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Fat effects in experimental tumorigenesis

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Carinogenesis, whether spontaneous, transplanted, or induced by any of numerous agents, in most organs responds to increased dietary fat with greater indices of tumor growth. While tumors of the pancreas and mammary gland have linoleic acid (EFA) requirements higher than those of the host, colon tumors do not exhibit this characteristic, and both lymphocytic leukemia and skin tumors seem to be inhibited by increased EFA. Antagonism of EFA metabolism by feeding ω-3 fatty acids or prostaglandin synthesis inhibitors usually reduces tumor growth. Most of the effect of dietary fat on tumorigenesis is during the promotion/progression phase, but it is unclear if this is simply due to a shorter duration of exposure surrounding the initiation phase. There are also other characteristics of fats that influence tumor growth; lard seems to enhance initiation as well as promotion while palm oil has a similar fatty acid composition but inhibits tumor growth, possibly due to its tocotrienol content. Despite the requirement of certain fatty acids for tumor progression in some model systems, it is unclear if the effect of dietary fat is due to some specific metabolic change or simply reflects increased energy intake and growth. Many studies have shown that energy intake is a far stronger determinant of tumor growth than is dietary fat, and the promoting effect of fat is only seen with ad libitum feeding. The real challenge for this field is to determine the mechanism(s) by which dietary fat and/or excess energy enhances tumorigenesis. This may involve altered immunity, hormones, DNA repair, cell–cell communication, peptide growth factors, cytokinetics, or other mechanisms. (J. Nutr. Biochem. 6:201–205, 1995.)

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The epidemiologic data linking dietary fat with increased development of certain cancers are derived from multiple types of studies. The strongest data are the international comparisons that relate the fat disappearance in a given country with its mortality rate from a specific type of cancer.[1] These data are the strongest because they show a 6-fold or greater difference in risk among countries. They suggest it might be possible to decrease rates of cancer to those countries with the lowest mortality. However, these data are at the same time the weakest of the types of studies because of their design. Fat disappearance is usually represented as equal to intake and this is not the case in most countries, particularly the more affluent ones where waste can be quite significant. This type of analysis assumes everyone in a population consumes the same level of the nutrient under study and this is clearly incorrect. Mortality data from less affluent countries is frequently unreliable because most people die outside hospitals and causes of death may not be determined. Many countries have different ethnic groups that may have markedly different rates of a specific cancer, due to genetics or nondietary environmental factors. Another problem is that this type of correlation analysis cannot prove causality; it can only demonstrate a statistical relationship. Any other measure of affluence will also give a significant, positive correlation with cancer incidence. While it can be understood that the number of department stores is not related to cancer incidence, the statistical correlation with cancer may be just as strong as with dietary fat.

To overcome some of the inherent weaknesses of correlation studies, individuals who have developed cancer are studied in a case-control paradigm. Although there have been conflicting results from these studies, Howe et al.[2]

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combined 12 case-control studies of diet and breast cancer and concluded that dietary fat, saturated fat, and total energy intake were significant risk factors for breast cancer, primarily in postmenopausal women. One weakness of this type of study is the potential for differential recall of diet. That is, women with breast cancer may focus on dietary fat more than controls if they are aware of the possible relationship between the two. Another type of study that avoids this potential bias is the prospective cohort study. The largest investigation of this type is being carried out by Willett and colleagues[3] and is the Nurses Health Study in which over 90,000 women are being followed for a variety of health outcomes and have responded to dietary questionnaires prior to the diagnosis of any disease. This study has not demonstrated any link between the amount or type of dietary fat and breast cancer but has implicated saturated fat derived from animal products as doubling the risk of colon cancer. One weakness of this type of study is the reliance on a semiquantitative food frequency questionnaire to process large numbers of surveys; although this instrument can probably differentiate between extremes of intake, it cannot reliably quantify nutrient intake and does not ask several relevant questions concerning dietary fat. These include how much fat is used in cooking, how much visible fat is trimmed from meat, and the portion size.

Because of the weaknesses inherent in studying human populations, more definitive conclusions concerning the effects of specific nutrients or foods on the carcinogenic process can be derived from studies with laboratory animals. However, extrapolation from animal studies to humans is fraught with many uncertainties. In experimental studies with animals, all variables can be controlled and diets can remain constant during the study period. While there has been research activity on diet and cancer since the early part of the twentieth century, a major impetus for the effects of dietary fat on breast cancer came from the work of Carroll and associates which began in the late 1960s. Carroll and Hopkins[4] reported that rats fed 3% sunflower oil had half the mammary tumor response to the carcinogen dimethylbenz[a]anthracene (DMBA) of rats fed 20% sunflower oil. When rats were fed 20% beef tallow or coconut oil the tumor response was equivalent to that seen in the group fed 3% sunflower oil. But when 3% sunflower oil was combined with 17% of one of the more saturated fats, the tumor response was the same as when the animals were fed 20% sunflower oil. The reason for this differential tumor response is that mammary tumor cells require linoleic acid for growth and this requirement is higher than that of the normal essential fatty acid (EFA) requirement.

The specific EFA requirement for DMBA-induced mammary tumors was determined to be 4% in a diet with 20% total fat.[5] For pancreatic tumors, the maximum response to azaserine occurred when diets contained 11 to 14% EFA when the total fat content was 20%.[6] The EFA requirement of colon tumors in rats was found not to exceed 0.6% when diets contained 5% total fat[7]; this finding suggests that in high-fat diets where the total is 20%, the EFA requirement could be as high as 2.4% although it may not be higher than 0.6%. In contrast to these tumors, lymphocytic leukemias and skin tumors appear to be inhibited by diets high in linoleic acid. When rats were intubated with corn oil by gavage and fed standard cereal-based diets, there was a significant reduction in the rate of spontaneous lymphocytic leukemia.[8] Mice fed diets high in corn oil also showed reductions in DMBA-induced skin papillomas.[9] It has also been demonstrated that normal and malignant human mammary epithelial cells have an EFA requirement for growth that is higher than the EFA requirement of the body.[10] The mechanism by which EFAs enhance tumor growth is not established but it is suspected that the metabolism to eicosanoids plays a critical role. Inhibitors of prostaglandin synthesis significantly reduce the growth of chemically induced tumors in rats,[11] and chronic aspirin use reduces the incidence of colon cancer in people by 50%.[12]

Although EFAs have specific potent effects on the growth of a variety of tumors, other classes of fatty acids and specific dietary fats also affect tumor growth. The effects of ω-3 fatty acids have been studied extensively in several models of chemically induced mammary and colon tumors. When fish oil is the sole fat source in the diet, there is a significant inhibition of tumorigenesis, presumably because there is EFA deficiency.[13,14] When adequate EFAs are provided and fish oil is fed, some investigators have reported a decrease in colon tumors,[15] but a significant increase in mammary tumorigenesis has also been reported.[16] The mechanism by which ω-3 fatty acids alter tumor growth may be via competition with linoleic acid for elongation and desaturation, thereby reducing production of prostaglandins. However, other mechanisms such as alteration of membrane microviscosity cannot be ruled out. Several specific dietary fats have been shown to have either promoting or inhibiting effects on the growth of experimental tumors that are distinct from the amount of EFA. Lard has been reported by several groups to increase the appearance of chemically induced mammary or colon tumors.[17] Palm oil, with a very similar fatty acid composition, has been reported to decrease the growth of mammary and stomach tumors[18–20]; this may be due to some minor constituents such as the tocotrienols or carotenoids.

One of the problems in interpreting the studies where different amounts of fat have been fed ad libitum is that the animals consume different amounts of total energy. This was recognized over 40 years ago and a number of investigators found that energy intake was a stronger determinant of tumor response than was dietary fat.[21] Some criticisms of the older experiments were that the nutrient requirements of rodents had not been fully elucidated nor satisfied in those studies and that most relied on total dietary restriction rather than energy restriction without a decrease in fat. The first studies to address the rebirth of this area were from our group which found that the effect of restricting total energy intake was far more important than the amount of dietary fat.[22] In rats treated with DMBA and fed diets containing 4% fat ad libitum, 58% of the animals developed an average of 2.8 tumors per tumor-bearing rat. The experimental group was fed 13.1% fat but 40% fewer calories and no animals developed any tumors. This study used diets containing a small amount of EFA-rich corn oil and coconut oil as the principal fat. A second study used diets where corn oil was the sole fat source and similar differences were observed.[23]

When dimethylhydrazine (DMH)-induced colon tumors

were studied with the same diets, there was a 50% reduction in rats fed the higher fat, lower calorie regimen.[23] We subsequently studied a variety of different dietary treatments and determined that a reduction of energy by 25% from ad libitum levels consistently reduced DMBA-induced mammary tumor growth significantly.[24,25] DMH-induced colon tumors are more sensitive to the effects of energy restriction, and 10% reduction from ad libitum is sufficient to induce a significant reduction in tumorigenesis.[26] The effects of high-fat diets were also studied by us,[27]and it was found that energy restriction by 25% significantly inhibited mammary tumor growth in rats fed diets where 54% of energy was derived from fat. Many other laboratories have subsequently confirmed our findings and extended them to a variety of tumor models[28–30]; almost all malignancies respond to energy restriction with reduced growth. It is this very effectiveness that has contributed to the controversy concerning the influence of energy restriction on tumorigenesis.

Part of the reason for the controversy surrounding energy restriction was pointed out by Doll and Peto[31] who stated that perhaps we should not consider restricted animals as small but the control rats as obese. This opinion is, in fact, supported by all the data we have generated on this subject. In addition to inhibiting chemically induced tumors, energy restriction has been found to prevent the appearance of tumors in rats that were allowed to live their normal life spans. Ross[32] allowed rats to eat ad libitum for their entire lives and necropsied them for appearance of any tumors. He found a strong correlation between maximum body weight and the frequency of tumors; in the lightest group 45% died with tumors, but in the heaviest group all animals died bearing at least one tumor. Albanes[33] summarized 82 experiments done in mice and rats where energy intake varied and concluded that energy intake was dominant over the amount of fat. In contrast, Friedman et al.[34] performed a meta-analysis on published studies where fat and energy intake varied and concluded that both fat and energy intake were independent contributors to the growth of tumors. In the latter study, few data are available for energy intake in rats, and the authors attributed an average energy consumption value to all the unknown study values that was derived from Sprague–Dawley rats which is probably not reliable for other strains of rat; this also greatly decreases the variability in the equation used to determine significance which weakened the strength of the conclusion concerning the effect of energy intake of tumor growth.

The major challenge for investigators studying the influence of dietary fat and energy on tumorigenesis is to determine the mechanism(s) by which these dietary manipulations affect the growth of tumors. Many potential mediators of tumor growth have been studied and these are summarized in *Table 1;* mechanisms less likely to influence tumor growth are at the bottom of the table. Those factors judged less likely candidates for growth regulating phenomena are at the bottom of the list either because they are more likely to influence initiation rather than promotion or because the information available does not support a strong causal relationship. While all of these factors have been shown to modulate the tumorigenic process, many of them are more effective during a specific phase of tumor growth, but none have been proven to be of consistent significance in modulating tumor growth in either humans or animals. The issue of tumor modulation during initiation versus promotion is complex. Dao and Chan[35] found that the time of exposure to a high-fat diet may be part, or all, of the explanation for the consensus that dietary fat primarily affects the promotion phase. Since initiation is a short, discrete phase in tumor development, and promotion can continue for an indefinite time until a tumor appears, the major effect of diet is seen during promotion.

Table 1 Potential mediators of tumor growth affected by dietary fat or energy

Eicosanoids	Alteration of membrane phospholipids
Bile acids—colon cancer only	Oncogenes—tumor suppressor genes
Autocrine/paracrine factors	Cytokinetics of target cells
Antioxidant status	Endocrine hormones and receptor activity
Immune system	DNA repair
Cyclic nucleotides	Carcinogen metabolism
Intercellular communication	

One unresolved issue in this area of putative mechanisms by which fat or energy modifies tumorigenesis is changes in metabolism by either the host or tumor itself. Sauer and colleagues[36,37] have found consistently that acute fasting stimulates the growth of tumors in their experimental model. This was found in adult rats but not in immature rats. The adults had lipolytic and ketotic responses to fasting that were not seen in young rats. It is likely that rats subjected to chronic energy restriction are more like young rats in that they have very little adipose tissue to affect these metabolic parameters. While Sauer and associates reported that acute underfeeding by 40% resulted in increases of plasma acetoacetate, 3-hydroxybutyrate, and free fatty acids, we did not confirm this in rats subjected to chronic energy restriction up to 40%, even in animals fed the majority of their calories from fat[24,27] (unpublished data).

Research in our laboratory has implicated changes in insulin and insulin-like growth factor I (IGF-I) as probable mediators of tumor growth. These peptides have been demonstrated to be mitogens for both normal and transformed mammary epithelial cells as well as other epithelial cell types. The potency of IGF-I over insulin as a mitogen is on the order of one to three logs. We have shown that fasting serum levels of these peptides are reduced in rats subjected to caloric restriction.[38] Others have shown a maintenance of significantly reduced IGF-I in postprandial serum samples from mice fed restricted energy diets for 13 months.[39] Perhaps more importantly, we have demonstrated marked changes in binding of these growth factors by mammary tumor membrane preparations from rats fed lower energy diets.[40] There is an anomalous upregulation of specific IGF-I receptors and, simultaneously, an ablation of the nonspecific binding of IGF-I in tumor membranes. These changes in binding affinity are specific for the tumors and are not seen in several normal tissues. A study of human tissue found that IGF-I receptors were overexpressed in breast cancer samples compared with normal breast tissue

and predicted a more favorable outcome of the breast cancer based on an inverse correlation with four well established prognostic indicators: estrogen receptors, progesterone receptors, aneuploidy, and percentage of cells in S phase.[41] This raises the related question of whether local production of growth factors is affected by energy restriction. The roles of autocrine and paracrine factors have been studied extensively in relation to tumor growth[42,43] but virtually nothing in this area has been published using nutritional studies.

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