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Calorie Restriction, *ad Libitum* Feeding, and Cancer[1] (42422)

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Abstract. The inhibition of cancer by calorie restriction was discovered over 50 years ago. By 1950 it had been well characterized and there existed sufficient data to propose a mechanism of action. For reasons that remain unclear, but are probably related to the perception of the calorie restricted rodent as "small" and the *ad libitum* feeding regimen as more "normal," the concept of calorie restriction has been largely ignored by investigators after this time. Hence, despite the fact that calorie restriction is one of the oldest, best-documented, and most effective ways known to reduce cancer risk in rodents, it has had little impact on modern cancer research. In this report the history of calorie restriction is briefly reviewed, and a mechanism of action is proposed that involves increased production of ACTH and decreased production of gonadotrophins. It is further proposed that these changes may come about in part from the restriction of the time during which feeding is permitted as well as from the restriction of food per se. There is renewed interest in calorie restriction due in part to the growing recognition that there are differences in the efficiency of utilization of various sources of energy, in particular that fat calories are utilized more efficiently and provide more usable energy than carbohydrate calories. New data are presented indicating that the apparent enhancement by dietary fat of mammary cancer in rats is really a manifestation of the caloric effect. Further, the effect is abolished by moderate calorie restriction of only 15–20%. The application of these findings to the prevention of cancer in humans is considered.

The purpose of this report is to

(1) provide a brief historical overview of calorie restriction as it relates to cancer prevention in rodents;

(2) discuss hormonal changes effected by calorie restriction vs *ad libitum* feeding in relation to the mechanism of cancer prevention;

(3) discuss emerging concepts with regard to efficiency of utilization of fats vs carbohydrates as energy sources;

(4) present new data indicating that the enhancement by dietary fat of mammary carcinogenesis in rats is in fact a manifestation of the calorie effect; and

(5) consider the application of these phenomena to the prevention of cancer in humans.

Calories and cancer development. MacCay and colleagues (1, 2) were the first to demonstrate that calorie restriction retards the onset of senescence, prolongs life, and lowers the incidence of spontaneous tumors in rats. Tannenbaum (3) systematically investigated this phenomenon, and in his first paper (4) on the subject showed that "underfeeding" mice (that is, feeding them less than they would consume if permitted free access to food) reduced the incidence of spontaneous mammary tumors from 40 to 2%, primary lung adenomas from 52 to 27%, and chemically induced skin tumors from 44 to 19%. In all three cases the mean induction time for tumor development was greatly prolonged in the "underfed" mice. This can be said another way: In all three cases, tumors developed much more rapidly in the "overnourished," *ad libitum*-fed mice.

Tannenbaum's data impressed a number of people in the cancer field, as summarized in Table I. Visscher *et al.* (5) employed spontaneous mammary tumors in C3H mice as a model, recording a reduction from 67% cancer incidence in the group allowed free access to food to no tumors in the group that was restricted. They also established that the inhibitory effect of "underfeeding" was a consequence of calorie restriction. Four members of the National Cancer Institute staff, White

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TABLE I. EXAMPLES OF INHIBITION OF TUMOR FORMATION BY CALORIE RESTRICTION

	Tumor incidence (%)	
	Freely fed (obese)	Restricted
Visscher *et al.* (5)	67	0
White and White (6)	100	20
Larson and Heston (7)	50	30
Saxton *et al.* (8)	65	10
Rusch *et al.* (9)	87	7
Rusch *et al.* (10)	85	70
Boutwell *et al.* (11)	82	18

and White (6) and Larsen and Heston (7) reported that calorie restriction inhibited spontaneous mammary and lung tumors. Saxton and co-workers (8) observed that spontaneous leukemia in AK mice was reduced by calorie restriction, and Rusch *et al.* (9) demonstrated that skin tumors induced in mice by ultraviolet light were reduced from 87 to 7%. In another experiment the incidence of sarcoma induced in C-strain mice by subcutaneous injection of a carcinogenic hydrocarbon was reduced only marginally, from 85 to 70%, probably because of the overwhelming carcinogenic stimulus used in this model (10).

Boutwell and co-workers (11), employing purified diets and the chemical induction of skin tumors in mice as a model system, reported that calorie restriction reduced papilloma incidence from 82 to 18%. Only the level of carbohydrates was reduced, the levels of protein, fats, minerals, and vitamins remaining constant. These studies also confirmed that calorie restriction resulted in the prolongation of cancer-free life.

Inevitably, one is led to the conclusion that calorie restriction is a remarkably effective inhibitor of cancer in a variety of animal models. In fact, calorie restriction appears to be the most effective inhibitor of cancer formation that we know of for the intact rodent. Having said this, we ought to consider why calorie restriction has come to be so poorly appreciated and remains so poorly understood.

The fact that the substantial body of data on calorie restriction has had little impact on modern cancer research is apparently more than anything else conceptual. Doll and Peto (12) observed that if the freely fed mice had been described as obese instead of the restricted mice being described as small, the phenomenon of calorie restriction might have had more impact. Roe (13) has lamented as an unfortunate accident of history our coming to regard *ad libitum* feeding as "normal."

Mechanism of action of the calorie effect. There are fundamental physiological differences between rodents fed *ad libitum* and those that are restricted. Mulinos and Pomerantz (14) coined the term pseudohypophysectomy to describe changes in ovarian function, uterine size, and mammary tissue that mimic hypophysectomy, which is a potent means of inhibiting cancer in experimental animals. Boutwell *et al.* (15) obtained experimental evidence indicating that these changes could explain the calorie effect. Specifically, they found that the adrenal glands are atrophied in freely fed mice whereas the ovaries and uteri are enlarged. Hence, a consequence of calorie restriction is increased production of ACTH and decreased production of gonadotrophins.

Boutwell (16) then demonstrated that skin tumor formation was inhibited by topically applied or systemically administered cortisone, thereby establishing a direct link between optimal adrenal gland function and the inhibition of tumor formation. More recently Roe (13) showed that serum prolactin levels rise to abnormally high values in freely fed rats as a function of age, and speculated that calorie restriction would be associated with smaller rises. It is known that prolactin acts as a promoter of mammary cancer. Hence, it is apparent that the hormonal imbalance resulting from *ad libitum* feeding is characterized by decreased levels of hormones that inhibit tumor formation and probably, at the same time, increased levels of those that stimulate tumor growth. Additional hormonal changes are also likely, and it is particularly important that this be thoroughly studied in rats subjected to moderate levels of calorie restriction in the range of 15–20%.

An intriguing possibility is that the physiological differences between *ad libitum*-fed and calorie-restricted animals may arise not only from the restriction of food but also from the restriction of the time during which feeding is permitted. Roe (13) suggested this possibility and it is supported by the data of others as well. For example, Yanagi *et al.* (17) showed

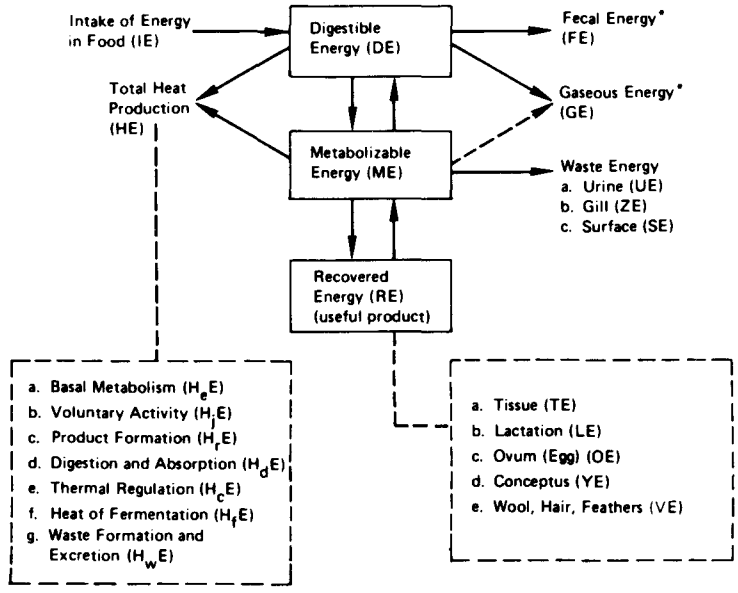

FIG. 1. Idealized flow of energy through an animal (NRC, 1981).

that the activity of the inducible short-lived liver enzyme ornithine decarboxylase rapidly increased eightfold following the onset of feeding in meal-fed rats, whereas this enzyme activity remained constantly very low in the livers of *ad libitum*-fed rats. This could only result from substantial meal feeding-induced hormonal changes that do not occur in *ad libitum*-fed rats because such animals do not eat meals but rather nibble at will (they never face an empty food cup). The extent to which this may affect cancer formation should be investigated.

Calorie effect vs. fat effect. Forbes and coworkers (18) were the first to report that the efficiency of utilization of a diet increases as the fat content increases. In the intervening years this observation has been confirmed and expanded upon and it is now clear that calories from fat are utilized more efficiently by the body than calories from carbohydrate or protein.

Figure 1 shows an idealized flow of energy through an animal. *Metabolizable energy* is differentiated from *recovered energy,* the difference being that energy is lost as heat during the conversion of metabolizable energy into recovered energy. The extent of the loss differs with energy sources, and more energy from carbohydrate or protein is lost during this conversion than occurs when fat is the energy source. Consequently, more energy is "recovered" or retained within the body from a calorie of fat than from a calorie of carbohydrate or protein.

This phenomenon has been studied extensively by animal nutritionists. Its basis is still not fully understood but it is related to the need to synthesize body fat from dietary carbohydrate when low-fat diets are fed, a process that is inefficient. By contrast, body fat is synthesized more efficiently from the additional dietary fat available in higher fat diets. In short, fat calories provide more usable energy than calories from other energy sources, and the additional energy is retained as excess body fat.

For example, Donato and Hegsted (19) compared the efficiency of dietary fat vs sucrose as energy sources for growing rats. They found that relative to sucrose, fat provided about 25% more energy than expected on the basis of the familiar Atwater values of 4 kcal/g of carbohydrate and 9 kcal/g of fat. It should be noted that the Atwater values refer to metabolizable energy. These values are appropriate for designing isocaloric diets, but they should not be extended too far when inter-

preting experiments, particularly when comparing data obtained from experiments using diets high in carbohydrate vs diets high in fat. For example, animals may retain more energy (recovered energy) from a high-fat diet in comparison to a low-fat diet even when the high-fat diet is restricted and provides 20% fewer calories based on metabolizable energy (Table II, see discussion below).

Boutwell *et al.* (11) were the first to apply the original observation of Forbes and colleagues concerning this issue to the fat and calorie effects on tumor formation. They calculated that the enhancing effect of fat on mouse epidermal carcinogenesis could be explained by the increased efficiency of utilization of fat vs carbohydrate calories. Unfortunately, the significance of this proposal was not appreciated.

Recently we began studying the role of calories in the apparent enhancement of carcinogen-induced breast cancer in rats by dietary fat (20). In designing our experiments we also incorporated the concept that calories from fat are utilized more efficiently by the body than calories from carbohydrate or protein.

Employing a commonly used breast cancer model system, female Fisher F344 rats were obtained as weanlings and fed a refined diet containing 5% corn oil. When the animals were 52 days of age they were treated with 7,12-dimethyl-benz[a]anthracene by gastric intubation to initiate mammary carcinogenesis and then switched to one of three dietary regimens, the 5% corn oil diet made freely available (designated the low-fat *ad lib* diet), an isocaloric diet containing 30% corn oil made freely available (designated the high-fat *ad lib* diet), or the high-fat diet fed at a slightly restricted level (about 16%).

TABLE II. BODY WEIGHT, BODY CONSUMPTION, AND MAMMARY CARCINOMA [FROM REF. (19)]

	Dietary regimen		
	High fat ad lib	Low fat ad lib	High fat, restricted
kcal consumption per day	41 kcal	42 kcal	34 kcal
Fat consumption per day	2.7 g	0.6 g	2.2 g
Body weight	217 g	190 g	182 g
Body composition			
% Body fat	24%	16%	25%
% Body protein	20%	23%	20%
Retained energy	752 kcal	532 kcal	634 kcal
Tumor incidence	73%	43%	7%

Table II shows the result of the experiment. The animals eating the diets *ad libitum,* whether high fat or low fat, consumed about the same number of calories per day. However, because fat calories are utilized more efficiently than carbohydrate calories the animals on the high-fat diet were significantly heavier. The rats fed the restricted diet consumed almost 20% fewer calories than the rats fed the low-fat diet *ad libitum,* but again because fat calories are utilized more efficiently than calories from carbohydrate the two groups did not differ significantly in weight at any time during the experiment. The rats fed the high-fat diet (*ad libitum* or restricted) were equivalent in body fat:protein ratio, whereas the rats fed the low-fat diet had 35% less body fat and 15% more body protein (lean body mass). This was also reflected in retained (recovered) energy, which was of the order high fat *ad libitum* > high fat restricted > low fat *ad libitum.*

At the conclusion of the experiment 73% of the rats fed the high-fat diet *ad libitum* had developed mammary neoplasms whereas 43% of the rats fed the low-fat diet *ad libitum* had similar tumors. Notably, only one rat in the group fed the high-fat restricted diet had developed one mammary carcinoma for an incidence of just 7% in that group. The high-fat restricted group consumed more than three times as much fat per day as did the low-fat *ad lib* group, yet the high-fat restricted group exhibited far fewer neoplasms. Moreover, obesity per se did not correlate with tumor incidence since the high-fat freely fed group and the high-fat restricted group were equally obese despite differences in tumor incidence.

Our data indicate that tumor appearance does not depend per se on the percentage of fat in the diet or even on the amount of fat or essential fatty acid consumed per day. Rather, mammary tumor development in this system depends on a complex interaction involving energy intake, energy retention within the body (body fat vs lean body mass), and ultimate body size.

We have developed a particular interest in the energy-restricted regimen, and have an experiment in progress that began with 120 rats divided into three groups and fed one of three isocaloric diets: a low-fat diet containing 5% corn oil, a medium-fat diet containing 17.5% corn oil, and a high-fat diet containing 30% corn oil. During the first 4 weeks following carcinogen treatment the animals were restricted slightly, but thereafter they were allowed free access to food.

The results, to be reported in full elsewhere, demonstrate that under this protocol there is no difference in tumor incidence among the groups. Hence, a slight calorie restriction for a relatively short time period is sufficient to abolish tumor enhancement by dietary fat.

We have also kept careful records on food intake for each individual rat from the day of arrival in the laboratory. Data calculations indicate that regardless of dietary fat level, the probability of developing a tumor is proportional to the calories that individual rats elect to consume.

It is important to note that to this point we have considered only one side of the equation, namely the input side. In future studies we will investigate the effect of energy expenditure as well as calorie intake. It is expected that exercise will markedly reduct tumor incidence. Perhaps the big eaters will also exercise the most, in which case the scenario derived from current data will be altered somewhat.

Extrapolation of calorie restriction from rats to humans. It is clear that we know how to reduce cancer risk for rodents: feed them a nutritionally adequate diet in moderation, and offer them the opportunity for adequate exercise (the latter is not yet proved conclusively but circumstantial evidence is very strong). Can such a simple formula be extrapolated to man?

At this time we do not know. However, hypotheses that focus on specific nutrients (e.g., fat) as cancer risk factors in man are not supported strongly or consistently by available epidemiologic data, suggesting the existence of underlying confounding factors. A growing number of epidemiologic studies have indicated a possible relationship between calorie consumption, energy expenditure, and cancer risk (21–24). The issue is complicated because of the difficulty in quantifying differences in efficiency of utilization of carbohydrate vs fat as energy sources.

Given the strength of the animal data on cancer prevention via calorie restriction it is clearly possible that the balance between energy consumption, retention, and expenditure, perhaps coupled with frequency and length of fasting periods between meals, represents an underlying and central link in the relationship between diet, nutrition, and cancer in Western society. What is needed is a thorough understanding of the mechanism of these phenomena as they relate to cancer prevention in rodents, and reliable methods for measuring them and their metabolic consequences in people. With such data in hand it may then be possible to consider extrapolation to man.

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