

Symposium: Diet, Anthropometry and Breast Cancer: Integration of Experimental and Epidemiologic Approaches

Diet and Breast Cancer: Studies in Laboratory Animals¹

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ABSTRACT Increasing dietary fat content increases mammary gland tumorigenesis in laboratory rodents. The effect can be attributed only in part to increasing energy intake, which itself increases tumorigenesis. Restriction of dietary or energy intake, sufficient to reduce body weight, reduces mammary gland tumorigenesis. Consideration of these effects has led to discussion of the possible need for changes in the feeding of laboratory rodents in carcinogenesis bioassays and other chronic studies. Studies of endocrine or other growth factors for the mammary gland have not identified specific effects of dietary fat or energy. In addition, tumorigenesis in other organs responds similarly to increased fat or decreased energy intake, indicating that the mechanisms are not, or not entirely, specific for the mammary gland. Extrapolations of results between species must always be made with caution, but the marked effects of dietary fat and energy in rodent tumorigenesis models must be considered in designing diet advice for humans. *J. Nutr.* 127: 933S–935S, 1997.

KEY WORDS: • fat • energy • mammary gland tumor • rats

The powerful enhancing effects of high dietary fat and suppressing effects of energy deprivation on mammary gland tumorigenesis in female rats and mice are undisputed, although the relative importance of increased fat and decreased dietary energy intake is a subject of extensive experimentation and discussion (Freedman et al. 1994, Rogers and Longnecker 1988, Simon 1991, Welsch et al. 1990, Welsch 1995). High fat diets and reduced energy diets influence tumorigenesis at other sites and in male as well as female rodents, but the largest body of data has been obtained in chemically induced breast cancer models in rats, and they will be the major subject of discussion here. Studies in mice, although less extensive, support the results of the studies in rats.

There is a large amount of evidence that nutritionally complete diets high (20–25% by weight) in corn oil or other (n-6) polyunsaturated fatty acid (PUFA²)-containing triglycerides increase 7,12-dimethylbenz(a)anthracene (DMBA)–or *N*-methyl,*N*-nitrosourea (MNU)-induced mammary gland carcinogenesis in rats compared with control (4–5% fat) diets. A similar effect of corn oil on 2-amino-methylimidazol[4,5-*b*]pyridine (PhIP) tumorigenesis was recently reported (Ghoshal et al. 1994). Enhanced carcinogenesis is manifested by reduced

tumor latency, increased tumor burden and, in some experiments, increased tumor incidence. Spontaneous and X irradiation–induced tumors also are increased in rats fed high fat diets (Welsch et al. 1990, Welsch 1995).

There is also a large amount of evidence that the dietary fat effect is exerted primarily after initiation of tumorigenesis and that it generally increases with the linoleic acid content of the fat at least up to about 5% linoleate in the diet (Ip 1993). Saturated fats are much less effective than (n-6) PUFA–containing fats; (n-3) PUFA–containing fats generally do not enhance carcinogenesis and may even inhibit the effect of (n-6) fats (Clinton et al. 1995, Rogers and Lee 1986, Welsch 1995).

This evidence has been obtained primarily in the DMBA model in Sprague-Dawley rats, but the conclusions are supported by results of studies of MNU tumorigenesis in Fischer 344 (F344) rats and Sprague-Dawley rats (Chan and Dao 1981). The relatively new PhIP model (Ghoshal et al. 1994), in which the carcinogen is given in 10 daily doses over 2 wk, seems to offer a promising opportunity to explore the mechanisms of dietary effects on tumor progression because the high fat diet was associated with a markedly increased incidence of malignant compared with benign tumors. Enhancement by fat in the DMBA and MNU models includes increased incidence and number of malignant tumors and also increased tumor size, which correlates with increased malignancy (Rogers and Conner 1990). Fat has not been reported to enhance malignant tumors preferentially over benign tumors, which are also increased in number. In the PhIP model (Ghoshal et al. 1994), the rats were given a large amount of corn oil (5 mL) with each of the 10 daily doses of PhIP, which may have influenced events at initiation (Clinton et al. 1984 and 1986), but all dietary groups were given the same treatment.

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² Abbreviations used: DMBA, dimethylbenz(a)anthracene; F344 rats, Fischer 344 rats; GnRH, gonadotropin-releasing hormone; MNU, *N*-methyl,*N*-nitrosourea; PhIP, 2-amino-methylimidazol[4,5-*b*]pyridine; PUFA, polyunsaturated fatty acid.

There is strong evidence also that reduced dietary or energy intake reduces mammary tumorigenesis. In studies in which diet design and intake control were well managed, reduction of energy intake reduced mammary gland tumorigenesis even in rats fed high fat diets (Klurfeld et al. 1989, Welsch et al. 1990). The smallest reduction in energy intake reported effective in reducing tumorigenesis is 10–12% (Klurfeld et al. 1989, Welsch et al. 1990). At 12% reduction of intake, rats fed a high fat diet did not show a statistically significant enhancement of tumorigenesis compared with rats fed a control fat diet in individual experiments (Welsch et al. 1990). However, statistical analyses of the data from the combined experiments and consideration of percent changes in tumor size and number rather than absolute changes demonstrated that the high fat diet did enhance tumorigenesis significantly in the restricted rats, although less markedly than in the rats given free access to feed (Freedman and Clifford 1991, Simon 1991).

In examining interactions of dietary fat and energy with mammary tumorigenesis in rats and mice in a large number of studies, Freedman and colleagues (Freedman and Clifford 1994, Freedman et al. 1990) concluded that dietary fat intake increases tumorigenesis independently of a contribution to increased energy intake, which also increases tumorigenesis. Their conclusions, in summary, are as follows:

1. In animals given free access to food, mammary tumor incidence increases with increased dietary fat. In rats, substituting 1 fat kcal for 1 nonfat kcal has 2/3 the effect on tumor incidence of increasing intake by 1 nonfat kcal/d; reducing dietary fat from 40% to 20% of total energy intake but keeping intake at 50 kcal per day, or reducing total energy intake from a 40% fat (energy content) diet by 7.7 kcal (15%), would reduce tumor incidence equally. An increased intake of 1 kcal/d (constant fat) increases tumor incidence from 50% to 53%; an increase of 1 kcal/d from fat (constant energy intake) increases tumor incidence from 50% to 52%.
2. Body weight increases by 1% per 10% increase in dietary fat content above control, but the associated increase in tumor incidence cannot be attributed to the increased weight.
3. Linoleic acid content contributes strongly to a fat's effect on tumorigenesis, but other fatty acids contribute also; fish oils and evening primrose oil are exceptions.

The effect on mammary tumorigenesis of cycling free access and restricted food intake or of initiating restricted intake at different times and for different periods has been studied. Kritchevsky et al. (1989) found that 25% energy restriction of a 10% corn oil diet for only 4 wk following DMBA exposure did not reduce tumorigenesis (or body weight after free access feeding was restored), but restriction for 8 wk reduced both tumorigenesis and body weight throughout the 16-wk experimental period. Restriction beginning later (4 wk, 8 wk) after DMBA exposure gave inconsistent results. Harris et al. (1995) studied DMBA-induced tumorigenesis in rats given free access to a 15% corn oil diet or 40% energy restricted throughout the experiment or given restricted feeding alternating with free access feeding in 48-h cycles. The cycled rats showed no reduction in indices of tumorigenesis, although their total energy intake and body weight were significantly reduced (by 19% and 15%, respectively). The rats restricted throughout the experiment had a significant reduction in tumorigenesis and in body weight (28%). Endocrine assays demonstrated significant reductions in serum estradiol and increases in serum corticosterone in both energy-restricted groups after 24 d. This raises an interesting question about the role of the endocrine system in mediating dietary effects on tumorigenesis.

The effect of free access feeding of rodents in long-term carcinogenesis and other bioassays is of increasing concern. There are numerous reports of increased incidences of spontaneous tumors and degenerative lesions and of decreased survival in such rodents compared with rodents fed amounts reduced by 25–50%. In extensive studies using Sprague-Dawley rats, Keenan et al. (1994) found that rats fed 65% of free access intake of a natural product diet have lower incidences of mammary gland tumor (females), pituitary adenoma (males and females) and pancreatic islet cell carcinoma (males) than rats given free access to food. Restricted feeding was associated with reduced white cell counts and serum cholesterol, triglycerides and glucose at 1 y; the differences did not persist to 2 y. There were no significant or persistent alterations of xenobiotic-metabolizing enzymes, although more severely restricted feeding can alter xenobiotic-metabolizing enzymes (Hart et al. 1995). Mammary gland tumor latency (time to 50% tumor incidence) was increased from 85 wk to 101 wk (Keenan et al. 1994). The investigators' subsequent studies showed that 25% restriction of food intake is similarly effective in reducing spontaneous tumors and increasing survival (Hart et al. 1995). Marked dietary restriction (50%) in female Sprague-Dawley rats was associated with more nearly normal patterns of estrous cycling at age 6 mo than either 25% restriction or free access to food (Keenan et al. 1996).

Investigators have raised the possibility that false positive carcinogenicity data could be derived from animals with free access to food if, for some reason, they ate more diet and gained more weight than their control group (Keenan et al. 1994 and 1996, Seilkop 1995). Conversely, in such studies, if the test compound decreases food intake and weight gain, false negative results might be obtained (Seilkop 1995). Proposals to address these problems include restricting diet intake of all groups by some percentage of previously observed free access intake and setting expected incidences of spontaneous tumors by consideration of tumor vs. body weight data obtained in previous control groups (Hart et al. 1995, Keenan et al. 1994, Seilkop 1995). Weight at 12 mo of age gives the best correlation with liver tumors in control B₆C₃F₁ mice. Body weight variance increases with age and can account for 20–50% of the variability in liver tumors in controls at 2 y of age (Hart et al. 1995). The best answer to these questions is not yet known; one must always be cautious in adjusting experimental results or methods (Haseman 1995).

In rodents fed high fat diets, increased chemical carcinogenesis in the mammary gland, pancreas and possibly colon makes it clear that the mechanisms of action of the high fat diets must include factors not specific for the mammary gland (Rogers and Longnecker 1988). Furthermore, the enhancing effect of dietary fat on mammary gland carcinogenesis is seen in ovariectomized rats (Clinton et al. 1995). Many factors related specifically to the mammary gland and fat intake have been investigated. Results in rats fed high fat diets include the following:

1. Serum estrogen and progesterone content and cyclic changes in them are not altered (Clinton et al. 1995, Wetsel et al. 1984). The effect of fat is seen in ovariectomized rats (Clinton et al. 1995).
2. Serum prolactin content and cyclic changes in it are not altered (Clinton et al. 1995, Wetsel et al. 1984). Pituitary prolactin and prolactin metabolism are not altered (Clinton et al. 1995).
3. Mammary gland epithelial cell proliferation is not altered (Lee et al. 1988).
4. Proliferation of cells bearing MNU-induced ha-ras codon 12 mutations is reported to be preferentially in-

creased (Hu et al. 1995) or decreased (Lu et al. 1995) compared with nonmutated cells.

In discussing the appropriateness of rodent models of mammary tumorigenesis for extrapolation to humans, many questions arise. Some questions are generic to all rodent models, arising from differences at all levels of molecular, biochemical, cellular, physiological and morphological organization and function. Others are more specific and arise from endocrine and other controls of growth, differentiation and functions specific for the gland.

Breast cancer development in women is clearly related to age, endocrine system function and reproductive history as well as to many other genetic and environmental factors. Chapin et al. (1996) summarized major endocrine changes with aging in the two rat strains in common use in mammary tumorigenesis studies. In Sprague-Dawley rats beginning at 8–10 mo of age, estrous cycling declines in association with failure of noradrenergic neurons to stimulate release of gonadotropin-releasing hormone when plasma estrogen rises. The consequent failure of release of follicle-stimulating hormone and luteinizing hormone and of ovulation results in persistent estrogen secretion, stimulation of tonic prolactin secretion and stimulation of the mammary gland and uterus; estrus days rise from 25% to about 45% of the cycle; plasma estradiol to progesterone ratios rise from about 4 at 3 mo to about 30 at 15 mo.

In F344 rats, the major endocrine change with aging is failure of control of daily prolactin surges, which extends luteal progesterone secretion. Persistent estrus is therefore not seen; plasma estradiol to progesterone ratios decrease from about 8 at 3 mo to about 4 at 15 mo, and the mammary gland is not stimulated.

Therefore, the F344 rat may be a better model than the Sprague-Dawley rat for the postmenopausal woman developing breast cancer. In the DMBA and MNU models in current use, however, rats are exposed to carcinogen at about 2 mo of age and develop tumors beginning at about 5 mo of age, so the endocrine changes with aging described above are not yet evident. In addition, the dietary effects on tumorigenesis discussed are reported in both rat strains. Dietary effects on spontaneous tumors that arise later in life might be influenced by the endocrine aging differences between strains; F344 rats develop fewer spontaneous mammary gland tumors than do Sprague-Dawley rats.

CONCLUSIONS

There is strong evidence from studies in rodent models that mammary gland tumorigenesis is significantly enhanced by high dietary levels of fat, particularly (n-6) PUFA. The evidence comes from many laboratories and many experiments and must be considered in setting dietary guidelines for people. There is similarly strong evidence that tumorigenesis is reduced by reduction in total energy intake from the level consumed by animals given free access to food. Fat has not been shown to affect mechanisms specific for the mammary gland, such as endocrine control, and high fat diets and restriction of food intake have similar effects on tumorigenesis in other organs. Therefore, dietary fat and energy intake levels may be assumed to alter carcinogenesis at least in part, by effects not specific for the mammary gland or other affected organs.

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