

# Dietary Energy Restriction in Breast Cancer Prevention

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Dietary energy restriction (DER) inhibits the development of spontaneous, chemically, genetically, and virally induced mammary cancer in rats and/or mice. DER inhibits the initiation and postinitiation stages of mammary carcinogenesis and the development of both ovarian-hormone-dependent and -independent mammary carcinomas. The predominant effect of DER appears to be suppression of the clonal expansion of transformed cells, and this effect is most likely mediated via the coordinated regulation of cell proliferation, apoptosis, and angiogenesis. The effects of DER on cell cycle regulation and apoptosis are consistent with the limitation of one or more cell survival factors. Evidence is presented that the chemical mediators of this effect, glucocorticoids, insulin, and/or insulin-like growth factors, are elicited in response to the limitation in glucose availability imposed by DER. Investigation of DER is highly relevant to the misregulation of body weight which has been identified as a human health problem of global proportion. Mechanistic studies hold the promise of leading to the identification of DER mimetic approaches that can be used in the prevention and control of breast cancer.

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**KEY WORDS:** dietary energy restriction; mammary carcinogenesis; cell proliferation; apoptosis; angiogenesis; glucocorticoids; insulin-like growth factors.

## INTRODUCTION

Dietary energy restriction (DER) is arguably the most potent physiological approach to the prevention of experimentally induced breast cancer that has been identified to date. However, the mechanisms by which DER exerts its effect on the carcinogenic process are only beginning to be elucidated, and the question of how to translate the findings from laboratory studies to the public health setting has received limited attention. The goal of this review is to provide a framework for understanding current developments in this field, to review candidate mechanisms by which the effects of DER are exerted, to consider how these findings may be translated, and to provide a perspective on key questions that remain to be addressed.

## DEFINITIONS

An energy-restricted state can be induced by either a reduction in energy intake when energy expenditure is held constant, or an increase in energy expenditure when energy intake is held constant, or some combination thereof. In the majority of experimental studies of the effect of energy restriction on mammary carcinogenesis, energy intake has been reduced with the presumption that energy expenditure remains constant. These studies are the focus of this review, and to maintain the distinction between these different approaches, we will refer to this type of energy restriction as DER.

In the literature, DER is also referred to as calorie restriction, energy restriction, dietary restriction, and food restriction (See Table I). These terms are not synonyms. In general, dietary or food restriction is used to refer to underfeeding of a complete diet such that less of all nutrients and dietary factors are

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*Abbreviations used:* CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; DER, dietary energy restriction; IGF, insulin-like growth factor; Rb, retinoblastoma.

**Table I.** Summary of Information Essential to Understanding the Meaning and Implications of Studies of Dietary Energy Restriction

Dietary energy restriction	<ul style="list-style-type: none"> <li>• Restriction of available energy due to limiting caloric intake in the absence of an effect on energy expenditure</li> </ul>
Dietary or food restriction	<ul style="list-style-type: none"> <li>• Reducing caloric intake by limiting the intake of all nutrients and dietary factors</li> </ul>
Caloric or energy restriction	<ul style="list-style-type: none"> <li>• Does not permit dissociation of effects due specifically to calories versus other nutrients</li> <li>• Diets are formulated so that when restricted amounts are fed, the only variable is the intake of calories</li> </ul>
Energy restriction: A relative term	<ul style="list-style-type: none"> <li>• Restriction of caloric intake does not necessitate the induction of weight loss</li> <li>• DER permits the evaluation of the effects of different rates of growth and adult body weight on the carcinogenic response</li> </ul>
DER as a model for excessive caloric intake	<ul style="list-style-type: none"> <li>• Ad libitum-fed animals are actually overfed</li> <li>• DER can serve as a model for evaluating the effects on cancer risk of excessive caloric intake and the resultant misregulation of body weight</li> </ul>
Three patterns of weight regulation that can be modeled by DER	<ul style="list-style-type: none"> <li>• Different rates of body weight gain resulting in corresponding differences in adult body weight; weight loss does not occur</li> <li>• Weight loss followed by maintenance of lower adult body weights than observed in ad libitum-fed animals</li> <li>• Weight cycling: Periods of weight loss followed by weight gain</li> </ul>

ingested. While such studies may provide valuable insights, this approach confounds the ability to detect effects due specifically to a limitation in dietary calories. Food restriction was frequently used in early studies (reviewed in (1)), but advances in dietary methodology make it unnecessary. Nonetheless, some investigators continue to evaluate the effects of food restriction and the results of such experiments must be interpreted with caution.

Calorie restriction and energy restriction are the terms generally applied to experimental approaches in which diets are formulated so that animals fed different numbers of calories still receive the same levels of other nutrients. Consequently, the only variable is the intake of energy. Studies which are designed on the basis of this dietary methodology provide the clearest understanding of the effects of limiting calories via dietary restriction.

While it would be desirable to limit this review and analysis to those experiments in which diets were formulated to study the specific effects of calories, there are in reality too few published studies for this option to be exercised. Nonetheless, it is important to bear in mind the methodological distinctions noted earlier, particularly in the design of new experiments.

### **ENERGY RESTRICTION: A RELATIONAL CONCEPT**

The general perception that energy restriction is equivalent to dieting is misleading because it implies that the energy restricted state must be accompanied

by weight loss, a view that is not supported by experimental evidence (reviewed in (2)). In reality what is usually investigated in DER experiments is the effect of limiting available calories relative to the amount of calories consumed when animals are ad libitum fed. The suggestion that DER stunts growth is inappropriate in that it implies that a greater rate of growth is "better," a suggestion that is not supported by studies of DER in a variety of research settings (3,4).

### **EFFECT OF DER ON THE CARCINOGENIC RESPONSE IN THE MAMMARY GLAND**

Carcinogenesis is characterized by a failure in the regulation of tissue size homeostasis in which a clone of transformed cells achieves growth-advantage due to an increased rate of cell proliferation and/or a decreased rate of cell death in comparison to neighboring populations of cells (5). The development of a carcinoma can be considered a failure of tissue size regulation attributed to the formation, selection, expansion, and progression of clones of transformed cells (5). Given this perspective, the published literature on DER and mammary carcinogenesis was evaluated to formulate responses to the following questions (see Table II).

*Does DER influence the formation of transformed cells in response to carcinogenic insult? The available evidence is limited but does indicate that DER during initiation, i.e., the time during which a carcinogenic insult is imposed, decreases the magnitude of the carcinogenic response (6–8). Whether*

**Table II.** Summary of the Effects of DER on the Carcinogenic Response in the Mammary Gland

- 
- Inhibits spontaneous, chemically, and virally induced mammary cancer models in rats and/or mice
  - Inhibits mammary carcinogenesis in genetically engineered mouse mammary model systems
  - Inhibits the initiation and postinitiation phases of chemically induced mammary carcinogenesis
  - The magnitude of protection is initiation < postinitiation < restriction throughout
  - Inhibits the occurrence of ovarian-hormone-dependent and -independent mammary carcinomas
  - In the range of 10–40% DER, inhibits mammary carcinogenesis in a dose-dependent mammary
  - Inhibitory effect is manifest as a reduction in cancer incidence and multiplicity, a decrease in tumor volume, and a prolongation of cancer latency
  - Inhibits the progression of premalignant mammary pathologies to carcinomas
- 

protection is due to direct effects on carcinogen metabolism and/or DNA repair, or is the result of indirect effects mediated by alterations in rates of cell proliferation and/or apoptosis in the target tissue, has not been determined, but these processes have been reported to be modulated by DER (reviewed in (9)).

*Does DER inhibit the processes of clonal selection, expansion, and/or progression?* These processes come into play during the postinitiation phase of carcinogenesis. As reported in (10), the loss of size regulation inherent in mammary carcinogenesis appears to be affected by energy restriction at the level of expansion of transformed cells to premalignant mammary pathologies and in the conversion of premalignant mammary pathologies to carcinomas.

*What else can be discerned about the inhibition of mammary carcinogenesis by DER?*

- DER is effective in preventing mammary cancer in different species and under diverse experimental conditions. DER inhibits the occurrence of spontaneous, virally induced, and chemically induced mammary cancer in rats and/or mice (see Ref. 9, pp. 172–174, for a tabular list of these publications).
- DER suppresses the occurrence of mammary tumors in p53-deficient mice (11).
- The greatest magnitude of protection is afforded when animals are energy-restricted during both the initiation and post initiation phases of the carcinogenic process (8).
- Depending on the degree of restriction imposed, which has ranged between 10 and 50%, DER reduces the incidence and multiplicity of mammary carcinomas by as much as 90% as well as the size of carcinomas that do emerge, and cancer latency is prolonged (10,12,13).
- On the basis of the magnitude of the inhibitory response to DER observed in rat models, it appears that both ovarian-hormone-dependent

and -independent mammary carcinomas are inhibited. This observation is also supported by studies of DER in mouse models, in which the mammary tumors are known to be ovarian-hormone-independent (see Ref. 9, pp. 172–174, for a tabular list of these publications).

- The effects of DER on the carcinogenic response have been shown to be dose-dependent. However, the magnitude of restriction often produces differential effects on cancer incidence versus multiplicity. It is likely that additional mechanisms become operative as the magnitude of restriction increases (13).
- DER works primarily by suppressing the development of cancer rather than by eliminating transformed cells (14).
- Studies conducted at the beginning of the twentieth century (reviewed in (1)) showed that DER inhibits the growth of transplantable tumors; this may indicate that DER affects the process of angiogenesis.

## MECHANISMS

The three most likely candidate cellular mechanisms by which DER exerts its effects on carcinogenesis are the inhibition of cell proliferation, the induction of apoptosis, and the modulation of tissue vascularization (Table III). The following sections focus on these candidate mechanisms and explore molecular targets and effector molecules by which DER may exert its effects on these cellular processes.

### Effects of DER on Cell Proliferation

DER has been reported to decrease the rate of cell proliferation in the mammary gland and in pre-malignant and malignant mammary pathologies (15,16). Emerging evidence indicates that DER

**Table III.** Hypothetical Mechanisms by Which DER Inhibits Mammary Carcinogenesis and for Which There is Emerging Evidence

	Proliferation	Apoptosis	Angiogenesis
Cellular effects	<ul style="list-style-type: none"> <li>• Cell proliferation reduced</li> <li>• Cell cycle progression arrested at the G<sub>1</sub>/S transition</li> <li>• Cells shifted into G<sub>0</sub></li> </ul>	<ul style="list-style-type: none"> <li>• Proapoptotic environment induced</li> </ul>	<ul style="list-style-type: none"> <li>• Vascular density in region circumscribing mammary pathologies reduced</li> <li>• Intratumoral vascular density unaffected</li> </ul>
Molecular events	<ul style="list-style-type: none"> <li>• Cyclins D and E (decreased)</li> <li>• CDKIs (Cip/Kip family and p16 family protein levels increased)</li> <li>• Activity of CDKs decreased (CDK 2 and 4)</li> <li>• Phosphorylation of Rb reduced</li> <li>• Binding of E2F-1 to Rb increased</li> </ul>	<ul style="list-style-type: none"> <li>• Caspases 9 and 3 activated</li> <li>• Bcl-2 and Bcl-XL reduced</li> <li>• Bax increased</li> <li>• IAP gene family expression reduced</li> </ul>	<ul style="list-style-type: none"> <li>• VEGF reduced</li> <li>• Flt-1 and Flk-1 reduced</li> </ul>
Chemical mediators	<ul style="list-style-type: none"> <li>• Insulin decreased<sup>a,b</sup></li> <li>• IGF-1 decreased<sup>a,c</sup></li> <li>• Glucocorticoids increased<sup>a</sup></li> <li>• Sex hormones<sup>d</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Insulin decreased<sup>a,b</sup></li> <li>• IGF-1 decreased<sup>a,c</sup></li> <li>• Glucocorticoids increased<sup>a</sup></li> <li>• Sex hormones<sup>d</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Insulin decreased<sup>a,b</sup></li> <li>• IGF-1 decreased<sup>a,c</sup></li> <li>• Glucocorticoids increased<sup>a</sup></li> <li>• Sex hormones<sup>d</sup></li> </ul>

<sup>a</sup> Change induced by the homeostatic response to the limited availability of glucose resulting from DER.

<sup>b</sup> Whether insulin and/or IGF-1 play the dominant role has not been investigated.

<sup>c</sup> Candidate target is a decrease in the activation of Akt/PKB via downregulation of cell signaling via the PI-3 kinase pathway.

<sup>d</sup> Involvement dependent on the magnitude of DER imposed. May influence the same pathways affected by changes in IGF-1 and glucocorticoids.

results in the arrest of the cycle cell at the G<sub>1</sub>/S transition (17). Mammary carcinomas that emerge in DER-treated rats are only 15% the size of age-matched carcinomas that occur in ad libitum fed control animals. Using these carcinomas to mirror the effects of DER on the carcinogenic process, levels of phosphorylated Rb and E2F-1 were observed to be significantly reduced by DER (18). Reductions in CDK2 and CDK4 kinase activity in DER carcinomas were likely to account for the observed effects on Rb and E2F-1. Both Cip1/p21 and Kip1/p27 and levels of these proteins complexed with CDK2 were significantly elevated in DER carcinomas, and levels of cyclin E were reduced. On the other hand, regulation of CDK4 kinase activity by DER was likely due to a reduction in cyclin D1 protein as well as increased binding of P16 and P19 to CDK4. The majority of changes induced were reported to be reversed when animals were released from DER. These observations are consistent with the hypothesis that DER exerts its profound cancer inhibitory activity, in part, by downregulation of cell cycle progression; the effects observed are consistent

with multifaceted regulation of cell cycle machinery by DER.

### Effects of DER on Apoptosis

DER has been reported to induce apoptosis in both pre-malignant and malignant mammary gland pathologies (15), and the pathway by which cell death was induced has been investigated (19) using the experimental approach reported in (14). Using caspase activity assays, it was shown that the activities of caspases 9 and 3 were elevated approximately twofold in carcinomas from DER rats compared to carcinomas from ad libitum-fed animals whereas caspase 8 activity was similar in carcinomas from both groups. This finding implies that DER induces the mitochondrial pathway of apoptosis activation, and is consistent with the finding that levels of Bcl-2 and Bcl-XL protein were significantly lower and levels of Bax and Apaf-1 were elevated in carcinomas from DER versus ad libitum-fed control animals. Expression levels of transcripts for IAP1, IAP2, X-linked IAP, and

survivin (AP14), proteins that can block the activity of activated caspases, were also found to be significantly lower in mammary carcinomas from DER versus ad libitum-fed animals. Collectively these data provide compelling evidence that apoptosis induction by DER is mediated via a pathway that is cell survival factor dependent.

### Effects of DER on Angiogenesis

To support new growth such as that represented by the development of a tumor, it is essential for neo-vascularization to occur, a process referred to as angiogenesis. Many findings point to the possibility that DER could inhibit angiogenesis; however, there are no published reports on this topic relative to the effects of DER on mammary carcinogenesis.

To pursue this issue, our laboratory has recently published a method that is appropriate for studying vascular density in rodent mammary gland (20) and mammary carcinomas (21). Applying those techniques, it was observed that pre-malignant development of transformed ductal mammary epithelial cells to DCIS does not require activation of the angiogenic switch (HJT, unpublished). Rather, pre-malignant lesions occur in regions with a preexisting blood supply. Whether this reflects a selection for the clonal expansion of cells in well-vascularized areas or the ability of pre-malignant lesions to induce the size expansion of existing vessels remains to be determined. On the other hand, chemically induced mammary carcinomas in the rat induce new blood vessel formation, i.e., they activate the angiogenic switch. On the basis of observations, the effects of DER on the vascularization of pre-malignant and malignant mammary pathologies were studied (HJT, unpublished). It was found that the density of blood vessels surrounding moderate and florid intraductal proliferations, DCIS, and mammary carcinomas was reduced in DER-treated rats in comparison to ad libitum-fed controls. However, there was no difference in blood vessel density within the carcinomas that emerged in DER or ad libitum-fed controls. These observations suggest that DER does affect vascularization, but the relationships are likely to be complex. Whether DER modulates vascular expansion and/or activation of the angiogenic switch remains to be determined. In this regard, preliminary experiments aimed at determining the molecular basis for the effects of DER on vascularity have shown that levels of VEGF protein and its receptors Flt-1 and Flk-1 are reduced

by approximately 50% in mammary carcinomas from DER-treated rats (HJT, unpublished).

## CANDIDATE CHEMICAL MEDIATORS

### Adrenal Cortical Steroids

As early as 1949, a role was hypothesized for the adrenal gland in accounting for the effects of DER in preventing tumor development (22), and as reported in (10), DER has been shown to increase urinary excretion of immunoreactive adrenal cortical steroids and levels of urinary corticosteroids were reported to be inversely associated with mammary carcinoma multiplicity. These observations were followed by a series of reports by the same laboratory (17,23,24). Briefly, it was shown both *in vivo* (17,23) and *in vitro* (24) that provision of supplemental corticosterone has effects on cell proliferation but not apoptosis that are similar to those observed in response to DER. In particular, corticosterone induced higher levels of the CDKI p27 and lower levels of cyclin D1, effects that would be expected to occur when cell cycle progression is arrested at the G1/S transition. However, two as yet unpublished observations from this laboratory raise questions about the degree to which increased adrenal cortical steroid activity alone accounts for the protective effects of DER against mammary carcinogenesis (HJT, unpublished). They are as follows: 1) in an animal study in which dietary corticosterone was fed to rats at a concentration that increased plasma corticosterone to levels comparable to those observed in animals that were 40% DER, mammary carcinogenesis was inhibited, but the degree of inhibition was markedly less than observed in response to DER; and 2) unlike observations reported in (25), adrenalectomy failed to negate the inhibitory activity of 40% DER against mammary carcinogenesis.

### Insulin and Insulin-Like Growth Factors (IGFs)

Studies in rodents have shown that DER prevented DMBA-induced mammary tumorigenesis in proportion to the degree of restriction imposed. In those studies, DER also resulted in a reduction in plasma insulin levels that was proportional to the degree of restriction imposed (13,26). The relevance of these observations is based in part on reports that the development of DMBA-induced mammary tumors was inhibited by alloxan-induced diabetes and that

alloxan- or streptozotocin-induced diabetes in rats caused a regression of 60–90% of DMBA-induced mammary tumors (27–30). Tumor growth was restored and tumor latency reduced upon insulin administration to diabetic rats.

Energy restriction has also been reported to prevent the development of DMBA-induced mammary tumors in genetically obese LA/N-cp female rats (31). In both the obese animals and their genetically normal lean controls, energy restriction resulted in low levels of plasma insulin. This led the authors to speculate that insulin might be mediating the effect of energy restriction on tumor occurrence in this model system.

The effects of levels of caloric restriction that inhibited mammary carcinoma development on IGF metabolism have been investigated. Ruggeri *et al.* (26) reported that a level of caloric restriction that inhibited DMBA-induced mammary tumorigenesis reduced circulating levels of insulin and IGF-1, but not IGF-II. Initially, levels of both insulin and IGF-1 were shown to be reduced, but only the effect of caloric restriction on insulin persisted. A causal role of IGF-1 in mediating the preventive effects of dietary restriction was hypothesized; in that paradigm, the effects of dietary restriction are mediated via a change in the availability of IGF-1, which in turn modulates tissue size homeostasis by decreasing cell proliferation and increasing the rate of apoptosis (32).

### **Adrenal Cortical Steroids and IGF: Should They Be Considered in Tandem?**

In the work reviewed to this point, effects of DER on either cortical steroid metabolism or insulin or IGF metabolism have been considered in isolation. However, the systemic changes in circulating levels of adrenal cortical steroids, insulin, and IGFs induced by DER occur in a coordinated response to maintain glucose homeostasis. Thus, *in vivo*, levels of IGF-1 were observed to be reduced and plasma levels of corticosterone were increased in response to DER (14). When animals were released from DER, plasma levels of IGF-1 and corticosterone changed rapidly and reciprocally and returned to the levels observed in ad libitum-fed animals. These changes were accompanied by a rapid emergence of mammary tumors. Such findings support the merit of studying the effects of DER on cellular and molecular events from the perspective of the concomitant changes in the metabolism of glucocorticoids and IGFs that are observed.

### **Other Hormones**

Of the hormones not already discussed and that could have an impact on the development of mammary cancer, the ovarian steroids estrogen and progesterone merit particular consideration. In this regard, the results of three studies are cited. Whereas Sylvester *et al.* (7) as well as Sarkar *et al.* (33) observed suppression of estrogen and prolactin secretion under conditions of energy restriction that inhibited mammary tumor development, Sinha *et al.* (34) failed to find differences in plasma estradiol levels at different stages of the estrous cycle between rats subjected to 20% calorie restriction or matched controls. Clearly, conditions of energy restriction can be defined that inhibit mammary carcinogenesis with or without an effect on the hypophyseal–pituitary–ovarian axis. Thus, while it appears likely that the cancer-preventive effects of energy restriction on hormone-sensitive target organs can be amplified by modulating the activity of this endocrine axis, effects on these hormones do not appear to be obligatory in accounting for the cancer-preventive activity of energy restriction.

### **New Views and Old Ideas**

As reported in (35), chronic energy restriction in rodents and nonhuman primates is associated with lower levels of plasma glucose and insulin. As noted earlier, levels of corticosterone are elevated and levels of IGF-1 are reduced in the plasma of energy-restricted rodents; this represents a metabolic adaptation to reduced glucose availability. However, to our knowledge, no one has determined how such systemic effects of energy restriction are “translated” locally. Interestingly, over seven decades ago, classical biochemical studies showed that tumors have altered metabolic profiles and display high rates of glucose uptake and glycolysis (36). Although these metabolic changes are not the fundamental defects that cause cancer, they might confer a selective advantage that allows some clones of transformed cells to survive, expand, and invade. This increased requirement for glucose might explain in part why the development of transformed clones of cells is suppressed by DER, and also may provide a rationale for targeting glucose metabolism using DER mimetic agents.

Consistent with early biochemical studies by Warburg, PET tumor imaging using the fluorinated analogue of 2-deoxyglucose on a large number of tumor types, including breast cancers, has provided

substantial documentation of the increased uptake of glucose by malignant cells (37–39). Those data also indicate that the degree of glucose uptake is positively correlated with histological grade and stage. This implies that a relationship exists between glucose utilization and the molecular profile of malignancies. In agreement with this is evidence from recent molecular studies that have revealed that several of the multiple genetic alterations that cause tumor development directly affect glycolysis, the cellular response to hypoxia, and the ability of tumor cells to recruit new blood vessels (40).

### **THE ORCHESTRATION OF DIFFERENT CELLULAR PROCESSES BY DER VIA A POTENTIAL COMMON MECHANISM**

The following working hypotheses are advanced in an effort to integrate what is currently known about the mechanisms underlying the cancer inhibitory activity of DER into a coherent framework upon which to formulate new research initiatives.

DER suppresses the development of the cancer phenotype by inhibiting clonal expansion. It does this by promoting cells to leave the proliferative compartment (i.e., to enter  $G_0$ ). For cells that remain in the cell cycle, DER slows cell cycle progression by arresting cells at the G1/S transition due to its effects on the phosphorylation of Rb and the consequent binding of E2F-1 to Rb. Hypophosphorylation of Rb is a consequence of the effects of DER on the activity of CDK-4 and CDK-2 per the mechanisms described earlier. In addition, DER promotes the maintenance of the cellular proapoptotic machinery by inducing changes in the metabolism of the Bcl-2 and IAP families of proteins. It is speculated that the effects of DER on cell proliferation and apoptosis affect not only the expansion of transformed clones of mammary epithelial cells, but also the ability of endothelial cells to respond to growth factors that induce vascular expansion. In addition, the synthesis of vascular endothelial growth factors, including those required for angiogenesis, is reduced. The coordinated regulation of proliferation, apoptosis, and vascularization account for the profound inhibitory activity of DER.

That the activity of three cellular processes is coordinately regulated suggests that a common molecular mechanism is at work. Again, we hypothesize that a primary consequence of DER is its effect on glucose homeostasis. In response to reduced glucose availability, levels of insulin and IGF-1 are reduced

and levels of glucocorticoids are increased. One outcome of these changes is the limitation within a tissue of intracellular survival factors. Evidence exists that is consistent with downregulation by DER of cell signaling via the PI-3 kinase pathway resulting in a reduction in levels of activated Akt. Low levels of activated Akt would favor cellular quiescence, increased apoptotic potential via modulation of the mitochondrial activation pathway, and a decreased propensity for expansion of the vascular compartment. Thus, it is hypothesized that the ultimate regulation of carcinogenesis by DER can be linked directly to glucose availability.

### **TRANSLATIONAL CONSIDERATIONS**

#### **DER as a Model for Studying the Health Consequences of Excessive Caloric Intake**

Studies of DER on the carcinogenic process conducted prior to 1980 frequently used dietary methods and experimental designs that created the perception that animals were starved, undernourished, and/or stunted in their growth (reviewed in (1)). At times these circumstances resulted in the criticism that there is little practical value in studying DER since as a global society we strive to relieve the world of starvation and undernutrition. However, most laboratory studies of the effects of DER on the carcinogenic process conducted since 1980 do not model either famine or chronic undernutrition. Rather, studies of energy restriction in the range of 10–40%, i.e., 90–60% of ad libitum intake, respectively, and using diets constructed so that the only variable is caloric intake, provide a paradigm for studying the relative effects of different levels of excessive caloric intake on the risk for cancer. As recently noted (2), properly designed DER studies do not necessitate the investigation of weight loss since energy-restricted animals can be maintained in positive energy balance throughout an experiment. While in many studies DER has been initiated in young growing animals, the evidence indicates that disruption of estrous cycle periodicity is not required for protection against cancer. Moreover, DER has been reported to inhibit carcinogenesis when initiated either in animals whose growth has plateaued, or at different stages of the disease process, including the growth and development of transplanted tumor cell lines, although the magnitude of cancer inhibitory activity is affected by the time of its initiation during the disease process and the duration

over which DER is maintained. Levels of energy restriction that inhibit carcinogenesis have been shown to increase longevity in the absence of deleterious side effects.

The idea that energy restriction provides a model for studying the effect of excessive caloric intake is not new. Over a decade ago Pariza and coworkers argued that caged, ad libitum-fed animals maintained in the laboratory are actually overfed, and at times obese (41,42). Given the recent publication of a working group report by the World Health Organization of the emerging global problem of increased risk for cancer due to excessive weight gain (9), there can be little question of the translational significance of the investigations of DER on the development of cancer.

## OTHER CONSIDERATIONS

### DER and Patterns of Body Weight Regulation

As reported in (2), three patterns of human weight regulation have been modeled experimentally. In this review, we have focused considerable attention on studies in which DER induced differences in the rate of body weight gain in the absence of weight loss. The second type of experimental approach is one in which weight loss occurs followed by maintenance of a lower body weight. To date, there are no reports of whether there is specific benefit against mammary carcinogenesis that can be attributed to weight loss *per se*, although the work reported in (31) was interpreted to indicate that the protective effects of DER are independent of its effect on body composition.

The third type of experimental approach models weight cycling (repetitive loss and regain of body weight), a common pattern of weight regulation observed in human populations. Again, while the number of studies conducted is limited and not all results are in agreement, the majority of work indicates that this pattern of weight regulation is associated with an increased carcinogenic response in the mammary gland (43–46).

### Energy Restriction: Intake Versus Expenditure

As noted in an earlier section, it is possible to induce energy restriction not only by reducing energy intake while holding energy expenditure constant but also by increasing energy expenditure while controlling energy intake. The most common way that

energy expenditure is increased is by increasing physical activity, usually via exercise. Recognizing that animals will increase energy intake when energy expenditure is increased, there have been few studies of the effects on mammary carcinogenesis of exercise-mediated energy restriction. Surprisingly, as reported in (47), exercise actually appeared to block the inhibition of mammary carcinogenesis induced by restricting intake alone. This provocative finding has significant translational implications and merits additional investigation.

## AREAS FOR CONTINUED INVESTIGATION

As noted in previous sections of this review, there are gaps of knowledge in both descriptive and mechanistic elements of the DER-breast cancer hypothesis. Key questions for continued investigation include 1) determining whether the signals that account for the cancer inhibitory activity of DER are systemic and/or local in origin; 2) identifying the molecular signaling pathway by which energy restriction mediates its cancer inhibitory activity, e.g., determining if cell signaling mediated via Akt/PKB plays a role in accounting for the effects of energy restriction; 3) establishing why (the molecular basis) some mammary carcinomas are inhibited by energy restriction, whereas other carcinomas emerge despite DER; and 4) evaluating whether the beneficial effects of energy restriction can be mimicked pharmacologically by agents that limit glucose availability. Progress in answering these questions will be facilitated by the discrete use of both genomic and proteomic interrogation tools, the expanded use of genetically engineered mouse models, and the investigation of the effects of DER in transplantable tumor systems in which human breast cancer cell lines with varying metastatic potential are used (Table IV). Finally, if DER mimetic agents are shown to inhibit mammary carcinogenesis *in vivo*, a rationale will be established for determining how these agents affect cell signaling using *in vitro* model systems.

## SUMMARY AND CONCLUSIONS

While the cancer inhibitory activity of DER was initially reported over 100 years ago, and the general perception may be that this topic has been extensively investigated, there are surprising few studies of the effects of DER on mammary carcinogenesis, and

Table IV. Summary of Opportunities for Continued Investigation

- 
- Determine whether the signals that account for the cancer inhibitory activity of DER are systemic and/or local in origin
  - Identify the molecular signaling pathway by which energy restriction mediates its cancer inhibitory activity
  - Establish why (the molecular basis) some mammary carcinomas are inhibited by energy restriction
  - Evaluate whether the beneficial effects of energy restriction can be mimicked pharmacologically by agents that limit glucose availability
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*Note.* Progress in pursuing these opportunities will be facilitated by the discrete use of genomic and proteomic interrogation tools, genetically engineered mouse models, transplantable human mammary carcinoma cell lines, and cell culture models.

significant gaps exist in the descriptive literature as well as the investigation of mechanisms. This situation is complicated by the fact that the dietary methodology used in some studies does not permit dissociation of the effects due to energy restriction per se from those that may be due to changes in the intake of other nutrients. Given that the effects of DER are not only profound in their magnitude but also physiologic, and associated with other health benefits, further development of this area is warranted since it may hold the key to identification of prevention strategies that have applicability to the general population as well as those at increased risk for the occurrence or recurrence of breast cancer.

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