Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research

Stephen D. Hursting1,2,*, Sarah M. Smith1, Laura M. Lashinger1,2, Alison E. Harvey1 and Susan N. Perkins1
1Department of Nutritional Sciences, The University of Texas at Austin, 103 West 24th Street, Austin, TX 78712, USA and 2Department of Carcinogenesis, The University of Texas M. D. Anderson Cancer Center, Smithville, TX 78957, USA

Introduction

Over the past 30 years, calorie restriction (CR), an experimental mode in which test animals receive a lower-calorie diet than ad libitum-fed controls, has emerged as the most potent, broadly acting dietary regimen for suppressing the carcinogenesis process, and many of the key studies in this field have been published in Carcinogenesis. Translation of the knowledge gained from CR research in animal models to cancer prevention strategies in humans is urgently needed given the worldwide obesity epidemic and the established link between obesity and increased risk of many cancers. This review synthesizes the evidence on key biological mechanisms underlying many of the beneficial effects of CR, with particular emphasis on the impact of CR on growth factor signaling pathways and inflammatory processes and on the emerging development of pharmacological mimetics of CR. These approaches will facilitate the translation of CR research into effective strategies for cancer prevention in humans.

Calorie restriction (CR) is arguably the most potent, broadly acting dietary regimen for suppressing the carcinogenesis process, and many of the key studies in this field have been published in Carcinogenesis. Translation of the knowledge gained from CR research in animal models to cancer prevention strategies in humans is urgently needed given the worldwide obesity epidemic and the established link between obesity and increased risk of many cancers. This review synthesizes the evidence on key biological mechanisms underlying many of the beneficial effects of CR, with particular emphasis on the impact of CR on growth factor signaling pathways and inflammatory processes and on the emerging development of pharmacological mimetics of CR. These approaches will facilitate the translation of CR research into effective strategies for cancer prevention in humans.

Abbreviations: AMPK, adenosine monophosphate-activated kinase; ATP, adenosine triphosphate; CR, calorie restriction; IGF, insulin-like growth factor; IL, interleukin; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferators-activated receptor; SIRT1, silent mating type information regulation homolog; TNF-α, tumor necrosis factor-alpha; TSC, tuberous sclerosis complex.

Translational potential of CR in humans

As we have previously reviewed, observational studies further support the notion that CR has beneficial effects on longevity and cancer risk in humans (4). For example, moderately reduced caloric intake decreased morbidity and mortality among Spanish nursing home residents (5). In addition, inhabitants of Okinawa, Japan, who until recently consumed significantly fewer calories than residents of the main Japanese islands, have lower death rates from a broad spectrum of cancers and other chronic diseases than inhabitants of the Japanese mainland (6). Furthermore, patients with early-onset anorexia nervosa, and hence periods of energy restriction, have reduced risk of breast cancer (7). Another population who may experience a similarly reduced risk of such cancers are women affected by the female athlete triad. The defining characteristic of this syndrome is insufficient calorie intake (which may reflect a psychopathological eating disorder like anorexia nervosa and/or bulimia or simple lack of sufficient nutritional knowledge on the part of the female athlete) in the face of high levels of physical activity; low energy availability leads to the other legs of the triad, menstrual dysfunction and decreased bone mineral density (8). While the true prevalence of the female athlete triad among both female athletes and the general active population is difficult to ascertain, the significant expansion in opportunities for American women to participate in sports after passage of Title IX legislation in 1972 implies that a larger proportion of this cohort may be affected than in previous generations. There are no published studies examining the effect of low energy intake in this population on cancer risk; however, affected women, while they are at increased risk of long-term health problems like osteoporosis, may also be expected to experience decreased risk of breast cancer, similar to the examples cited above.

Data from countries that experienced varying degrees of energy restriction during World War II are also informative. For example, a cohort of Norwegians showed reduced breast cancer risk in the face of acute (<1 year) energy restriction (~50% reduction in calorie intake without significant changes in diet quality) (9). In contrast, survivors of the Dutch Famine of 1944, during which energy restriction (~70% reduction in rations for adults; 50% reduction in rations for children) was more severe than the Norwegian exposure, experienced higher breast cancer rates but no apparent change in risk of any other cancer (10). Cohorts exposed to even longer term and more severe (>80% reduction in usual energy intake) energy restriction, such as European Jewish survivors exposed to the Holocaust (11) or Russian survivors of the Siege of Leningrad (12), actually show increased risk of cancer (10). Cohorts exposed to even longer term and more severe (>80% reduction in usual energy intake) energy restriction, such as European Jewish survivors exposed to the Holocaust (11) or Russian survivors of the Siege of Leningrad (12), actually show increased risk of cancer (10).
CR studies in animal models that consistently show anticancer effects. CR is often referred to as ‘undernutrition without malnutrition’, and CR experiments typically involve 10–40% reduction in calories relative to controls coupled with adequate nutrition and a controlled physical environment (4). CR regimens administered throughout life are generally more protective than adult-onset CR (4). However, both early-onset and adult-onset CR prevent adult-onset obesity, significantly extend life span, and suppress tumorigenesis, prompting many investigators to suggest that obesity prevention may be a key underlying factor in the anticancer effects of CR (1,4). Rodents allowed to consume food freely become overweight, even obese, depending on the strain and diet. As Pariza et al. noted, the long-time underappreciation of CR in cancer research was probably due to ‘a problem in terminology’ (13) in that ‘more interest might have been aroused…if the freely fed mice had been described as obese instead of the mice on the restricted diet being described as small!’ (14).

There are several National Institute of Aging-funded clinical trials underway to test the effects of CR in humans under controlled conditions on biomarkers of age-related diseases processes, including cancer, to determine the similarities and differences of CR responses in humans relative to the well-characterized effects of CR in rodents. Although each of these long-term trials is in the early stages, a preliminary report of biomarker responses in one of these studies (termed the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy Study conducted at the Pennington Biomedical Research Center) indicates that many of the same metabolic and endocrine changes observed in rodents and monkeys may be also occurring in humans in response to CR (15,16).

Consistent with these preliminary findings in the National Institute of Aging trials of the effects of CR on biomarkers of age-related disease processes are findings from Biosphere 2 (17) and a Netherlands Toxicology and Nutrition Institute study (18). Biosphere 2, which took place in a closed ecosystem in Arizona from 1991–1993, involved four men and four women who experienced, on average, a 30% restriction in calorie intake relative to their usual energy intake prior to the 2 year Biosphere project. Although this sample was too small and uncontrolled to allow clear conclusions, many of the physiological parameters associated with the anticancer effects of CR in rodents and non-human primates were observed in these subjects (17). A TNO Toxicology and Nutrition Institute study in the Netherlands was more controlled, with ad libitum control subjects and 16 subjects on a 20% CR regimen relative to their usual intake for 10 weeks. As in Biosphere 2, the TNO study subjects on the CR regime, relative to the controls, displayed positive health effects, including decreased fat mass, lowered blood pressure and improved blood lipid and other chemistry values (18).

Obesity is an important risk factor for several chronic diseases, including many cancers. Translation of the CR phenomenon to human chronic disease prevention is especially critical considering that obesity is increasing alarmingly throughout the world (19). Given these trends, the development of intervention strategies that either prevent obesity or disrupt the mechanistic link underlying obesity and carcinogenesis will become increasingly critical in the coming years. Important insights into this problem can be found in the extensive literature on CR and carcinogenesis, which we have attempted to summarize over the past several years. Unfortunately, the mechanisms responsible for the observed effects of CR on cancer and other chronic diseases are not yet clearly elucidated.

Selected potential mechanisms

Energy balance-related hormones and growth factors

Energy balance-related physiological processes, such as energy expenditure, appetite regulation, metabolism and thermogenesis, are all under hormonal control. Recent evidence, particularly from several mutant mouse models in which specific hormonal factors have been altered, have provided evidence that insulin, insulin-like growth factor (IGF)-1, glucocorticoids and several adipose-derived factors (such as leptin and adiponectin) associated with inflammation and energy metabolism, may be key factors in the anticancer effects of CR (20). These factors will be the primary focus of the following mechanistic discussion.

Insulin. Insulin particularly under conditions of chronic hyperinsulinemia and insulin resistance increases risk for cancer at several sites (21), although it is unclear if the tumor-enhancing effects of insulin are due to direct effects involving the insulin receptor on preneoplastic cells or alternatively due to indirect effects on IGF-1, estrogens or other hormones. Certainly, high circulating levels of insulin promote the hepatic synthesis of IGF-1 and decrease the production of IGF binding protein-1, thus increasing the biologic activity of IGF-1 (22). Furthermore, both insulin and IGF-1 act in vitro as growth factors to promote cancer cell proliferation and decrease apoptosis (23). Insulin resistance, a state of reduced responsiveness of tissues to the physiological actions of insulin, results in a compensatory rise in plasma insulin levels and is affected by both adiposity and physical activity. Intra-abdominal obesity is associated with insulin resistance (24), whereas physical activity improves insulin sensitivity (25). A growing body of epidemiologic evidence suggests that type 2 diabetes, which is usually characterized by hyperinsulinemia and insulin resistance for long periods, is associated with increased risks of endometrial, colon, pancreas, kidney and postmenopausal breast cancers (22).

IGF-1. IGF-1 is a mitogen so named because of its sequence homology to pro-insulin. IGF-1 plays a central role in regulating cell cycle progression from G1 to S phase by activating the phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction pathway and modulating cyclin-dependent kinases (26,27). IGF-1 can also significantly suppress apoptosis in a variety of cell types, and cells overexpressing IGF-1 receptor show decreased apoptosis (28,29). IGF-1 is thus a major endocrine and paracrine regulator of tissue growth and metabolism. IGF-1’s involvement in cancer was first suspected when in vitro studies consistently showed that supplementation of culture media with IGF-1 enhances the growth of a variety of cancer cell lines (30–33). There is also abundant epidemiologic evidence supporting the hypothesis that IGF-1 is involved in several types of human cancers (34–40). IGF-1 may be acting either directly on cells via its receptor, IGF-1 receptor, or indirectly through interaction with other cancer-related molecules such as the tumor suppressor p53 (41,42). Levels of circulating IGF-1 are determined primarily by growth hormone-regulated transient synthesis, which is influenced by dietary intake of energy and protein (43). To a lesser extent, IGF-1 synthesis can also occur in extrahepatic tissues, but this involves a complex integration of signals involving growth hormone, other hormones and growth factors and IGF binding proteins, which determine the local availability of IGF-1 and systemic half-life (44).

There is increasing evidence that reduction in serum levels of IGF-1 mediates many of the antiproliferative, pro-apoptotic and anticancer effects of CR through its role in an evolutionarily conserved regulatory pathway that is responsive to energy availability (4,45). In fact, restoration of IGF-1 levels in CR mice has been shown to abolish the antitumor effects of CR in multiple preclinical models (43,46,47). Conversely, we have shown that diet-induced obesity can lead to insulin resistance, with increased IGF-1 and decreased IGF binding protein-1, all of which can result in enhanced IGF-1 signaling (48).

Downstream targets of the IGF-1 receptor and insulin receptor comprise a signaling network that regulates cellular growth and metabolism predominately through induction of the PI3K/Akt pathway (recently reviewed in ref. 49,50). The importance of this signaling cascade in human cancers has recently been highlighted by the observation that it is one of the most commonly altered pathways in human epithelial tumors (49,51–53). Engagement of the PI3K/Akt pathway allows both intracellular and environmental cues, such as energy availability and growth factor supply, to affect cell growth, proliferation, survival and metabolism.

Activation of receptor tyrosine kinases and/or the Ras proto-oncogene stimulates PI3K to produce the lipid second messenger, phosphatidylinositol-3,4,5-trisphosphate. Phosphatidylinositol-3,4,5-trisphosphate...
recruits and anchors Akt to the cell membrane where it can be further phosphorylated and activated (51,53). Akt is a cyclic adenosine 3',5'-monophosphate-dependent, cyclic guanosine monophosphate-dependent protein kinase C that when constitutively active is sufficient for cellular transformation by stimulating cell cycle progression and cell survival as well as inhibiting apoptosis (54,55). Frequently associated with the aberrant Akt signaling commonly seen in human cancers is an elevation in mammalian target of rapamycin (mTOR) signaling. mTOR is a highly conserved serine/threonine protein kinase which is activated by Akt and also inhibited by an opposing signal from adenosine monophosphate-activated kinase (AMPK). At the interface of the Akt and AMPK pathways, mTOR dictates translational control of new proteins in response to both growth factor signals and nutrient availability through phosphorylation of its downstream mediators, S6K and eukaryotic translation initiation factor 4E-binding protein-1 (56–58). Ultimately, activation of mTOR results in cell growth, cell proliferation and resistance to apoptosis.

An important convergent point for these signaling cascades is the tumor suppressor, tuberous sclerosis complex (TSC) (reviewed in ref. 59–61). Briefly, the TSC binds to and sequesters Rheb, a G-protein required for mTOR activation, thus inhibiting mTOR and downstream targets. However, phosphorylation of the TSC elicits inactivation and Rheb is released, allowing for direct interaction with adenosine triphosphate (ATP) and subsequent activation of mTOR (62,63). Alternatively, when the TSC is inhibited, Rheb is able to phosphorylate and activate mTOR.

Energy balance can influence both the Akt and AMPK pathways of mTOR activation. For example, overweight and obese states are positively associated, as previously mentioned, with high serum levels of IGF-1. We and others have found that obesity is associated with enhanced induction of the PI3K/Akt pathway (64,65). In contrast, CR reduces steady state PI3K/Akt signaling as a result of decreased circulating levels of IGF-1 (64,66). Furthermore, genetic reduction of circulating IGF-1 mimics the effects of CR on tumor development and PI3K/Akt signaling (67). Additionally, the literature suggests that elevated cellular amino acid, glucose and ATP concentrations, as are present during high-energy conditions, signal for mTOR activation (68). Conversely, it has been shown that low glucose availability, high adenosine monophosphate:ATP ratios and decreased amino acids, such as those achieved during CR, can lead to growth arrest, apoptosis and autophagy through an AMPK-induced repression of mTOR (69).

**Leptin.** Leptin is a peptide hormone secreted from adipocytes that is involved with appetite control and energy metabolism through its effects on the hypothalamus. In the non-obese state, rising leptin levels result in decreased appetite through a series of neuroendocrine changes. The obese state is associated with high circulating levels of leptin (62,63,70,71), suggesting that the obese may develop leptin resistance. This resistance appears to explain much of the inability of exogenous leptin administration to prevent weight gain and may result in a higher ‘set point’ for body weight (72). The limited number of studies to date are suggestive of an association between circulating leptin levels and cancer risk, with the most consistent findings thus far for colon (73) and prostate cancer (particularly progression of prostate cancer, as suggested by Chang et al. (74) and Saglam et al. (75)). In vitro, leptin stimulates proliferation of multiple types of preneoplastic and neoplastic cells (but not ‘normal’ cells, as reported by Fenton et al. (33)), and in animal models appears to promote angiogenesis and tumor invasion (76).

The primary physiologic role of leptin may be the regulation of energy homeostasis by providing a signal to the central nervous system regarding the size of fat stores, as circulating leptin levels correlate strongly with adipose tissue levels in animals and humans (77). The canonical pathway that transduces leptin’s signal from its receptor is the Janus kinase 2/signal transducer and activator of transcription 3 pathway (78). Leptin may also exerts its metabolic effects, at least in part, by activating AMPK in muscle and liver, thus decreasing several anabolic pathways (including glucose-regulated transcription and fatty acid and triglyceride synthesis) and increasing several ATP-producing catabolic pathways (79). In addition, there is emerging evidence of cross talk between the Janus kinase/signal transducer and activator of transcription family of transcription factors, the insulin/IGF-1/Akt pathway and AMPK (80). Furthermore, leptin plays a role in regulating the hypothalamus/pituitary/adrenal axis and thus influences IGF-1 synthesis (79). Finally, leptin functions as an inflammatory cytokine and appears to influence immune function, possibly by triggering release of interleukin (IL)-6 and other obesity-related cytokines (33,81). Thus, although not well-studied to date, leptin is certainly positioned as a central player in the energy balance and cancer association.

**Adiponectin.** Adiponectin is a 28 kDa peptide hormone produced by adipocytes and intimately involved in the regulation of insulin sensitivity and carbohydrate and lipid metabolism. The link between adiponectin and cancer risk is not well-characterized, although there is a report that adiponectin infusion inhibits endothelial proliferation and inhibits transplanted fibrosarcoma growth (82). PLasma levels of adiponectin, in contrast with other adipokines, are decreased in response to several metabolic impairments, including type 2 diabetes, dyslipidemia and extreme obesity. This obesity-related decrease can be partially reversed by weight loss, although recent reports suggest these changes are relatively small unless there are drastic weight changes, such as occurs following severe CR or surgical intervention (83,84). Recent findings suggest leptin and adiponectin interact antagonistically to influence carcinogenesis (85,86), although this interaction has not been well established.

**Steroid hormones.** Steroid hormones including estrogens, androgens, progesterone and adrenal steroids, reportedly play a role in the relationship between energy balance and certain types of cancer. Adipose tissue is the main site of estrogen synthesis in men and postmenopausal (or otherwise ovarian hormone deficient) women, through the ability of aromatase (a P450 enzyme present in adipose tissue) to convert androgenic precursors produced in the adrenals and gonads to estrogens (22). In addition, adipose tissue is the second major source of circulating IGF-1, after liver. The increased insulin and bioactive IGF-1 levels that typically accompany increased adiposity can feedback to reduce levels of sex hormone-binding globulin, resulting in an increased fraction of bioavailable estradiol in both men and women (22). The epidemiologic literature clearly suggests that the increased bioavailability of sex steroids that accompanies increased adiposity is strongly associated with risk of endometrial and postmenopausal breast cancers (87) and may impact colon and other cancers as well. Adrenal glucocorticoid hormones may also play a role in the anticancer effects of CR, especially at restriction levels >30% CR, which markedly increase corticosterone levels in rodents (88–90). Glucocorticoid hormones have long been known to inhibit the anticancer effects of CR, especially at restriction levels. Birt et al. (88) have shown that the CR induction of corticosterone can inhibit protein kinase C and mitogen-activated protein kinase signaling, including reduced extracellular signal-regulated kinase-1 and -2 signaling and activator protein 1-DNA binding.

**Inflammation.** The association between chronic inflammation and cancer is well established (92), as is the link between obesity and inflammation (4). We and others have shown that CR decreases several markers of inflammation, including multiple cytokines (4,93). In general, acute inflammation is a process that is beneficial to the host by providing protection from invading pathogens and initiating wound healing. In the acute phase response, the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and IL-1β are produced locally at the site of infection by macrophages. These cytokines stimulate the release of IL-6, which has been shown to have both pro-inflammatory and anti-inflammatory effects (94), and the secretion of C-reactive protein by the liver into the blood (95). Following
clearance of the infection, the production of IL-1β and TNF-α is dampened by the production and release of IL-1 receptor antagonist and soluble TNF-α receptors, respectively, thus altering signal transduction via these receptors (96). Additionally, IL-10, an anti-inflammatory cytokine produced by T-lymphocytes, works by inhibiting the production of IL-6 and by deactivating pro-inflammatory macrophages (97,98).

In contrast to the acute inflammatory response, chronic (low grade) systemic inflammation, which typically accompanies obesity, has been described as a perpetual inflammatory process in which there is a 2- to 3-fold increase in the circulating levels of TNF-α IL-1β, IL-6, IL-1 receptor antagonist, soluble TNF-α receptors and C-reactive protein. Unlike acute inflammation, the origin of the cytokine cascade that accompanies obesity is not believed to be due to the presence of a foreign pathogen. The cause of elevated cytokines with chronic systemic inflammation is not well understood, although adipocytes as well as immune cells (such as macrophages) within adipose tissue of obese subjects are known sources of pro-inflammatory mediators such as IL-6, IL-1β and TNF-α. In addition, the type of macrophage present in the adipose tissue may also affect the extent and degree of the inflammatory response. Adipose tissue macrophages are recruited in response to the adipocyte enlargement that occurs due to excessive nutrient intake (99). Activated macrophages can polarize into either a classic pro-inflammatory M1 phenotype or an immunosuppressive M2 phenotype depending on the cytokine and chemokine environment. In the context of obesity, adipose tissue is associated with an increase in M1 and a decrease in M2 macrophages; however, these phenotypes exhibit a high degree of plasticity and are reversible given environmental changes within the adipose tissue (100,101). It is unknown at this time if CR can reverse the inflammatory M1 macrophage phenotype toward an anti-inflammatory M2 phenotype.

Sirtuins

Sirtuins are a family of proteins that have been implicated in the regulation of aging (102), transcription (103), endocrine signaling (104), stress-induced apoptosis (105) and most recently in metabolic changes associated with obesity (reviewed in ref. 106). Sirtuins were originally studied in the budding yeast Saccharomyces cerevisiae (107,108) and nematode Caenorhabditis elegans (109), where CR was shown to increase life span as well as increase the levels and activity of the Sir2 protein. In mammals, it has been shown that the levels of sirtuin type 1 (SIRT1), a mammalian homologue of Sir2, also rise during CR and promote long-term survival of cells. SIRT1 is a nicotinamide adenine dinucleotide+-dependent deacetylase that acts on Ku70, which in turn sequesters the pro-apoptotic factor Bax from the mitochondria, thus inhibiting stress-induced apoptotic cell death (105). Additionally, SIRT1 has been shown to repress peroxisome proliferators-activated receptor (PPAR)-γ by docking with its cofactors and thereby ultimately repressing PPAR-γ-responsive genes. This results in lipolysis upon CR and SIRT1 upregulation (110). Decreases in sirtuin levels during obesity, specifically SIRT1 levels, have been shown to regulate many other metabolic alterations linked to obesity. SIRT1 has been shown to play a role in regulation of adiponectin (111,112), insulin secretion (113), plasma glucose levels and insulin sensitivity (114) and regulation of oxygen consumption and mitochondrial capacity (115,116). Recent studies with thiazolidinediones, a class of synthetic antidiabetic drugs acting primarily through activation of PPAR-γ, have shown thiazolidinediones suppress breast carcinoma in vitro and in vivo. Reactivation of PPAR-γ in obese patients may interfere with estrogen receptor signaling and signal transducer and activator of transcription5 and nuclear factor-kappaB signaling cascades and up-regulate SIRT1 in diabetic patients (117,118). Another yeast Sir2 homologue, mammalian SIRT3, has been shown to be selectively downregulated at both the gene and protein levels in a mouse model of type 2 diabetes but not in a model of insulin deficiency without diabetes. In this study, insulin-deficient mice lacked muscle insulin receptor (muscle insulin receptor knockout mice) but maintained normal levels of insulin, glucose and insulin-regulated genes. The same muscle insulin receptor knockout mice with streptozotocin-induced diabetes, however, modeled the metabolic changes associated with type 2 diabetes, including downregulation of Sirt1 (119). These findings further suggest that sirtuins may be involved in the control of important downstream transcriptional regulatory mechanisms involved in glucose metabolism.

While CR has long been shown to have a dramatic effect on lifespan and tumor suppression in almost every tumor type tested, the specific role of sirtuins in cancer development/progression has yet to be elucidated (120). Studies have shown conflicting data as to whether SIRT1 can act as a tumor suppressor gene or an oncogene. SIRT1 is upregulated in several tumor types and can inhibit apoptosis and downregulate the expression of tumor suppressor genes to extend the longevity of epithelial cancer cells (121). SIRT1 is upregulated in tumors and cancer cells lacking the tumor suppressor gene, HIC1 (122), and upregulated in mouse and human prostate cancers (123). In addition, deleted in breast cancer 1-mediated repression of SIRT1 was shown to increase p53 function (124,125). However, there is also evidence that SIRT1 can act to suppress polyP formation in the adenomabut polyposis coli/intestinal tumor model (126). Additionally, preclinical studies of resveratrol, a phytochemical shown to activate sirtuins, have suggested that activation of SIRT1 may be a viable cancer prevention or therapy strategy (127). Conversely, it should be noted that histone deacetylase inhibitors, which regulate the expression of many protumor and antitumor genes, including sirtuins, have shown some success in preclinical studies and in combination therapy trials (128) and reviewed in ref. 129).

Calorie restriction mimetics

The findings mentioned above with resveratrol reinforce the notion that the identification and development of natural or synthetic agents that mimic some of the protective effects of CR may constitute a new strategy for cancer prevention. Given how difficult it is for many people to adopt a low-calorie diet for an extended period, the identification of drugs that could either complement or even reproduce the anticancer effects of CR without drastic changes in diet and lifestyle is a goal for many pharmaceutical companies. Numerous studies have used microarray analyses to profile the molecular targets responding to CR and other dietary energy balance modulations (130–135). Most of these studies were focused on understanding CR effects related to aging, and they revealed that the extent to which CR modulates the transcriptionome is species specific, tissue specific and dependent on the duration and intensity of CR. Nonetheless, some patterns from these studies have emerged, suggesting that transcripts involved in inflammation, growth factor signaling (particularly related to the insulin and IGF-1 pathways), oxidative stress and nutrient metabolism are commonly altered by CR (4). Application of the emerging field of metabolomics to this question should accelerate the identification of additional targets.

As described above, the IGF-1 and Akt/mTOR pathways have emerged as potential key mediators of CR’s anticancer and anti-aging effects and are initial targets for possible CR mimetics. Our studies with A-Zip/F1 mice, which lack white adipose tissue (and hence have very low adipokine levels) but are diabetic, display high levels of insulin, IGF-1 and inflammatory cytokines and are highly susceptible to cancer development, support the hypothesis that components of the insulin/IGF-1 and inflammatory pathways may be key targets breaking the obesity-cancer link (20). Agents or interventions that reduce IGF-1 or inhibit one or more components of the signaling pathways downstream of IGF-1 and other growth factors (including Akt and mTOR) without requiring drastic dietary changes, may provide an effective physiological or pharmacological mimetic of those effects, which could be readily adopted by a large proportion of the population, particularly those at high risk for cancer or other chronic diseases associated with obesity. Small-molecule inhibitors of IGF-1 (136), as well as antisense inhibitor approaches (137) and anti-IGF-1 antibody therapies (138), are under development. In addition, a wide variety of agents with demonstrated cancer chemopreventive or chemotherapeutic activity have recently been reported to inhibit the IGF-1
pathway, including retinoids, soy isoflavones, flavonoids and somato-
statin analogues (139–142). mTOR inhibitors have also emerged as potential CR mimetics. In particular, a recent report that rapamycin treatment, even when started late in life, extends life span and delays cancer in mice suggests mTOR is indeed a target for mimicking the effects of CR (143). Genetic induction of the Sir2/SIRT1 family of nicotinamide adenine dinucleotide-dependent deacetylases has been shown to mimic some of the effects of CR (126). Sirtuin modulators, including resveratrol and its analogues (144), as well as pharmacolo-
logic modulators of SIRT1 (145), have also shown some anticancer activity, although much of this work has been limited to in vitro systems and awaits verification in vivo.

Conclusions
This review discusses promising molecular targets for cancer prev-
vention, particularly components of the IGF-1/Akt/mTOR pathway, adi-
pokine pathways, inflammatory pathways and sirtuin pathway, based on the lessons learned from many years of CR research. Clearly, no single pathway accounts for all of the anticancer effects of CR. As with most chronic disease intervention strategies, combination ap-
proaches that target multiple pathways (and that maximize efficacy and minimize adverse effects) will probably be most successful for preventing cancer. An emerging issue in this area is the relative effects of nature versus nurture, i.e. the contributions of systemic factors (which has been the focus of this review) in the context of cell autonom-
ous effects. The recent observation by Kalanay et al. (146) of cancer cells with constitutively activated PI3K mutations are prolif-
erative in vitro in the absence of insulin or IGF-1 and form CR-
resistant tumors in vivo illustrates this issue. These findings suggest that cell autonomous alterations, such as activating PI3K mutations, may influence the response of cells to CR or CR mimetics. Future studies aimed at further elucidating the mechanisms underlying the anticancer effects of CR and that exploit this mechanistic information to target CR-responsive pathways through combinations of dietary and pharma-
cologic approaches, will facilitate the translation of the past 30 years of CR research into effective cancer prevention strategies in humans.

Acknowledgements
Conflict of Interest Statement: None declared.

References
491.
4. Hursting,S.D. et al. (2003) Calorie restriction, aging, and cancer prev-
13. Pariza,M.W. et al. (1987) Historical perspective: calories and energy expend-
15. Heilbronn,L.K. et al. (2006) Effect of 6-month calorie restriction on bio-
markers of longevity, metabolic adaptation, and oxidative stress in over-
weight individuals: a randomized controlled trial. JAMA, 295, 1539–1548.
18. Velthuis-ite Wierik,E.J. et al. (1994) Energy restriction, a useful interven-
26. Sara,V.R. et al. (1990) Insulin-like growth factors and their binding pro-
29. Resnicoff,M. et al. (1995) The insulin-like growth factor I receptor pro-
cer, 65, 311–320.
32. Singh,P. et al. (1996) Proliferation and differentiation of a human colon cancer cell line (CaCo2) is associated with significant changes in the expression and secretion of insulin-like growth factor (IGF) IGF-II and IGF binding protein-4: role of IGF-II. Endocrinology, 137, 1764–
1774.
33. Fenton,J.I. et al. (2005) Leptin, insulin-like growth factor-1, and insulin-