Chronic Energy Restriction Versus Energy Cycling and Mammary Tumor Promotion

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Abstract. **Chronic energy restriction significantly inhibits mammary tumor promotion in rodents. The present work studied the effect of short-term, intermittent energy restriction or energy cycling on mammary tumor promotion since this feeding paradigm mimics the dieting habits of humans. Female Sprague-Dawley rats were given 7,12-dimethylbenz[a]anthracene (DMBA) at 50 days of age (5 mg ig). One week later, rats were randomly divided into three dietary groups. One group was fed *ad libitum* throughout the study (AL), another (ER) was fed 40% fewer calories than the AL group, and a third, energy-cycled group (EC) was fed in repeated cycles of 2 days of feeding at a level comparable to that of AL rats followed by 2 days of 40% energy restriction. At 10 weeks post-DMBA, the mammary tumor incidences in the AL and EC groups were the same, but incidence in the ER group was significantly lower. A second experiment examined serum levels of three hormones thought to play a role in mammary tumorigenesis. After 12 or 24 days on diet, ER rats had lower insulin levels compared with the other groups. Serum insulin levels in AL and EC rats were the same. After 24 days on diet, estradiol levels were significantly lower and corticosterone levels higher in the ER and EC groups compared with the AL group. Although energy cycling is a type of energy restriction that lowers overall weight gain and energy intake, it does not inhibit mammary tumor promotion as does chronic energy restriction. These data also suggest that feed efficiency and serum insulin levels correlate with susceptibility to mammary tumor promotion.** [P.S.E.B.M. 1995, Vol 209]

Dietary restriction has been shown to extend longevity in many animal species (1–3) and may be achieved either by reducing the total level of diet fed (food restriction) (4–5) or by decreasing overall energy intake (energy restriction) (6). Both food restriction and energy restriction involve an overall reduction in energy intake and both can significantly inhibit spontaneous and chemically induced mammary tumors (4–8). Energy restriction, however, provides a better paradigm for studying tumorigenesis than true food restriction because diets used in energy restriction studies are formulated in such a way that the intake of macro- and micronutrients are similar between restricted rats and *ad libitum* controls. This eliminates the possibility that a nutrient deficiency rather than energy restriction is responsible for the effect on tumorigenesis. Energy restriction has been shown to inhibit growth of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumors even in rats fed high-fat diets (9, 10). High-fat diets have consistently been shown to increase significantly the incidence of mammary tumors in rodent models (11–13). A reduction in energy intake of only 12% (88% of *ad libitum* intake) has been shown to significantly reduce tumor incidence and tumor burden compared with rats fed high-fat diets *ad libitum* (14). Greater levels of restriction dramatically reduce the appearance of mammary tumors (15, 16).

Behavior patterns indicate that most human dieters do not chronically reduce their energy intake but rather fast or significantly reduce calories for short periods of time, often in an effort to lose weight, and

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then resume their former eating habits. For many this is a recurrent cycle of weight loss followed by weight gain. This phenomenon of weight cycling or "yo-yo" dieting may have significant health implications since body weight variability has been consistently linked to all-cause mortality and mortality from coronary heart disease (17). The effect of continued weight or energy cycling and thus weight fluctuations on breast cancer occurrence in humans is unknown. Previous studies on energy restriction and mammary tumorigenesis used energy restriction that was maintained for the duration of the study (i.e., usually more than 3 months). One study (18) that examined the effect of a shorter period of energy restriction on DMBA-induced mammary tumors showed that 25% energy restriction instituted for 4 weeks during tumor promotion could significantly inhibit tumor growth, even if rats had previously been fed *ad libitum*.

In the present study, we examined whether energy restriction could maintain its anti-tumor-promoting effects if restriction is regularly interrupted by periods of *ad libitum* feeding. We subjected a group of rats to repeated cycles of 2 days of 40% energy restriction followed by 2 days of feeding at a level comparable to that of *ad libitum* fed rats. Comparisons of mammary tumorigenesis were made to rats fed *ad libitum* throughout the study and rats subjected to 40% energy restriction throughout the study. In order to assess the effects of energy cycling on endocrine status, we performed a separate study using control (no DMBA) rats. The same three dietary regimens used in the first study were examined for their influence on three hormones known to affect mammary tumor growth: insulin, estradiol, and corticosterone.

Materials and Methods

Tumor Induction and Dietary Regimen. *Study 1.* Virgin female Sprague-Dawley rats (Charles River, Raleigh, NC) were received at 40 days of age and housed individually on cob bedding throughout the study. At 50 days of age each received by gavage 5 mg of DMBA (Sigma Chemical Co., St. Louis, MO) dissolved in 0.5 ml corn oil. Rats were fed semipurified AIN-76 diet prior to and 1 week following DMBA treatment. At 1 week post-DMBA rats were randomized into 3 groups of 30 rats each and transferred to the specially formulated diets shown in Table I. One group (AL) was allowed free access to food, a second group (ER) was fed 40% fewer calories than the AL group, and the third group (EC) was fed in repeated cycles of 2 days of feeding at an equivalent level to the AL group followed by 2 days of feeding at a level equivalent to the 40% restricted rats (i.e., energy cycling). The diets were prepared by ICN Biomedicals, Inc. (Cleveland, OH), cold pressed into jumbo pellets, sealed under nitrogen, and shipped frozen. Cold pressing into jumbo pellets allows a more accurate determination of food intake. All rats were fed fresh diet daily approximately 2 hr prior to the end of the light cycle. Food intake was measured daily by weighing the uneaten food per cage.

Table I. Composition of the Semipurified Diets (g/100 g)[a]

Ingredient	AL[b]	ER[b]
Sucrose	48.0	34.7
Casein	21.6	26.5
Corn oil	15.0	20.0
Cellulose	10.1	10.1
AIN-76A vitamins	1.0	1.7
AIN-76 mineral mix	3.8	6.3
DL-methionine	0.3	0.4
Choline chloride	0.2	0.3
Total	100.0	100.0
Density (kcal/g)	4.1	4.2

[a] This diet was formulated as reported by Ruggeri *et al.* (15).
[b] AL = *ad libitum;* ER = 40% energy restricted. The 40% restricted diet provides identical micro- and similar macronutrient intake since adjustment of all ingredients would have required reducing sucrose to 13%. The energy cycled group was fed the AL diet for the first 2 days and the ER diet for the next 2 days of the 4-day cycle.

Study 2. In the second study, 24 non-DMBA-treated rats were divided into three groups and placed on the same three dietary regimens (AL, ER, and EC) used in Study 1. Serum levels of insulin, estradiol and corticosterone were measured after 12 and 24 days on the dietary regimens, 69 and 81 days of age, respectively. Body weights were measured every 2 days to define the effect of varying energy intake on weight gain.

Animal usage elements for both studies were reviewed and approved by the Institution's Animal Care and Use Committee according to the policy of the United States Public Health Service and regulations of the Animal Welfare Act.

Hormone Measurements, Necropsy, and Histopathology. In Study 1 prior to the day of euthanasia at 10 weeks post-DMBA, rats were fasted overnight and euthanized the following morning by thoracotomy after induction of narcosis with CO_2 gas. Mammary glands, mammary tumors, and livers were removed. Sections of each tissue were placed in 10% neutral buffered formalin. Tumors were described histologically (19) and only adenocarcinomas were used in data analysis. In Study 2, after 12 and 24 days on diet and prior to the day of euthanasia, rats were fasted overnight. Approximately 5 ml of whole blood was collected by closed cardiac puncture after induction of narcosis with CO_2. Serum was separated from clotted blood by centrifugation and stored at −80°C. All blood

was collected between 9 and 11 A.M. Serum insulin, estradiol-17β, and corticosterone were measured by radioimmunoassay (ICN Biomedicals, Costa Mesa, CA) according to the supplier. Estrous cycles were monitored daily by vaginal smears and only rats in diestrus at the time of euthanasia were used for hormone analyses.

Statistical Analysis. Tumor incidences were compared by chi-square analysis without continuity correction. Comparisons of body weight, food consumption, feed efficiency, and hormones were by one-way analysis of variance followed by Fisher's LSD with $P < 0.05$.

Results

Study 1 showed that significant differences in body weights occurred after only 1 week on the dietary regimens (Fig. 1) and continued for the duration of the study. Rats in the ER group showed continued growth but at a much slower pace than the other two groups. There was minimal variation in average daily food intake in the AL group throughout the study (15.6 ± 0.5). Mean weight gain over the 9 weeks of feeding was 126 g for AL, 78 g for EC, and 37 g for ER. On average, EC rats received 81% and ER rats 61% of the energy intake of rats in the AL group, and at the end of the study weighed 15% and 28% less than AL rats, respectively. Feed efficiency, which is a measure of energy utilization and is calculated by the body weight gained per unit calorie consumed and the calorie is based on actual food intake, was highest in AL rats and lowest in ER rats.

Four weeks after DMBA administration, mammary tumors appeared in all groups (Fig. 2). Tumor

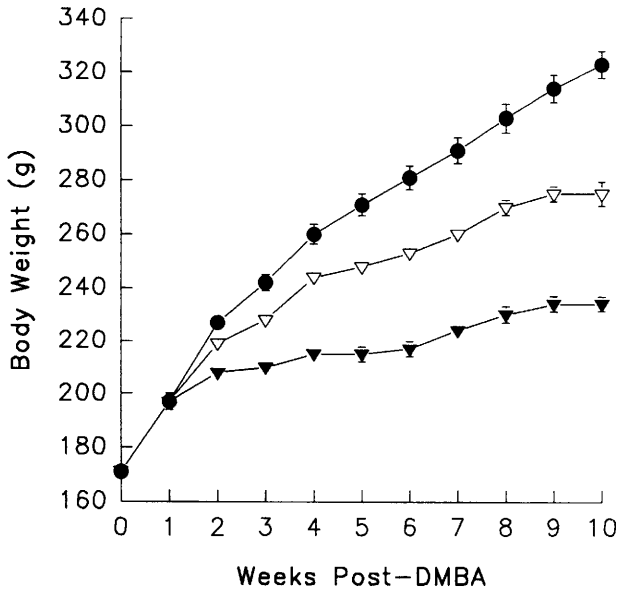

Figure 1. Body weight of female Sprague-Dawley rats given DMBA at 50 days of age and fed *ad libitum* (●), energy cycled (▽), or 40% energy restricted (▼) beginning 1 week post-DMBA.

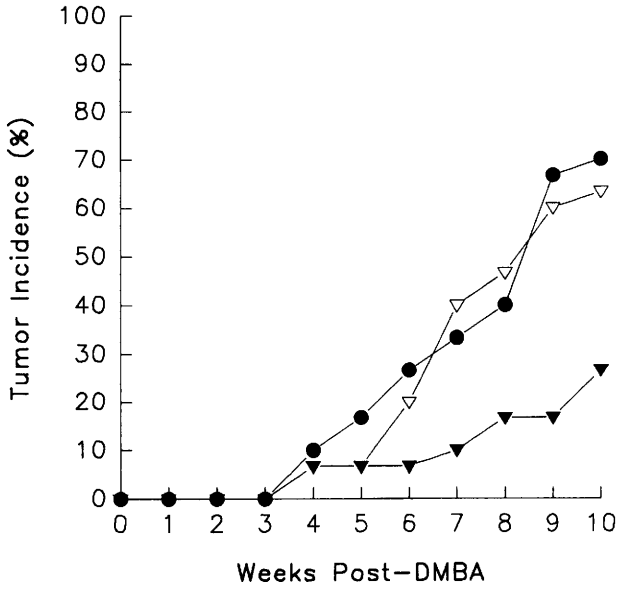

Figure 2. Incidence of mammary tumors in female Sprague-Dawley rats following DMBA treatment at 50 days of age. Diets were *ad libitum* (●, energy cycled (▽), or 40% energy restricted (▼), and were begun 1 week post-DMBA. Tumor incidence reflects only palpable mammary tumors that were confirmed as adenocarcinomas at necropsy.

incidence sharply increased in subsequent weeks in both the AL and EC groups but only gradually in the ER group. Final tumor incidences were not significantly different between AL and EC groups, while incidence in the ER group was significantly lower (Table II). Tumor burden was slightly, but not significantly lower in ER rats compared with the other groups. Both weight gain and energy intake correlated with tumor incidence ($r = 0.92$ and $r = 0.93$, respectively), but the strongest correlation was between feed efficiency and tumor incidence ($r = 0.98$).

A second study was performed to assess changes in hormone levels and body weights in response to energy cycling. Serum insulin levels were lower in the ER group at both time points compared with the other groups (Table III). No differences existed between AL and EC insulin levels. All groups exhibited an age-dependent increase in insulin levels. Estradiol levels were not different after 12 days on any of the dietary regimens but were significantly decreased by energy restriction (ER or EC) after 24 days on diet compared with *ad libitum* fed rats. Serum corticosterone levels were increased in the ER and EC groups but these elevations were not significant at 12 days. By 24 days on diet, corticosterone was significantly lower in the AL group compared with the other groups.

By measuring body weight every 2 days, we were able to detect dramatic effects on body weight gain and feed efficiency in the EC group depending on the feeding mode (Table IV). During the 2 days of *ad libitum* feeding, rats in the EC group gained significantly more weight and were more feed efficient than rats in the

Table II. Effect of Energy Cycling on Mammary Tumor Promotion

Diets[a]	Tumor incidence[b]	Tumor latency[c]	Tumor burden[d] (g)	Number of carcinomas per rat			
				0	1	2	3+
AL	70%[1]	8.0	4.69 ± 2.04[1]	9	5	4	12
ER	27%[2]	9.0	2.06 ± 0.84[1]	22	4	2	2
EC	63%[1]	7.0	3.39 ± 0.95[1]	11	7	3	9

[a] AL = *ad libitum;* EC = energy cycled; ER = 40% energy restricted. $n = 30$ for each group.
[b] Tumor incidence reflects the percentage of rats with histologically confirmed adenocarcinomas at 10 weeks post-DMBA. Values that are significantly different ($P < 0.05$) are followed by different numerical superscripts.
[c] Tumor latency is shown as the median cancer-free time in weeks and included only the rats with adenocarcinomas.
[d] Tumor burden reflects the mean weight of mammary tumors per tumor-bearing rat. Values are mean ± SEM.

Table III. Serum Insulin, Estradiol, and Corticosterone at 12 and 24 Days on Diet

Diet[a]	Insulin (μU/ml)		Estradiol (pg/ml)		Corticosterone (ng/ml)	
	12 Days[b]	24 Days[b]	12 Days[b]	24 Days[b]	12 Days[b]	24 Days[b]
AL	28.3 ± 2.3[1]	42.7 ± 3.0[1]	59.6 ± 8.4[1]	82.8 ± 7.1[1]	262 ± 122[1]	242 ± 48[2]
ER	19.1 ± 2.5[2]	26.5 ± 1.9[2]	57.3 ± 7.0[1]	60.0 ± 5.6[2]	380 ± 100[1]	448 ± 83[1]
EC	37.9 ± 6.0[1]	43.3 ± 6.1[1]	53.5 ± 6.8[1]	65.1 ± 4.0[2]	475 ± 72[1]	540 ± 42[1]

[a] AL = *ad libitum;* ER = 40% energy restricted; EC = energy cycled.
[b] Values are mean ± SEM with $n = 4$ for each time point. Rats were 69 and 81 days of age, respectively, at sampling times. Means which are significantly different ($P < 0.05$) are followed by different numerical superscripts.

Table IV. Effect of Various Dietary Regimens on Energy Utilization

Diet[a]	Day 1–2[b]			Day 3–4[b]		
	Food intake	Weight gain	Feed efficiency[c]	Food intake	Weight gain	Feed efficiency[c]
AL	16.2 ± 0.9[1]	4.6 ± 0.6[2]	3.4 ± 0.4[2]	17.4 ± 0.6[1]	5.9 ± 1.4[1]	4.3 ± 0.8[1]
ER	10.0 ± 0.5[2]	2.2 ± 1.8[2]	2.7 ± 0.8[2]	10.5 ± 0.7[2]	2.7 ± 1.0[1]	3.1 ± 1.0[1]
EC	16.2 ± 0.9[1]	7.6 ± 1.1[1]	5.8 ± 0.9[1]	10.5 ± 0.7[2]	−1.7 ± 0.7[2]	−1.9 ± 0.7[2]

[a] AL = *ad libitum;* ER = 40% energy restricted; EC = energy cycled.
[b] Days 1–4 refer to a typical 4-day feeding period during the 24-day study. All values are mean ± SEM with $n = 4$. Means which are significantly different ($P < 0.05$) are followed by different numerical superscripts.
[c] Feed efficiency was calculated as percentage of grams of body weight gained per kcalorie consumed.

other groups. Conversely, during the 2 days of 40% energy restriction, EC rats did not gain weight or often lost weight, and thus had a significantly lower feed efficiency.

Discussion

The present studies used diets that mimic the high fat content of a typical North American diet. They examined a common dieting pattern in which energy intake of the dieter is reduced for a short period of time, often in an effort to lose weight, and then is followed shortly thereafter by resumption of normal eating patterns and rebound weight gain. Our study showed that energy cycling, unlike the antipromotional effect of extended periods of energy restriction, had significant mammary tumor-promoting ability in the DMBA-induced rat mammary tumor model. Even though the EC rats received significantly fewer total calories and weighed significantly less than *ad libitum* fed rats throughout the study, mammary tumor promotion observed in rats fed the energy cycling regimen was very similar to that of the *ad libitum* group.

While the physiological effects of repeated cycles of short-term energy restriction followed by *ad libitum* feeding in rats has been examined in other disease syndromes (see Ref. 17 and 20 for reviews), this feeding regimen has been studied very little in regard to experimental mammary tumorigenesis. Over 40 years ago, Tannenbaum and Silverstone (21) showed that mice fasted for 24-hr periods twice weekly from 34 weeks of age to death had an equivalent incidence and latency of spontaneous mammary tumors as mice fed *ad libitum* for the duration of the study. Others (18) have demonstrated in the DMBA-induced rat mammary tumor model that energy restriction over prolonged periods of time (4 or 8 weeks) either preceding

or following similar prolonged periods of AL feeding reduces mammary tumor incidence. Thus, a clear distinction exists between the effects of long-term energy restriction, intermittently prolonged energy restriction and the short-term energy restriction as used in this study.

Mechanisms to explain the effects of energy restriction on tumorigenesis include altered immune status (22), altered circulating mammotrophic and glucocorticoid hormones (23–25), reduced growth factor responsiveness (26), reduced oncogene expression (27), and reduced cellular proliferation (28). It has been suggested that some factor, possibly insulin or insulin-like growth factor (IGF-I), that is responsive to acute changes in energy intake and/or body weight may play a role in the inhibition of mammary tumor promotion by energy restriction (15, 18). Insulin is a known mitogen for breast cancer cells (29) and insulin administration in streptozotocin-induced diabetic rats reverses the tumor regression observed with streptozotocin treatment alone (30). Treatment of rats with DMBA does not affect insulin levels compared with untreated rats (15). Therefore, the combined data from the current studies support a correlation of insulin levels with mammary tumorigenicity since there were no differences in tumor incidences in AL and EC rats treated with DMBA, and no differences in insulin levels between non-DMBA-treated AL and EC rats. Insulin levels were lower in 40% energy restricted rats and support the results of previous studies (15).

Ovarian steroids, particularly estrogen, appear to be critically important for the growth and development of human breast cancers (31) and carcinogen-induced rat mammary tumors (32). Initial growth of DMBA mammary tumors in rats is dependent on prolactin since these tumors grow in ovariectomized rats (33), but estrogen may be required for prolonged growth of these neoplasms even in rats with increased secretory rates of prolactin (34). Therefore, a reduction in serum estradiol by energy restriction could influence mammary tumor development. Our second study showed that chronic 40% energy restriction as well as energy cycling could reduce estradiol levels after 24 days on these feeding regimens. Thus, if energy cycling reduces estradiol levels compared with *ad libitum* feeding, then estradiol levels do not explain why tumor incidence, number, and size were not different in EC versus AL rats.

The role of glucocorticoid hormones in experimental mammary cancer is not well defined. Early studies on adrenalectomy (ADX) and mammary tumor promotion yielded conflicting reports showing either enhanced growth (35) or no effect on growth (36) of the tumors. More recently, ADX has been shown to enhance the growth of DMBA-induced mammary tumors (37). This effect is abolished by daily administration of hydrocortisone. Our study showed that corticosterone levels tended to be higher in rats subjected to energy restriction either continuously or on a energy cycling regimen. Levels of this hormone in the three groups did not correlate with tumor incidence, but instead probably indicates an effect of metabolic or behavioral stress on the animals in the ER and EC groups. Energy-restricted rats exhibited a foraging behavior and were more active during the light cycle than rats fed *ad libitum*.

Studies examining weight cycling have shown that multiple metabolic changes occur in response to energy restriction including decreased fatty acid synthesis and insulin levels, increased lipolysis, and decreased basal metabolic rate (38, 39). Upon refeeding, lipogenesis and insulin sensitivity increase, basal lipolysis and hormone sensitive lipolysis decrease, and there is a temporary increase in energy efficiency (i.e., energy-cycled rats are more efficient in converting grams of food into grams of body weight than noncycled rats). The present study supports these observations since energy cycled rats were more feed efficient than either AL rats or ER rats during the AL feeding phase. However, the EC rats were much less energy efficient during the subsequent energy-restricted period.

The efficient utilization of energy during periods of reduced energy intake is dependent on a number of hormones including glucagon, epinephrine, and the glucocorticoids. Adrenalectomized rats subjected to a starvation/refeeding paradigm do not have an increase in feed efficiency (27), indicating that the glucocorticoid hormones play a catabolic role during energy restriction and an anabolic role when the restriction is lifted. One possible explanation for the lack of an antipromotional effect of energy cycling would be that the mammary gland becomes "sensitized" to tumor promoters (e.g., growth factors, hormones, calories, fat) during energy restriction. Upon resumption of normal feeding, these promoters more efficiently stimulate the growth of mammary tumors. This hypothesis is supported by Kritchevsky *et al.* (18), who showed that there is an accelerated appearance of mammary tumors when rats are fed *ad libitum* following energy restriction compared with rats continuously fed *ad libitum*.

In summary, energy restriction is an effective method of decreasing body weight and has been shown to decrease mammary tumor incidence in DMBA-treated rats. In our study, 9 weeks of 40% energy restriction significantly decreased body weight and mammary tumor incidence compared with rats fed *ad libitum*. However, the feeding mode we called energy cycling also decreased body weight compared with *ad libitum* fed rats but did not afford protection from mammary tumor promotion. The explanation for this phenomenon may involve alterations in energy utilization and the endocrine influences of insulin and corti-

costerone. This study also emphasizes the need for further research on the effects of energy and weight cycling on disease states including cancer. This subject has received little attention despite the recommendation by the *Surgeon General's Report on Nutrition and Health* that "the health consequences of repeated cycles of weight loss and gain" receive "special priority" (40).

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