Reduction of Enhanced Mammary Carcinogenesis in LA/N-cp (Corpulent) Rats by Energy Restriction (43202)

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Abstract. **Restriction of energy intake significantly reduces mammary tumorigenesis in normal rats exposed to carcinogens. Genetically obese LA/N-cp (corpulent) female rats were given 7,12-dimethylbenz[a]anthracene and fed purified diets *ad libitum* or restricted to 60% of the *ad libitum* caloric intake. Phenotypically lean littermates were also fed *ad libitum*. Obese animals developed large mammary tumors more rapidly than genetically normal rats so that 100% of the animals had tumors in less than 16 weeks. Only 21% of the lean animals developed tumors; the energy restricted obese animals had a tumor incidence of 27%. Although obese rats fed the restricted diet weighed significantly less than those fed *ad libitum*, percent body fat was not reduced, indicating that lean tissue was affected more. Obese animals were markedly hyperinsulinemic (1003 ± 193 microunits/ml) and energy restriction reduced this to 328 ± 41; the lean animals had insulin levels of 12 ± 2. Tumor-bearing rats had higher insulin levels than rats without tumors. These data suggest that body fatness is not directly associated with risk of carcinogenesis. Lean body mass, adipose tissue mass, and their interaction with insulin in its capacity as a growth factor rather than body fatness *per se* may be determinants of tumor promotion.** [P.S.E.B.M. 1991, Vol 196]

The relationship of obesity with risk of developing breast cancer is of interest for proposing rational dietary recommendations that might reduce the incidence of various types of cancer as well as for understanding potential tumor growth regulatory factors. Lew and Garfinkel (1) reported one of the earliest systematic studies on cancer mortality in relation to body weight. They found that there was a positive linear trend in risk of death from breast cancer with increasing body weight. Brisson *et al.* (2) found that both body weight and nodular densities of the breast were directly related to breast cancer risk, suggesting that excess weight correlates with growth of benign and malignant mammary epithelium. Whereas other studies have found increased risk of breast cancer with increased body weight (3, 4), not all studies have been able to find such an association (5, 6); one report cited no relation of baseline body mass with subsequent breast cancer but did find a significant risk with weight gain during adult life (6).

Studies with animal models of breast cancer have shown that there is increased development of spontaneous or induced tumors with increasing body weight (7–10). Studies in mice with obesity secondary to gold thioglucose treatment or genetic selection have established that obese animals are significantly more likely to develop mammary tumors than lean mice (11, 12). However, these treatments result in diabetes, which is known to affect tumor development in rats (13). Most genetically obese strains of rat become overtly diabetic except the LA/N-corpulent strain; the obese phenotype of this strain of rat is hyperinsulinemic and hyperlipidemic and has normal development of lean tissue, which contrasts with other genetically obese animals (14). Since our previous work showed that restricted feeding could significantly inhibit tumorigenesis in strains of normal rats given carcinogens (7, 8, 15, 16), study of obese animals was expected to increase our knowledge of whether body mass and body fat were

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important determinants of mammary tumor promotion.

Materials and Methods

Female LA/N rats were identified as phenotypically lean or obese around 1 month of age and were fed a standard commercial diet. At 65 days of age, each animal was given 5 mg of 7,12-dimethylbenz[*a*]anthracene (DMBA) dissolved in 0.5 ml of corn oil by gavage. One week later, the rats were fed one of two diets (Table I). Fifteen pairs of obese rats and 19 lean rats were used. One group of obese animals was fed *ad libitum* as were the lean rats; the other obese rats were individually pair fed to the other obese group so that these animals received 60% of the calories of the first group but without reduction in absolute intake of any nutrient except carbohydrate. Rats were fed daily, weighed and checked for tumors weekly, and necropsied after 16 weeks. All animals were fasted overnight prior to killing by overdose of sodium pentobarbital, and blood was obtained for measurement of glucose, insulin, triglycerides, and cholesterol. Glucose was determined using a glucose oxidase kit (Sigma Chemical Co., St. Louis, MO), insulin by radioimmunoassay (Amersham Corp., Arlington Heights, IL), triglycerides, and cholesterol using enzymatic kits from Sigma. Tumors were dissected, weighed, and processed for histologic examination. Total tumor burden is defined as the total weight of tumors per tumor-bearing animal. Carcasses were frozen for determination of body composition. Total body water was determined by lyophilization, body fat by gravimetric determination on Soxhlet extracted samples, and protein by the microKjeldahl method, and the remainder of body weight (less water, fat, and protein) was assumed to be ash.

All statistical tests were performed using NWA Statpak software (Northwest Analytical, Portland, OR). Palpable tumors were compared using multiple linear regression. Final tumor incidence was analyzed using the chi-square test. All other tumor parameters were found to be distributed nonparametrically using Bartlett's test for homogeneity of variance so the Kruskal-Wallis test was employed followed by the Newman-Keuls test on ranked samples for pairwise comparisons. Serum metabolites and body composition were analyzed using a completely randomized one-way analysis of variance followed by the Newman-Keuls test. All data except tumor incidence are expressed as mean ± SE.

Table I. Percent Composition of Purified Diets

	Ad libitum	Restricted[a]
Sucrose	58.0	30.1
Casein	21.6	36.0
DL-Methionine	0.3	0.4
Corn oil	5.0	8.3
Cellulose	10.1	16.8
Mineral mix	3.8	6.3
Vitamin mix	1.0	1.7
Choline dihydrogen citrate	0.2	0.3

[a] The restricted diet provides identical absolute nutrient intake, except carbohydrate, when fed at 60% of the *ad libitum* diet.

Results

The obese rats were 60% heavier than the lean animals at the time of DMBA administration. When averaged over the 16-week feeding period, the lean rats consumed 39.5 kcal/day while the obese rats fed *ad libitum* ate 74.2 kcal/day and the restricted obese rats consumed 43.9 kcal/day. Despite the similarity in energy intake by the lean rats and obese restricted rats, there was a significantly greater (2.4 times as much) weight gain in the latter group. At every time point, all three groups were significantly different ($P < 0.001$) in body weight except for the two obese groups at Week 0 (Fig. 1). This means the obese rats received a much lower dose of carcinogen per unit body mass than the lean animals. Nevertheless, the obese rats fed *ad libitum* exhibited very early development of mammary tumors compared with the lean rats (Fig. 2). The lean animals grew very little over the course of the study despite having free access to a palatable diet that supports maximal growth of rats. This was associated with a low incidence of tumors in these animals. The incidence of palpable tumors in the obese energy-restricted rats was similar to that of the lean group.

At necropsy, tumor incidence was significantly higher in the obese rats fed *ad libitum* than in the other

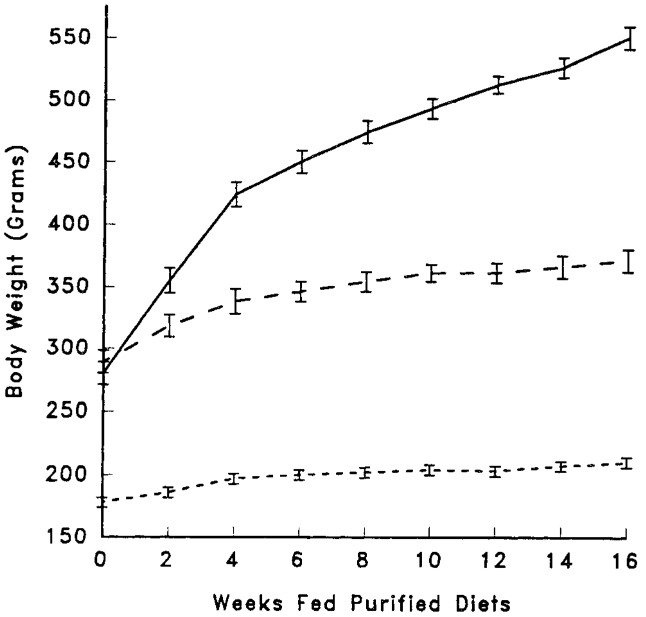

Figure 1. Body weights of LA/N rats. Obese rats fed *ad libitum* (solid line), obese rats fed restricted energy (long dashed line), or lean rats (short dashed line).

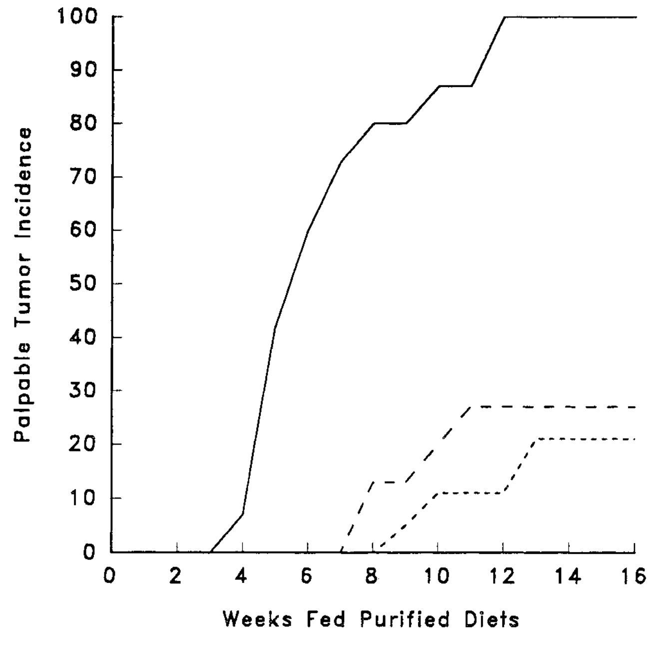

Figure 2. Cumulative incidence of palpable mammary tumors in LA/N rats. Obese rats fed *ad libitum* (solid line), obese rats fed restricted energy (long dashed line), or lean rats (short dashed line).

two groups (Table II). None of the other measures of tumor multiplicity or size was significantly different, but there was a clear trend toward more tumors in the obese rats fed *ad libitum* and larger tumors in both groups of obese animals.

Serum metabolites are shown in Table III. The obese animals were hyperlipidemic, hyperglycemic, and hyperinsulinemic. The abnormalities were only partially corrected by restricted feeding. Analysis of serum insulin levels versus tumor-bearing status was performed in the lean and obese restricted groups. Among the lean animals, tumor-bearing rats had a mean serum insulin level of 29 ± 9 microunits/ml, whereas the tumor-free animals had a concentration of 11 ± 2 microunits/ml ($P = 0.072$); in the obese restricted rats, tumor bearers had insulin levels of 429 ± 72 microunits/ml, whereas the insulin levels in non-tumor bearers were 283 ± 43 microunits/ml ($P = 0.065$). Since the number of tumor-bearing rats in each of these treatment groups was only four, it is likely that a larger sample size would have shown a highly significant difference. This type of analysis was not possible on the *ad libitum*-fed obese rats, since all of these animals developed tumors.

Analysis of body composition revealed that the lean animals had very low body fat (7.4%) (Table IV); non-obese strains of rat (Fischer 344 and Sprague-Dawley) of comparable age and fed a similar purified diet would have 20–30% body fat. The obese rats were comprised of approximately half lipid, and dietary restriction showed no effect on lipid deposition when expressed on a percentage basis. Changes in lean body mass accounted for most of the difference observed in total body weight between the two obese groups.

Discussion

This study has shown that obese rats fed *ad libitum* are significantly more sensitive to mammary carcinogenesis than lean animals. Wolff *et al.* (11) found that genetically obese yellow mice were more responsive to the tumorigenic effects of DMBA and proposed that this was due to earlier formation of hyperplastic alveolar nodules. One potential mechanism addressed in the present study is the role of insulin, which is a mitogen for mammary cells (17). This relationship may also explain the better correlation of lean body mass with tumorigenesis rather than that of body fat with tumors in the present study.

Waxler (18, 19) reported that C3H mice that had become obese following gold thioglucose treatment developed mammary tumors in a shorter time than normal weight controls. Some mice that developed obesity had food withheld until they were the weight of normal controls, and these animals had a delay in mammary tumor development that was significantly longer than that seen in controls.

In studies of spontaneous tumors in relation to calorie intake, Ross *et al.* (9) found that the level of calorie intake proved less informative than the efficiency with which the diet consumed was used for growth. This effect may be mediated via insulin and/or insulin-like growth factor I, both of which have anabolic effects and are mitogens for mammary epithelium. Both growth factors are elevated in rats fed *ad libitum* when compared with rats fed energy-restricted diets (20). Furthermore, there may be abnormal regu-

Table II. Tumor Data from Necropsy of LA/N Rats Given DMBA

	Obese *ad libitum*	Obese restricted	Lean
Tumor incidence	15/15 (100%)	4/15 (27%)	4/19 (21%)
Tumors/tumor-bearing rat	4.1 ± 0.6	1.5 ± 0.4	1.0 ± 0.0
Mean tumor weight (g)	5.3 ± 1.4	6.4 ± 3.2	1.7 ± 0.4
Mean tumor burden (g)	22.0 ± 5.0	9.6 ± 4.0	1.7 ± 0.4

Table III. Concentration of Plasma Metabolites in LA/N Rats[a]

	Obese *ad libitum*	Obese restricted	Lean
Insulin (microunits/ml)	1003 ± 193	328 ± 41	12 ± 2
Glucose (mg/dl)	360 ± 27	315 ± 18	119 ± 10
Triglycerides (mg/dl)	1028 ± 111	297 ± 21	120 ± 15
Cholesterol (mg/dl)	197 ± 19	165 ± 11	88 ± 9

[a] For each obese group $n = 15$; for lean group $n = 19$. Within each row, all values (except glucose in the two obese groups) are significantly different ($P < 0.001$).

Table IV. Percent Body Composition of LA/N Rats[a]

	Obese *ad libitum*	Obese restricted	Lean
Lipid	50.5 ± 1.7	47.7 ± 2.4	7.4 ± 0.9
Water	33.9 ± 1.4	35.5 ± 1.7	66.6 ± 0.7
Protein	13.3 ± 1.0	15.1 ± 0.9	20.8 ± 1.3
Ash	2.3 ± 0.7	1.7 ± 0.2	5.2 ± 0.8

[a] For each group $n = 6$. All values for lean group are significantly different ($P < 0.001$) from those for both obese groups, which are not significantly different from each other.

lation of receptors for these growth factors in tumors from rats subjected to energy restriction (21) that interfere with substrate utilization. Yet another mechanistic possibility in affecting tumor growth rates in these rats involves production of transforming growth factor α, which is an angiogenesis factor for these tumors and is elevated in tumors from the obese animals (22). Although we did not obtain serum insulin samples at the time that tumors began to develop in this study, previous experiments from our laboratory have documented that there are significant differences in fasting insulin levels from rats fed *ad libitum* or restricted energy throughout the study period (20).

The LA/N-cp rat strain is exquisitely sensitive to the carcinogenic effects of DMBA. This sensitivity can be ameliorated by feeding less energy; this occurs even though body fatness is not reduced, suggesting that some correlate of lean body mass has significant regulatory activity toward mammary tumor growth. Results presented here indicate that insulin is a potential mediator of these effects.

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