obesity has been linked with the risk of development of various cancers, including breast cancer. Dehydroepiandrosterone (DHEA) is an over-the-counter dietary supplement used as an immunomodulating, anti-depressant, anti-aging, anti-cardiovascular disease, and anti-cancer agent and anti-obesity supplement. The objectives of this study were to investigate the long-term effects of obesity and DHEA treatment on body weight gain and on 7,12-dimethylbenz(a)-anthracene (DMBA)-induced mammary tumor development. Forty-three six-week-old obese female Zucker rats were used. Rats were randomly assigned and had *ad libitum* access to water and a diet of either chow (2016) as a control diet or chow with the addition of DHEA at a concentration of 6 g/kg of chow as a DHEA diet. All rats were orally gavaged at age 50 days with 65 mg DMBA/kg body weight. Rats were weighed and palpated twice weekly for detection of mammary tumors and sacrificed 155 days post-DMBA treatment. Obese rats fed the DHEA diet gained significantly less weight than obese control diet rats (P<0.001). At the end of the experiment, 55% of the control diet group developed mammary tumors, while no tumors were detected in the DHEA diet group (P<0.001). Our results suggest that DHEA treatment can reduce body weight gain and protects against DMBA-induced mammary tumor development in the obese Zucker rat model.

Introduction

Breast cancer is the most common malignant tumor among women, and of all cancers, it is the second leading cause of cancer-related mortality in women in the US. In 2009, an estimated 192,370 women will be diagnosed with invasive breast cancer, and 40,170 women will eventually die from this disease (1).

Obesity has been an epidemic in the US for more than two decades, and the proportion of overweight and obese adults in the population continues to grow. Results from the 2005-2006 National Health and Nutrition Examination Survey (NHANES), using measured heights and weights, indicate that an estimated 32.7% of US adults 20 years and older are overweight, 34.3% are obese, and 5.9% are extremely obese (BMI ≥40 kg/m^2) (2). Most countries are experiencing similar dramatic increases in obesity (3). Worldwide, more than one billion adults are overweight and over 300 million are obese. This trend has alarming health implications, as obesity is associated with serious health conditions, including type 2 diabetes, cardiovascular disease, and certain type of cancers, such as breast cancer (4).

A recent study investigated the role of overweight and obesity in carcinogenesis (5). This study not only documented an association between the BMI and mortality from several types of cancer, but also provided a reliable estimate of the contribution of overweight and obesity to the total mortality from cancer. They reported that women with the highest BMI of 40 kg/m^2 had death rates from all cancers combined that were 62% higher for women (with relative risk of death of 1.62) than the rates in women of normal weight. They reported a significant trend of increasing risk with higher BMI for death in women from cancers of the breast, uterus, cervix, and ovary (5).

Since the Dietary Supplement and Health Education Act (DSHEA) was passed in 1994, sales of dietary supplements have increased nearly 2-fold, from $8.8 billion in 1994 to $14.7 billion in 1999 (6). From 2000 to 2006, over $21 billion

is spent annually on dietary supplements, with sales increasing consistently by approximately 4% each year (7). Dehydro-epiandrosterone (DHEA), a naturally occurring substance that is changed in the body to the hormones estrogen and testosterone, is a widely available over-the-counter dietary supplement used for weight loss. DHEA is also reported to have immunomodulating, anti-depressant, anti-aging, anti-cardiovascular disease, and anti-cancer effects, but considerably more evidence is required to substantiate these claims (8).

Zucker rats (fa/fa) are the best known, most widely used rat model for early onset obesity. Obesity in the Zucker rat is inherited as an autosomal recessive trait caused by a mutation in the leptin receptor gene (fa) (9,10). Animals homozygous for the fa allele become noticeably obese by 3 to 5 weeks of age, and by 14 weeks of age their body composition is more than 40% lipid (11). The most valuable contribution of the Zucker rat has been its utility as a model of human early-onset, hyperplastic-hypertrophic obesity. Many investigators have used this model to study the development, etiology, associated pathologies, possible treatment, and putative mechanisms of severe obesity (12).

We have shown previously that obesity increases the susceptibility to DMBA-induced mammary tumors (13). The main objective of this experiment was to investigate the effects of DHEA supplementation on DMBA-induced mammary tumor development using the obese Zucker rat model.

Materials and methods

Experimental design. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas for Medical Sciences. Female obese Zucker rats (fa/fa) were purchased at 6 weeks of age from Harlan Industries (Indianapolis, IN). Rats were housed two per cage in the animal facilities at the Arkansas Children's Hospital Research Institute. Rats were randomly assigned to a diet of either chow (2016, Harlan Teklad, Madison, WI) as a control diet or chow with the addition of DHEA (Sigma Chemical Co., St. Louis, MO) at a concentration of 6 g/kg of chow (0.6% by weight) as a DHEA diet. The 2016 diet contains minimal isoflavones and natural ingredients (corn, corn gluten meal, wheat, wheat middlings, soybean oil). Rats had *ad libitum* access to water and assigned diet. All rats were weighed twice weekly. At age 50 days, all rats received, via gavage, 65 mg DMBA/kg body weight (Sigma Chemical Co.) in sesame oil. Beginning 4 weeks after DMBA treatment, all rats were palpated twice weekly for mammary tumor detection. The detection date and location of each mammary tumor were recorded for each rat. Rats were sacrificed 155 days post-DMBA treatment. All mammary tumors were excised and counted. Rats with tumor masses exceeding 2.5 cm in diameter were euthanized according to an IACUC-approved animal protocol. Sections of all tumors were placed in 10% neutral buffered formalin for histopathologic analysis. Sections (4 μ) of the paraffin-embedded tumors were stained with hematoxylin and eosin for light microscopic evaluation.

Pathology. A board-certified anatomic pathologist (SK) evaluated tumors in a blinded protocol. Tumors were classified as benign (essentially unremarkable breast parenchyma); intraductal proliferation (IDP), characterized by benign
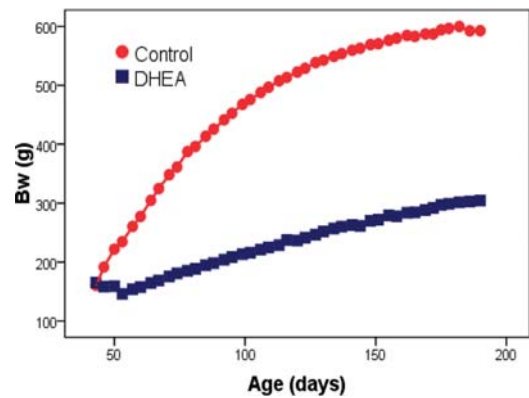

Figure 1. Body weights of control and DHEA-fed obese female Zucker rats.

Table I. Body weight (g) at different days of age during the experiment (mean ± SE).

Groups	Day 43	Day 99	Day 151	Day 195
Control	160±3	468±9	571±12	593±12
DHEA	165±3	213±5[a]	272±8[a]	304±8[a]

[a]P<0.001 (Mann-Whitney test).

epithelial proliferation or presence of ductal hyperplasia of the usual type, or multiple papillomas; ductal carcinoma *in situ* (DCIS); or invasive ductal carcinoma (IDC).

Statistical analysis. Mann-Whitney test was used to compare the body weight of the two groups. Kaplan-Meier analysis of tumor latency was performed. The log-rank test was used to compare median tumor-free times. Fisher's Exact test was used to compare percentage of rats with tumors and tumor histology in each group. Statistical significance was set at P<0.05. For the few rats that were sacrificed early due to tumor burden, we assumed that the number of tumors remained constant until the end of the study. Data analyses were generated and plots were constructed using SPSS© version 17.0 for Windows (SPSS Inc., Chicago, IL) and SAS version 9.2 (SAS Inc., Cary, NC).

Results

Body weights. All rats gained weight during the course of the experiment (Fig. 1). The average body weights (mean ± SE) at different ages during the experiment are shown in Table I. There were no differences in body weight of control and DHEA-fed rats at the beginning of the experiment (age 43 days), but as the experiment continued the DHEA-fed rats gained less body weight than control rats (P<0.001) (Fig. 1 and Table I). The DHEA diet group showed a significant reduction in weight gain compared to the control diet rats (P<0.001).

Time course for tumor formation, latency, and multiplicity. The time course of palpable mammary tumor detection is shown in Fig. 2, and data are presented in Table II. The tumor
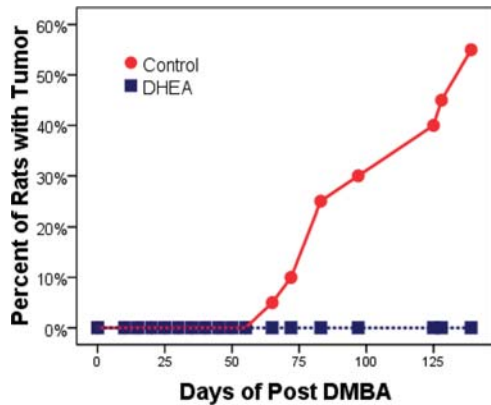
Figure 2. Mammary tumor incidence (percent of rats with tumors) of female rats (days post-DMBA treatment).

latency (the number of days post-DMBA treatment until the detection of the first mammary tumor) was shorter in control rats than the DHEA-fed group (P<0.001). The first mammary tumor detected in a control animal was 65 days post-DMBA treatment. In addition, 25% of the control rats had developed mammary tumors by 83 days post-DMBA treatment. By the end of the experiment, 55% of the control rats developed mammary tumors compared to no tumors detected in DHEA-fed rats (P<0.001). The median number of mammary tumors per tumor-bearing rat (multiplicity) was compared. In regards to multiplicity, the obese control rats had a range of one to five tumors per rat compared to zero tumors in DHEA-fed rats (Table II).

Mammary tumor characteristics. Mammary tumor histology data are presented in Table II. A total of 18 mammary tumors were detected in the study. Table II shows the light microscopic classification of these mammary tumors. All of the 18 tumors were in the control diet group; no tumors were detected in the DHEA diet group. Pathologic analyses revealed tumors of the following classifications: 1 (6%) benign, 2 (11%) IDP, 11 (61%) DCIS and 4 (22%) IDC. Fig. 3 presents photomicrographs of tumors from each classification for this study.

Discussion

The major objectives of this study were i) to investigate the effects of DHEA and obesity on body weight gain and ii) to compare the effects of long-term DHEA intake and obesity on DMBA-induced mammary tumor development. In the present study, we found that i) obese DHEA-fed rats gained significantly less weight than obese control rats (P<0.001) and ii) 55% of DMBA-treated obese control rats developed mammary tumors compared to none of the DHEA-fed rats.

Obesity is associated with higher mortality and morbidity such as diabetes, cardiovascular disease, and several types of cancer including breast cancer. Several epidemiological and animal studies have shown the link between higher body weight and risk of breast cancer development (13-21). In fact, it has been shown that obese breast cancer patients, regardless of their menopausal status, have higher mortality and
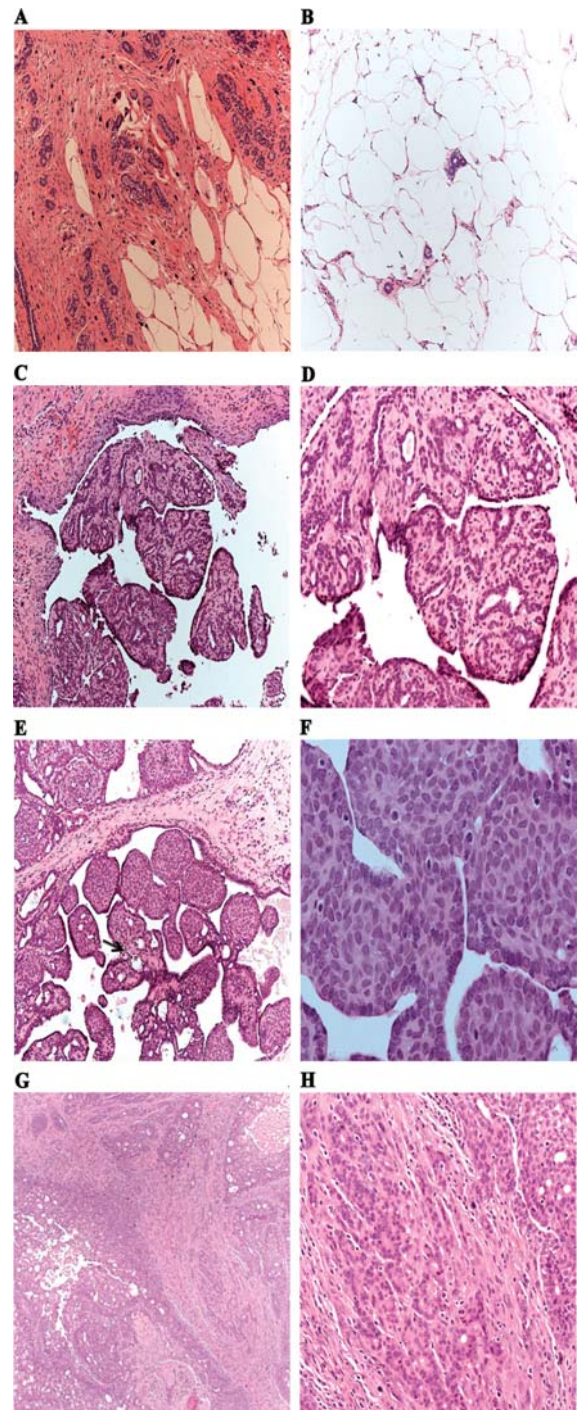

Figure 3. Mammary tumor histology. Normal breast tissue, original magnification, x10 (A and B): benign ductal and lobular structures were either surrounded by fibrous stroma or mature adipose tissue. No proliferation of epithelial cells noted in these images. Intraductal proliferation (IDP), papilloma, original magnification x10 (C); original magnification x20 (D). Ductal carcinoma *in situ* (DCIS), original magnification, x10 (E); original magnification, x40 (F): majority of ductal carcinomas showed micropapillary pattern. Arrow (E) shows an area within DCIS with numerous apoptotic bodies; note uniform proliferation of epithelial cells. Invasive ductal carcinoma (IDC), original magnification, x4 (G); original magnification, x40 (H): image shows infiltrating ductal carcinoma arising from a background of DCIS.

poorer outcome (5,19,22-27). Also, obese breast cancer patients have higher tumor grade and tumor burden (24,28). A recent study has evaluated the prognostic effects of obesity

Table II. Characteristics of DMBA-induced mammary tumors in control and DHEA-fed obese female Zucker rats.

	Control (n=20)	DHEA (n=23)
Tumor onset		
Day of first tumor[a]	65	No tumors present[b]
Day at 25% tumors[c]	83	No tumors present[b]
Tumor incidence		
% of rats with tumors[c]	55	0[b]
Multiplicity	1 (1-5)	0
Total tumors = 18[d]	18 (100%)	0[b]
Benign	1 (6%)	0
IDP	2 (11%)	0
DCIS	11 (61%)	0
IDC	4 (22%)	0

[a]Post-DMBA day at which the first mammary tumor was detectable by palpation. No tumors were detected in the DHEA-fed rats by the end of the experiment. [b]$P<0.001$ (Fisher's Exact test). [c]Post-DMBA day at which the probability of tumor development was 25%. No tumors were detected in the DHEA-fed rats by the end of the experiment. [d]Number and percentage of total mammary tumors graded as benign, IDP, DCIS, or IDC as described in Materials and methods.

and weight change after breast cancer diagnosis and found obesity and weight change after diagnosis are inversely associated with breast cancer prognosis. The authors suggested that weight control is important among women with breast cancer (29).

Recently, our laboratory has shown that obesity is a major risk for breast cancer development using an obese Zucker rat DMBA-induced mammary tumor model. In these experiments, obese rats developed more mammary tumors at earlier time points compared to their lean littermates (13). In agreement with the present data, other studies using the obese Zucker rat model showed that treatment with DHEA resulted in reduced body weight gain (30-35). In laboratory studies, higher body weight has been associated with increases in both spontaneous and chemically induced mammary tumors in various strains of mice (36,37).

The obesity pandemic is a very serious health problem, and obesity has been linked to breast cancer, especially in older women. Permanent weight reduction through diet and exercise is found to be difficult or impossible for most individuals (38). In regards to weight reduction and breast cancer development, a dietary supplement intervention that can help to reduce body weight and consequently reduce the risk of breast cancer development would be beneficial. Since DHEA is marketed as a supplement with purported health benefits, including weight loss, we used DHEA as a dietary supplement to investigate its effects on both body weight and its effects on breast cancer. The use of DHEA is controversial, with some reports indicating that DHEA may promote breast tumors in women (39,40), while other reports suggest that DHEA will protect against breast cancer (41). One of the major goals of this investigation was to determine whether dietary supplementation with DHEA in obese Zucker rats would affect the development of DMBA-induced mammary tumors. To our knowledge, this report is the first to demonstrate that DHEA

protects obese female Zucker rats against DMBA-induced mammary tumor development.

In the case of DHEA supplementation for anti-obesity, it is not known if DHEA decreases abdominal obesity in humans as has been shown in laboratory animal studies (33,42-44). The results of epidemiologic studies of the relationship between DHEA and abdominal fat have been conflicting. In some studies, levels of serum DHEA or a DHEA metabolite, DHEA sulfate (DHEA-S), were significantly inversely related to abdominal obesity (45,46), while in other studies in postmenopausal women, DHEA-S did not protect against obesity (47). In rats and mice, DHEA feeding reduces fat accumulation in both genetic- and diet-induced obesity models and has protective effects against insulin resistance induced by a high-fat diet (33,42). Studies have reported an association between low levels of serum or urine DHEA or DHEA metabolites and increased breast cancer risk in premenopausal women (48-53). The relationship between endogenous androgens, such as DHEA, and breast cancer risk is less clear with both epidemiological and experimental data providing conflicting results. High serum DHEA-S has been linked to increased breast cancer risk in postmenopausal women (54). In postmenopausal women, obesity is predominantly abdominal and is reported to be positively associated with higher serum DHEA concentration (45,46). In contrast, abdominal obesity has been associated with lower DHEA concentrations in premenopausal women (55). The reason for these differences is not clear, but several studies have shown abdominal obesity to be a marker for breast cancer risk in postmenopausal women (56).

Our present study has demonstrated that DHEA supplementation resulted in decreased weight gain in the obese rat model, as well as protection against DMBA-induced mammary tumors in DHEA-fed rats. A number of mechanisms may contribute to our observation that DHEA prevented mammary

tumors in DMBA-treated obese Zucker rats. In the absence of estrogens, DHEA has been shown to have stimulatory effects on the growth of mammary tumors, due to the interaction of DHEA or its metabolites with the estrogen receptor. However, in the presence of estrogens, such as in intact Sprague-Dawley (non-obese) rats, DHEA has been reported to inhibit mammary tumor growth (57). DHEA also has chemopreventive activity against carcinogens such as DMBA, as well as UV-induced DNA damage. In a Sprague-Dawley model of DMBA-induced mammary tumors, DHEA treatment decreased the incidence of tumors, increased tumor latency, and significantly decreased tumor volume (58,59).

DHEA has also been shown to inhibit the DMBA-mediated induction of hepatic carcinogen activating enzymes, including cytochrome P450 1A1 (CYP1A1), CYP1A2, and CYP1B1 (60). These authors concluded that DHEA regulated the *in vivo* expression levels of these enzymes, decreased basal expression of CYP1A1 and CYP1A2, and reduced the induction of their expression by DMBA. This reduction in DMBA-activating activity may be a major factor in the chemopreventive activity exhibited by DHEA in our obese rat DMBA-induced mammary tumor model.

In conclusion, we have demonstrated that DHEA treatment can reduce body weight gain and protects against DMBA-induced mammary tumor development in the obese Zucker rat model. The mechanism for this protection remains to be defined and will be the subject of future investigation.

Acknowledgements

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