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Review Article

The manner in which calories are restricted impacts mammary tumor cancer prevention

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Abstract

Although treatments for breast cancer have improved and long-term survival after diagnosis is now common, prevention of the disease is the ultimate goal. Weight loss or weight maintenance is one approach that has been recommended to reduce the risk of breast cancer, particularly for peri/postmenopausal women. This approach is supported by decades of data indicating that calorie restriction prevents spontaneous and chemically induced mammary tumor development in rodents. In most cases, calorie restriction was implemented by a consistent daily reduction of calories, i.e. chronic calorie restriction (CCR). There have also been several studies where periods of reduced caloric intake were followed by periods of refeeding, i.e. intermittent calorie restriction (ICR), resulting in the prevention of spontaneous mammary tumorigenesis. In most of the early studies, there were no direct comparisons of CCR to ICR. One study using moderate calorie restriction in a chemically induced breast cancer rat model found a slight increase in mammary tumor incidence compared with *ad libitum* fed and CCR rats. However, recently, it has been demonstrated in several transgenic mouse models of breast cancer that ICR consistently provided a greater degree of protection than CCR. This review will provide a detailed comparison of ICR and CCR for breast cancer prevention. It will also examine potential mechanisms of action that may include periods of reduced IGF-I and leptin as well as an increase in the adiponectin:leptin ratio. Application of this approach to at-risk women may provide an approach to lower the risk of breast cancer in overweight/obese women.

Keywords: Animal models, breast cancer, calorie restriction, intermittent calorie restriction

INTRODUCTION

The past several decades have seen significant advances in the treatment of breast cancer, with long-term survival now an expected outcome for most women.[1] Clearly, however, the ultimate goal is the prevention of this disease. The complete etiology of breast cancer is multifactorial and remains poorly understood. Some of the factors are genetics,[2] exposure to increased levels of certain hormones,[3] inflammation,[4] radiation exposure[5] and exposure to carcinogens.[6] In order to better understand the role of these factors in breast cancer etiology, a number of different animal models have been developed. Two different types of animal models are commonly utilized. In the first model, the tumors arise spontaneously due to transgenic modifications leading to overexpression of the genes related to inflammation[7] or growth factors and their receptors.[8] In the second model, mammary tumors are induced by exposure to high doses of carcinogens.[9] Both types of models can be useful tools for

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investigating a disease as complex as breast cancer provided they are utilized properly and their limitations are taken into account.

The risk factors for breast cancer are better understood than the etiology, and can be utilized to identify at-risk women based on specified criteria. Effective preventative approaches, including medications and/or lifestyle interventions, can then be determined based on an individual's risk. The Gail Model has been used as one means to identify women at increased risk for breast cancer. This approach uses a number of factors, including age, number of pregnancies and family history. Women considered at high risk can be offered chemoprevention with tamoxifen or raloxifene.[10] However, these interventions carry significant risks and additional options to reduce breast cancer risk are desired by the women affected and their advocates.

Tamoxifen acts by directly binding to the estrogen receptor (ER), inhibiting proliferation of ER-positive cells in the breast.[11] Several large, long-term studies have shown that tamoxifen significantly prevents breast cancer relapse and is now a first-line adjuvant therapy for postmenopausal women with ER-positive breast cancer.[12] In addition, tamoxifen treatment has been shown to prevent the initial occurrence of ER-positive tumors in high-risk individuals.[13] However, the actual use of this compound by at-risk women has been low due to the potential side-effects.[14] Raloxifene has been more recently approved for prevention, but the long-term acceptance rates are not yet clear. Low acceptance rates for tamoxifen appear to be a result of a variety of factors, including hot flushes as well as potentially life-threatening events such as increased risk of endometrial cancer, venous thromboembolism, hepatic steatosis and stroke.[11,15]

Aromatase inhibitors (AIs) block the synthesis of estrogens from androgens by inhibiting the cytochrome P450 enzyme aromatase.[16] Recently, AIs have been introduced as another treatment option for breast cancer and may also have a potential for chemoprevention. However, the side-effects of AIs, particularly osteoporosis and a higher number of fractures compared with control groups, may limit their use for the chemoprevention of breast cancer.[17]

Elevated body mass index [BMI = weight (kg) ÷ height (m^2)] was not originally included as part of the Gail Model. However, data obtained during the past 20 years have shown that body weight increase and overweight/obesity can now be considered as additional risk indicators of breast cancer for peri/postmenopausal women.[18] This subject has been reviewed periodically.[19-23] Recently, there has been increasing support for either weight maintenance or loss in women approaching menopause as strategies to reduce postmenopausal breast cancer risk.

Calorie restriction in humans

Although few in number, there are studies in humans which support that voluntary weight loss through calorie restriction will lower the risk of postmenopausal breast cancer diagnosis.[24-26] These three studies utilized data collected through interview and body weight information primarily based on recall. Using data from the cancer registries from several states, Trentham-Dietz *et al.* [26] reported that women between the ages of 50 and 79 years, with a weight loss greater than 15 kg in adulthood reduced breast cancer risk by 20% compared with the reference group that gained 5–9.9 kg or women whose weight change was between loss of 5 kg to 4.9 kg gained. Analysis of data obtained from the Iowa Women's Health Study indicated the lowest rates of breast cancer were found for women who either maintained or lost weight in adulthood.[25] In a Japanese cohort, women who lost more than 5 kg in adulthood had a hazard ratio for development of breast cancer of 0.35 when compared with women who lost or gained less than 2 kg.[24] It has also been reported that weight loss following gastric bypass surgery reduced the risk of developing breast cancer.[27] However, this is an extreme measure and not likely to be widely implemented. Interest has also focused on data from Okinawa, where individuals who eat in the traditional manner until they are 80% full have very low incidence rates of breast cancer relative to other Japanese.[28]

Several short-term studies have evaluated the consequences of weight loss on estrogen metabolites as possible explanations for how reduced calorie intake would affect factors that may impact breast cancer development.[29,30]These have tended to use premenopausal women and combined exercise with reduced caloric intake, making it unclear what was actually responsible for the hormonal changes. Also, these were fairly short-term studies. In one study, it was found that although overall there was no effect on either 2-hydroxyestrone, 16α-hydroxyestrone or their ratio, if the subjects were divided by tertiles of starting levels, the ratio was improved in women who initially had a low level.[29] In the second study, estrogen and progesterone exposure was reduced by exercise plus calorie restriction compared with women who did only light conditioning.[30]

One report has presented data on overweight/obese premenopausal women who lost weight over 1 month consuming 864 kcal/day.[31] These women lost weight (7 kg) and had significant reductions in serum insulin, leptin, cholesterol and triacylglycerol levels. Analyses of breast tissue biopsies before and after the intervention indicated changes

in genes associated with glycolysis and lipid synthesis.

Chronic calorie restriction in rodents

Calorie restriction (also termed energy restriction and/or food/diet restriction) has consistently been reported to prevent mammary tumor development in spontaneous and chemically induced breast cancer rodent models.[32-39] With respect to the development of spontaneous mammary tumors in mice in relation to calorie restriction, Dirx, *et al.* performed a meta-analysis of 14 studies published between 1942 and 1994.[40] Calorie intake was reduced by 23–50% compared with control mice and the pooled estimate of reduction in mammary tumors was 55%.

Previous work has illustrated several important points concerning mammary tumor inhibition by calorie restriction. In general, the approach was to provide the same reduction in calories on a daily basis, i.e. chronic calorie restriction (CCR). In addition, in most cases, the intervention was initiated in fairly young animals and then maintained throughout the course of the experiment. The results obtained over many decades clearly illustrate that inhibition of mammary tumorigenesis by CCR is possible. Even when initiated in older animals, a protective effect of calorie restriction has been documented. For example, over 70 years ago, Tannenbaum reported that severe calorie restriction (50–67% reduction) beginning at 23 weeks of age reduced spontaneous mammary tumor development from 40% in *ad libitum* fed mice to 2% in the calorie-reduced group.[41] This age is well beyond the timing of reaching sexual maturity, which, for mice, is 6 weeks of age. In most cases within a specific study, investigators applied only one level of calorie restriction. However, a few reports utilizing two or more levels of restriction in carcinogen-induced mammary tumorigenesis suggested that as the degree of restriction increases, so does the degree of prevention.[32,33]

Despite the overwhelming evidence that CCR can prevent and/or delay the development of mammary tumors, it has not been embraced for the prevention of breast cancer in humans. This may be partially due to that fact that although it is easy to implement this intervention in controlled preclinical studies, application to human subjects may have limited long-term compliance. Further, weight loss, if it does occur in humans, is frequently followed by weight regain. This raises the question, what is the impact of regaining this weight on breast cancer initiation and progression?

Intermittent calorie restriction studies

As indicated above, the usual approach when implementing calorie restriction has been to do so in a chronic fashion providing the same reduced intake daily. However, several investigators reported that periods of intermittent calorie restriction (ICR) followed by periods of refeeding also reduced the incidence of spontaneous mammary tumors in rodents.[42-46] A summary of these five studies is presented in Table 1. It should be mentioned that in several of these papers it is only clear upon reading the papers that the calorie restriction intervention was implemented in an intermittent fashion. This is because they provided the mice with food only twice a week, which results in the mice consuming all their available food in the first day or two and then having little or no food for the remaining time.

Table 1: Summary of intermittent calorie restriction on spontaneous mammary tumor development

animal model	Diet fed (date started)	Type of intervention	Study length (final age; weeks)	Outcome	Reference
Wistar Institute rats	Omnivorous –high fat? (6 weeks of age)	Alternate-day feeding Every third day fasted Every fourth day fasted	Until death (98–104)	Every other day rats 7% MT incidence (1/15) Other groups 29–37% MT weight reduced in all ICR groups	Carlson and Hoelzel 1946[42]
C3H/He mice	Commercial laboratory food (5 weeks of age)	Alternate-day feeding	Until death [median survival AL mice = 78 days versus 88 ICR ($P < 0.005$)]	83% MT incidence AL mice 53% MT incidence ICR mice	Shankariah *et al.* 1984 [45]
C3H/Ou mice	7.5% fat or 67% fat (6–8 weeks of age)	Fed two-times/week 40% restriction	~160 cycles (90)	100% MT incidence AL mice <10% MT incidence ICR mice (most in the 67% fat group) MT latency greatly delayed by ICR	Chen *et al.* 1990[44] Engelman *et al.* 1990 [46]
C3H/Bi mice	4.5% fat or 68.2% fat (16–20 weeks of age)	Fed two-times/week 40% restriction	~80 cycles (60)	70% MT incidence AL mice 20% MT incidence ICR mice	Shao *et al.* 1990[43]

ICR: Intermittent calorie restriction; AL: *Ad libitum*; MT: Mammary tumor

In contrast to the findings presented in Table 1 showing consistently that spontaneous mammary tumors are reduced by calorie restriction, carcinogen administration-induced mammary tumor studies found that mammary tumors were only reduced under certain circumstances by calorie restriction. It is important therefore to carefully examine the studies using carcinogens to induce mammary tumors to identify those that are most relevant to human breast cancer. The carcinogen was generally given for short periods of time at a relatively young age. In several cases, calorie restriction/refeeding was initiated at the time of carcinogen administration at 8 weeks of age.[47-49] The amounts of carcinogen administered were considerably higher than most humans would receive unless they were exposed to some type of industrial accident. The high dose of carcinogen and early age of exposure led to mammary tumor development at a relatively early age compared with the age that humans would normally be expected to develop carcinogen-induced breast cancer.

Specific examples of the effect of ICR in carcinogen-induced mammary tumors include two studies during which high-fat diets were fed and body weight levels were maintained but not decreased in the ICR groups. The *ad libitum* fed rats gained weight during the experiments. In the first study, calorie restriction of 40% alternated with two days of *ad libitum* feeding resulted in a 20% reduction in calorie intake.[47] There was no effect on mammary tumor incidence of these ICR rats compared with *ad libitum* fed rats as a result of this intervention. In the second study, ICR was implemented 1 week a month for 4.5 months.[48] In this case, mammary tumor incidence was slightly and significantly higher in the ICR rats compared with those fed *ad libitum*.

In a more recent study when four periods of 50% calorie restriction (maintained until rats lost 20% body weight) were introduced 2 months after carcinogen administration,[49] mammary tumor incidence was 8.8% compared with 17.6% for the *ad libitum* fed rats.[49] In addition, mammary tumor burden was reduced by 80% in the ICR rats. This suggests that loss of weight during ICR may be required for inhibition of mammary tumorigenesis. A comparison of these three studies is shown in Table 2. The different results obtained may also be due to timing with respect to the administration of the carcinogen in relationship to the calorie restriction intervention. For example, it was reported that when rats were fasted for 3 days starting 1 week after carcinogen administration, decreased mammary tumor latency was reported as well as a 100% tumor incidence, while nonfasted rats had a 80% incidence.[50] In another study of rats, three periods of fasting/refeeding following carcinogen administration increased mammary tumor number compared with rats either fed *ad libitum* or subjected to only one period of fasting/refeeding.[51] We speculate that the periods of refeeding at the time of high-level carcinogen administration may enhance tumor development due to the release of many growth factors as well as the hormonal milieu present in young rats as they become sexually mature. The relevance of this to the etiology of most human cancers is not known.

Comparison of chronic versus intermittent calorie restriction

Only one of the above studies directly compared CCR with ICR. In that case, rats injected with NMU were calorie restricted for 1 week a month for 4.5 months, resulting in a reduced calorie intake of 14%.[48] Mammary tumor incidence was 66% for the ICR rats compared with 54% in the control and 57% in the CCR rats. To further investigate how ICR affected mammary tumors, we conducted a study directly comparing CCR and ICR using the MMTV-TGF-α transgenic mouse strain. This mouse strain overexpresses human TGF-α. These mice develop tumors later in

Table 2: Summary of intermittent calorie restriction cycles on carcinogen-induced mammary tumor development

Animal model	Diet	Type of intervention	Study length (final age; weeks)	Outcome	Reference
Sprague-Dawley rats DMBA	30% fat calorie (from 8 weeks of age)	CCR 40% restriction ICR 2 days AL/2 days 40% restriction resulting in 20% reduction in calorie intake	~17 cycles (17)	ICR rats had same MT incidence of AL ~60% CCR had 25% incidence	Harris *et al.* 1995[47]
Sprague-Dawley rats MNU	40% fat calorie (from 8 weeks of age)	Two meals/day AL ICR two meals/day with 33% restriction 1 week/month Both groups ~14% reduction in calorie intake	4.5 cycles (26)	54% MT incidence AL 57% MT incidence for two meals/day AL 66% for two meals ICR	Tagliaferro *et al.* 1996[48]
Wistar rats DMBA	60% fat calorie (from 8 weeks of age)	50% restriction until 20% weight loss/20% regain	4 cycles (different lengths) 1st cycle started at 16 weeks of age (~52)	17.6% MT incidence in AL 8.8% MT incidence in ICR MT burden reduced by 80% for ICR compared with AL	Buison *et al.* 2005[49]

CCR: Chronic calorie restriction; ICR: Intermittent calorie restriction; AL: *Ad libitum*; MT: Mammary tumor

life,[52] which makes them highly relevant as a model of postmenopausal breast cancer. The interventions were initiated at 10 weeks of age. A control group, *ad libitum* fed, had free access to AIN-93M diet. The ICR group was calorie restricted at 50% of *ad libitum* for 3-week intervals with a diet formulated to provide the same nutrients as consumed by *ad libitum* fed mice except for restriction of carbohydrates. Each restriction period was followed by 3 weeks of free access to the AIN-93M diet. A third group, CCR, was calorie restricted by daily pair-feeding to age-matched ICR mice for each 6-week cycle. Mice were followed until 80 weeks of age (1 week of refeeding in the 12th cycle). Overall, the reduction in calorie intake for the ICR and CCR mice was approximately 21%. As expected, mammary tumor incidence was reduced to 44% by calorie restriction in the CCR mice compared with 77% for *ad libitum* fed mice. In addition, the ICR protocol resulted in a mammary tumor incidence of only 3%.[53] This was quite a spectacular development with only one tumor in one ICR mouse discovered upon necropsy at the termination of the study. This single tumor weighed only 0.063 g compared with an average tumor weight of over 1.000 g for those obtained from *ad libitum* fed mice and 0.688 g for CCR mice. Overall, these results illustrate a large degree of mammary tumor inhibition by ICR as compared with CCR.

In a second experiment, Study 2, also using MMTV-TGF-α mice, the degree of protection from ICR was not quite as great as that described above for Study 1. Mammary tumor incidence for ICR mice of 15% was obtained[54] compared with 3% in Study 1. We hypothesized that a possible explanation was that during refeeding in Study 2, the ICR mice consumed more calories than did *ad libitum* fed age-matched mice. This resulted in only an 11% reduction in calorie intake compared with the *ad libitum* fed mice. Perhaps this overeating during the refeeding period contributed to a greater tumor cell proliferation allowing some recovery? This led us to conduct a third experiment where the ICR mice were carefully matched to the food intake of *ad libitum* fed mice during the refeeding periods, preventing the opportunity for overeating. As seen in Figure 1, this resulted in a mammary tumor incidence rate of 9%, which was intermediate between the values found in the two earlier experiments.[55]

In all three experiments (Studies 1–3, Figure 1), when calorie intake was chronically reduced ~20–25%, mammary tumor incidence of CCR mice was significantly lower compared with *ad libitum* fed mice.[53-55] However, when the same degree of restriction was implemented using 3 weeks of 50% restriction followed by 3 weeks of refeeding, this ICR protocol provided a greater degree of prevention than the CCR regimen. The mammary tumor incidence for ICR mice was reduced by 82–96% compared with a 41–68% reduction for CCR mice in comparison with *ad libitum* fed mice. A cross-sectional study where mice in the three diet groups were euthanized at different time points further supported the findings of a greater protective approach for ICR versus CCR.[56] Overall, these experiments provided similar results, indicating that greater protection was provided by ICR versus CCR.

In addition to studies using MMTV-TGF-α mice, we have conducted experiments in a second transgenic mouse model of breast cancer, MMTV-neu. These mice develop estrogen receptor-negative mammary tumors that overexpress HER2/

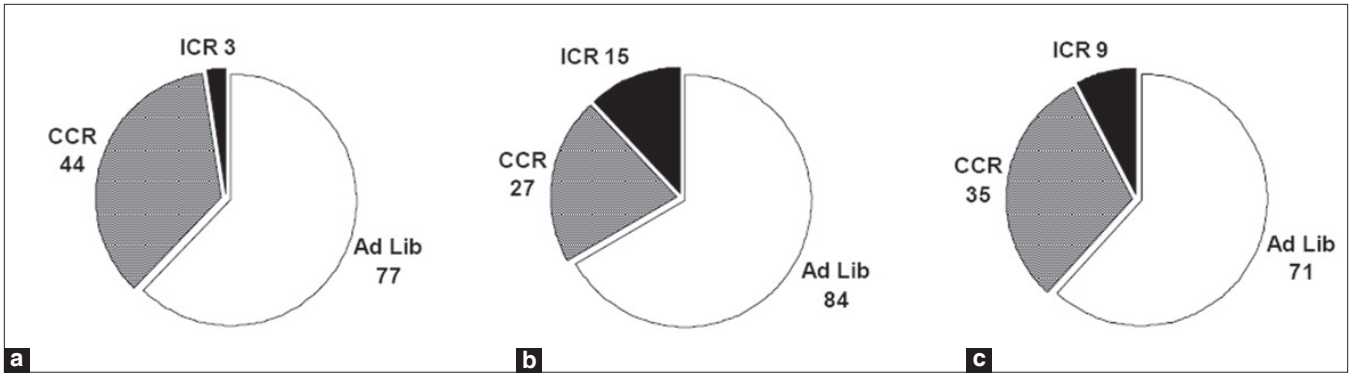

Figure 1: Comparison of *ad libitum* feeding versus chronic calorie restriction (CCR) feeding versus intermittent calorie restriction (ICR) feeding on mammary tumor incidence (%) in MMTV-TGF-α mice. (a) Study 1: intervention started at 10 weeks of age. ICR mice calorie restricted by 50% for 3 weeks followed by 3 weeks of *ad libitum* refeeding until 80 weeks of age (1 week of refeeding). Calorie intake for ICR and CCR mice reduced ~20%.[41] (b) Study 2: intervention started at 10 weeks of age. ICR mice calorie restricted by 50% for 3 weeks followed by 3 weeks of *ad libitum* refeeding until either 79 (after final restriction period) or 80 weeks of age (1 week of refeeding). Calorie intake for ICR and CCR mice reduced 11% and 14%, respectively.[42] (c) Study 3: intervention started at 10 weeks of age. ICR mice calorie restricted at 50% for 3 weeks followed by 3 weeks of controlled refeeding (i.e., matched to *ad libitum* fed mice's intake) until 79 (after final restriction period) or 82 weeks of age (after the final 3 weeks of refeeding). Calorie intake for ICR and CCR reduced ~25%.[43] For each study, the values are all different by Chi square analyses

neu. In the first study, CCR heterozygous MMTV-neu mice exhibited no reduction in mammary tumor incidence while the same ICR protocol described above reduced mammary tumor development by 50% compared with the *ad libitum* fed mice.[57] In a recent experiment, mice homozygous for the neu gene were utilized. There was a higher overall incidence of mammary tumors as compared with mice heterozygous for the neu gene (86.7% at 60 weeks versus 37.5% at 80 weeks of age), suggesting more aggressive tumor formation (ME Grossmann and MP Cleary, unpublished). There was significant mammary tumor inhibition by CCR versus *ad libitum* fed (47.2% versus 86.7%) mice. ICR also had a significant effect verses *ad libitum* (59.6% versus 86.7%), but did not show a significant difference versus CCR, suggesting that in very aggressive mammary tumor models such as homozygous neu mice and the early life carcinogen-induced rat models, the effects of ICR and CCR may be similar. This remains to be studied in greater detail in the future.

Effects of intermittent calorie restriction on other cancers and diseases

Other types of cancer have been shown to be affected by various ICR protocols. For example, ICR implemented in the TRAMP mouse model of prostate cancer indicated that this intervention delayed prostate tumor detection and death while there was little effect of CCR in comparison with *ad libitum* fed TRAMP mice.[58,59] A similar protocol was utilized as described above for the MMTV-TGF-α and MMTV-Her2/neu mice, but the ICR restriction phases for the TRAMP mice were for 2 weeks followed by 2 weeks of refeeding. This was done because of the shorter time span for disease development in the TRAMP mouse. In another approach, several different ICR regimens were evaluated in mice inoculated with a human prostate cancer cell line.[60] Survival of mice was improved by either 1 or 2 days of fasting per week with controlled refeeding for the remainder of the week. In several different lymphoma models, ICR reduced disease incidence even when the intervention was initiated in older animals.[61,62]

Other diseases have also been shown to be responsive to the protective effects of ICR. This includes reports that various ICR protocols have cardioprotective and antidiabetic effects in rodents and humans.[63-68] Body fat distribution was reported to be improved and adiponectin levels increased by fasting/refeeding of female mice.[69] Asthma-related symptoms were reported to improve following a fasting/refeeding regimen, which also resulted in significant weight loss in overweight human subjects.[70]

Potential mechanisms of action of intermittent calorie restriction

One way that calorie restriction may impact tumor development is through changes in body weight/fat, which in turn alters the production and secretion of adipokines. Adiponectin is of particular interest as *in vitro* studies have shown that the addition of adiponectin inhibits human breast cancer cell proliferation.[71-73] In addition, reduced serum adiponectin levels have been associated with the diagnosis of breast cancer.[74,75] However, in female MMTV-TGF-α mice, adiponectin levels were not altered by either CCR or ICR nor were their levels related to the presence of mammary tumors.[76,77] Further, serum adiponectin levels were not predictive of later development of mammary tumors when samples were obtained in a longitudinal protocol.[77] Other studies have not reported consistent changes in serum adiponectin levels of rodents in response to different dietary interventions.[58,59,69,78-80] However, with respect to humans with breast cancer, reduced serum adiponectin levels have been reported for postmenopausal women with breast cancer.[74,75,81]

Leptin is another adipokine that has been considered to potentially play a role in breast cancer development. *In vitro* studies have indicated that addition of leptin enhances the proliferation of human breast cancer cell lines.[82-84] *In vivo* studies found that MMTV-TGF-α mice crossed with either leptin-deficient or leptin receptor-deficient mice did not develop mammary tumors in contrast to their sisters without these homozygous genetic defects.[85,86] In the MMTV-TGF-α mice, we have recently found that serum leptin levels were reduced 30% by CCR compared with *ad libitum* fed mice while after 3 weeks of ICR intervention, the reduction was 80%.[77] Interestingly, after 3 weeks of refeeding, the ICR values were still 65% lower than *ad libitum* fed mice and still lower than those of CCR mice. However, for individual mice, there was no association of serum leptin levels with the presence of mammary tumors nor were leptin levels at a younger age predictive of the eventual development of mammary tumors. Measuring leptin levels alone in association with breast cancer diagnosis has given inconsistent findings in humans. Some studies have shown leptin to be increased in women with breast cancer, but other studies have found leptin to be decreased or unchanged.[87,88] This suggests that the role of leptin in breast cancer tumorigenesis is complex and not fully understood.

The ratio of adiponectin to leptin may be the key to understanding the role these two adipokines play in breast cancer initiation and progression. This is because these two adipokines share a unique relationship, whereby leptin rises with increasing body weight and in humans, serum adiponectin decreases.[89] Thus, as body weight increases, the ratio of these two factors changes drastically. Interestingly, in women, a low adiponectin:leptin ratio was associated

with breast cancer, and *in vitro* studies have shown this ratio to impact breast cancer cell proliferation.[90] Calculation of the adiponectin:leptin ratio in relationship to the protective effect of ICR indicates that following restriction periods of 50%, there is a marked increase in this ratio compared with *ad libitum* fed, CCR or ICR mice after refeeding.[77] However, there was no association with individual mice and their mammary tumor status. In humans, a study of breast cancer patients found that a decreased adiponectin:leptin ratio appeared to indicate the presence of aggressive breast cancer independent of the effect of BMI.[91]

Insulin-like growth factor I (IGF-I) is another growth factor that has been associated with the development of various cancers, and calorie restriction has been reported to reduce the circulating levels of this protein. In particular, the reduction in mammary tumor incidence following 40% CCR in an NMU model was reported to be associated with lower IGF-I levels.[92] In CCR rats that were allowed to refeed, the IGF-I levels were rapidly restored to the levels of control rats. At the termination of the study, mammary tumor incidence for CCR-refed rats was similar to that of the controls. In MMTV-TGF-α mice following 20–25% calorie reduction by CCR protocols, the IGF-I levels were reduced as were levels in ICR mice following 3 weeks of 50% calorie restriction.[54,55] Refeeding for either 1 or 3 weeks resulted in IGF-I levels approaching the range of the *ad libitum* fed mice.[53-55]

Evidence for the role of IGF-I in human breast cancer remains elusive. The apparent conflict between animal models and human studies may in part be related to the effects of IGF-I on breast cancer initiation and development. This further illustrates that development of breast cancer may depend on a complex interplay between a number of different growth factors that remain to be fully elucidated. In addition, what happens to the levels of various receptors and how intracellular signaling is effected remains largely unexplored. A recent report provided additional data of other ways that ICR may affect inflammation.[93] Serum was collected from rats subjected to alternate-day fasting. The serum was added to rat C6 glioma cells and a variety of measurements were made. Notable effects included significant reduction of interleukin-6 secretion and matrix metalloproteinase-2 activity as well an increase in TGF-β1 secretion in cells exposed to the ICR serum versus *ad libitum* fed serum, indicating that the serum contained anti-inflammatory factors. This suggests that additional pathways and growth factors may be important at the cell level.

Humans and intermittent calorie restriction

Whether ICR impacts human breast cancer development has not been prospectively studied. A retrospective study of postmenopausal women with a history of weight cycling found no association with breast cancer development.[26] However, several studies suggest that women who have suffered from anorexia nervosa and presumably experienced periods of weight loss and regain were at reduced risk for breast cancer.[94,95] In addition, women who have experienced weight loss through gastric bypass surgery,[27] which leads to significant calorie restriction, were found to have a reduced incidence of breast cancer. Whether these women had a history of weight-cycling is not known, but one could speculate that they had as previous attempts at weight loss is frequently a criterion before surgery is implemented.[96] This illustrates the potential for ICR to reduce the breast cancer incidence in the human population.

A recent study was designed to directly compare the physiological impact of ICR versus CCR in overweight women considered to be at high risk for breast cancer. A 6-month overall 25% restriction was utilized with a number of outcomes assessed.[97] Compliance to the interventions was good and similar in both study arms. In addition, weight reduction was similar between the groups, as were most of the measurements made. One highly significant finding was that the homeostatic model assessment (HOMA) results that quantify insulin resistance and beta-cell function were reduced to a much greater extent compared with starting values in the ICR versus CCR group. This indicates that the ICR group had a reduction in insulin resistance compared with the CCR group. A slight decrease in the leptin:adiponetin ratio was also found. In another report using modified alternate-day fasting in human subjects, successful weight loss was obtained.[68] Thus, this intervention is feasible to apply to humans and in fact may be physiologically relevant from the stand point of what the human body was accustomed to up until recently, i.e. periods of calorie restriction interspersed with periods of adequate food.

CONCLUSIONS

Although the mechanism(s) of action for CCR and ICR are not known, understanding and identifying critical time points when the intervention can be implemented and be successful will provide a more focused approach toward application of this intervention to at-risk women. On a practical note, it may be easier for human subjects to tolerate short discrete periods of caloric restriction than a lifetime of chronic restriction. Here, we have presented data of the potential contributing roles of several growth factors that may mediate the effect of ICR. However, there are still many additional potential pathways and mechanisms to evaluate. Effects on apoptosis, production of reactive oxygen species and autophagy may also be involved, but have not been investigated to any degree.

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