Macronutrient Intake and Cancer: How Does Dietary Restriction Influence Tumor Growth and Why Should We Care?

Perspective on Rogozina et al., p. 712

Michael Pollak

Abstract This perspective on the report by Rogozina and colleagues (beginning on page 712 in this issue of the journal) reviews mechanisms that may underlie inhibitory effects of dietary restriction on tumor growth in the mouse mammary tumor virus transforming growth factor alpha (MMTV-TGF-alpha) breast cancer model and comments on the potential clinical relevance of these mechanisms. The inhibitory effect of caloric restriction on carcinogenesis and tumor growth in rodent models is a classic finding that is receiving increasing attention. In some experimental models, the magnitude of the effect is significant, rivaling what can be achieved by pharmacologic approaches to cancer prevention or treatment. Major challenges include defining the underlying mechanisms and determining the clinical relevance of laboratory models of caloric restriction.

Early reports showing that dietary restriction has an inhibitory effect on the growth of experimental cancers were published nearly 100 years ago (1), and these results have been confirmed in dozens of subsequent studies. Recently, there has been a resurgence of interest in this field (2), and progress is being made in defining the underlying mechanisms. Key points are briefly reviewed below in the context of the report by Rogozina et al. (3).

Does Dietary Restriction Limit Nutrient Supply to Cancers?

The nutrient supply available to cancers in relation to host dietary restriction is a fundamental issue: does starving the host reduce energy supply to cancer cells? In diet-restricted mice (and humans), physiologic mechanisms maintain adequate nutrients in the circulation at times of reduced food ingestion, not only by maximizing hunger-stimulated food-seeking behavior but also by breaking down glycogen and, if necessary, by reducing muscle and fat mass. Because these mechanisms are effective, hypoglycemia does not occur until starvation is advanced. Therefore, moderate dietary restriction is unlikely to inhibit carcinogenesis or tumor growth by causing a reduction in nutrient supply to at-risk or already transformed cells. It must be acknowledged, however, that this is a plausible assumption rather than a proven fact, because studies of caloric restriction in relation to carcinogenesis, including the report by Rogozina and colleagues (3) in this issue of the journal, generally do not include measurements of the impact of variations in diet on levels of circulating nutrients such as glucose, fatty acids, or amino acids.

If moderate dietary restriction does not reduce the concentration of nutrients in the circulation, how can it influence carcinogenesis or neoplastic proliferation? It is likely that the mechanism is indirect and involves an influence of diet on hormones and, secondarily, of hormones on carcinogenesis and/or neoplastic behavior. There are many candidate hormones whose levels vary with nutritional state, including insulin, insulin-like growth factors (IGFs), glucagon, leptin, fgf21, adiponectin, and others. Changes in these hormone levels can be sensed by a subset of at-risk and transformed epithelial cells, and this effect can influence cancer biology.

In tissue culture, it is easy to create conditions of suboptimal nutrient availability because the whole-organism control systems designed to maintain stable circulating nutrient levels are absent. Cellular consequences of reduced energy supply are sensed by AMP kinase (AMPK), which is activated when the ratio of AMP to ATP increases, and by mammalian target of rapamycin (mTOR), which only transduces signals from upstream pathways when intracellular nutrients are adequate. In untransformed and some transformed cells, these control systems downregulate processes that consume energy, such as protein translation and proliferation, and promote autophagy. The physiologic purpose of this regulation is to maximize chances of cell survival at times of energy stress by avoiding a prolonged cellular energy deficit that would result in cell death. Therefore, in certain tissue culture models, a moderate restriction in nutrient supply reduces proliferation as a consequence of a physiologically regulated reduction in energy consumption, which is secondary to activation of AMPK and/or inhibition of mTOR. This effect must be

Author's Affiliation: Division of Cancer Prevention, Department of Oncology, McGill University and Segal Cancer Centre, Montreal, Quebec, Canada
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Requests for reprints: Michael Pollak, 3755 Cote Sainte Catherine, Montreal, Quebec, Canada H3T 1E2. Phone: 514-340-8222, ext. 4139; Fax: 514-340-8600; E-mail: michael.pollak@mcgill.ca.

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distinguished from the cell death caused by nutrient restriction severe enough to cause depletion of cellular energy reserves.

Dietary Restriction Can Lead to Endocrine Changes That Reduce Proliferation

For complex organisms, it is interesting that diet-induced changes in levels of certain circulating hormones perturb intracellular signaling systems in ways that are similar to perturbations resulting directly from a decreased environmental nutrient supply to single cell organisms, and also are similar to perturbations caused by reducing nutrient concentration in tissue culture media of cells propagated *in vitro*. As examples, a low circulating insulin level, which is seen at the whole-organism level when nutrient consumption is reduced, leads to decreased activation of mTOR, and reduced mTOR activation is also seen in association with reduced nutrient levels in tissue culture. In a similar way, a high circulating adiponectin level, which occurs in lean (as compared to obese) subjects, activates AMPK, which also can be activated *in vitro* directly by a high cellular AMP-ATP ratio occurring at times of reduced energy supply (4). Therefore, endocrine signals can substitute to some extent for direct changes in nutrient supply in controlling cellular energy consumption. This substitution can allow cellular energy utilization to be regulated in relation to varying dietary intake by the host, even when circulating nutrient levels vary minimally.

The influences of each diet-regulated hormone on cancer biology have not been fully characterized, but considerable data are available about the most-studied hormones, such as insulin and IGF-I. These hormones are considered to be likely mediators of the effects of macronutrient intake on neoplasia because (a) their receptors are present on neoplastic cells (5–7), (b) they have important pro-proliferative and anti-apoptotic actions (reviewed in ref. 8), and (c) insulin and IGF-I levels vary substantially with energy intake, the former from hour to hour and the latter over longer time frames. Strong circumstantial evidence from both population (8, and reviewed in refs. 9, 10) and experimental (refs. 11–15, for example) studies indicates that these hormones influence cancer risk and cancer behavior. As their levels vary with nutritional status, they are candidate mediators of the effect of varying macronutrient intake on neoplasia. The existing evidence, however, falls short of a formal demonstration of causality, which would require strategies such as measuring differences in the effect of diet on tumor behavior between a control cancer model and the same model engineered to lack receptors for these hormones. There is evidence (16) that the transcription factor NF-E2-related factor (Nrf2) is mechanistically involved in mediating the effects of caloric restriction in certain chemical carcinogenesis models.

Do All Cancers and Cells at Risk for Transformation Respond Equally to Dietary Restriction?

The recent demonstration that cancers with mutations that result in constitutive activation of the phosphatidylinositol-3-kinase (PI3K)-AKT pathway are relatively resistant to the growth-inhibitory effects of caloric restriction (17) is consistent with prior evidence (11) that caloric restriction acts to restrict neoplastic proliferation by reducing signaling through insulin-IGF receptors. One would predict that constitutive activation of the critical pathway downstream of the receptors would make any reduction in ligand levels by dietary measures irrelevant, and this is what was observed.

The lack of efficacy of caloric restriction in models of mutational activation of the AKT pathway (17) contrasts with the observations of Rogozina and colleagues in the mouse mammary tumor virus (MMTV) transforming growth factor alpha (TGF-alpha) model (3), in which dietary restriction led to a reduction in mammary tumor number and size. The basis for this difference is a matter of speculation, as neither tumor-signaling endpoints nor tumor-growth curves were presented in the Rogozina study. It is possible (a) that the degree of overexpression of TGF-alpha in the MMTV-TGF-alpha transgenic model is sufficient to cause transformation but inadequate to maximize AKT pathway activation and (b) that further signaling from the insulin-IGF-I receptor family can increase pathway activation and tumor aggressivity, thus providing an opportunity for the observed effects of diet-induced reduction in ligand levels to reduce cancer growth.

It is plausible that dietary restriction may be more effective in inhibiting carcinogenesis than in reducing the growth of established tumors because the molecular mechanisms implicated in resistance to caloric restriction, such as constitutive activation of the AKT pathway, are generally not present in untransformed cells.

The observation (3) that tumor-growth inhibition was higher with intermittent than with continuous caloric restriction is intriguing and correlates with the different effects of these dietary regimes on the hormones that may mediate the effects of diet on cancers. Describing the basis for the different effects of the two dietary restriction regimes on the circulating ligand levels will be an important next step, and it also will be necessary to determine if the different effects of the dietary regimes can be correlated with different degrees of inhibition of the PI3K-AKT pathway.

What about Overfeeding?

If caloric restriction can inhibit the growth of some cancers, can overfeeding accelerate neoplastic growth? If we postulate that similar mechanisms are involved, we would need to know if overeating can raise insulin and/or IGF-I levels and if such changes would increase AKT pathway activation in tumors above that seen with a control diet.

This question is more complex than it seems, both for mice and humans. The evolutionary pressure to develop control systems to conserve energy during starvation was strong; no such pressure existed to increase energy consumption during times of plenty, although energy storage in specialized cells such as adipocytes during times of plenty is clearly advantageous. Behavioral responses to the availability of excess macronutrients differ between individual humans and different mouse strains to a much greater extent than do the responses to restriction. Nutrient restriction leads uniformly to hunger that drives food-seeking behavior. Excess nutrient availability may or may not lead to increased consumption, as determined by genetic and/or cultural factors that influence hunger and appetite. Furthermore, between-person variability in endocrine response is greater with overfeeding than with starvation. Notwithstanding these complexities, there is epidemiologic (10, 18, 19) and experimental (ref. 13, for

example) evidence that overfeeding can promote aggressive neoplastic disease, and it is likely that this effect involves the same mediators as those involved in the effects of caloric restriction.

Does This Research Have Clinical Implications?

Because obesity is associated with hyperinsulinemia, there is reason for concern that the "obesity epidemic" may lead to an increased prevalence of a hormonal profile associated with elevated cancer risk and/or an adverse cancer prognosis. Therefore, in addition to its well-known general health benefits, maintaining an ideal body weight is also important in the specific contexts of cancer prevention and improving the prognosis of cancer patients (20).

Severe macronutrient restriction is likely an impractical cancer-control strategy. An area that deserves investigation, however, is pharmacologic interventions that mimic endocrine effects that mediate the benefits of dietary restriction. The possibility of developing "caloric restriction mimetics" has been explored in longevity research because it is known that modest caloric restriction extends life expectancy in animal models (21). It now deserves rigorous study in the specific contexts of cancer prevention and treatment.

Compounds that target members of the insulin-IGF receptor family are already in clinical trials (see refs. 8, 22 for a history of this field), and early results have been sufficiently encouraging to justify expanding this research; more than 50 ongoing clinical trials of more than 10 drug candidates are now in progress. An important related project underway in many laboratories is a comparison of the efficacy of these agents with that of macronutrient restriction in animal models of carcinogenesis or cancer progression. At present, these compounds are being studied clinically in the context of treatment rather than prevention.

Metformin is one of the most widely prescribed drugs in the world. Its current indications include type II diabetes and polycystic ovary syndrome. In view of its insulin-lowering properties (reviewed in ref. 8), it also deserves consideration in the context of drugs that mimic beneficial aspects of dietary restriction. It is convenient and safe and deserves investigation in both the prevention and treatment contexts.

The mechanisms of action of metformin and related compounds are complex and only now are being defined in detail (23–26), although the drug has been widely prescribed for decades. Major consequences of metformin administration include reduced gluconeogenesis and decreased hepatic glucose output. These actions reduce circulating glucose levels, which results in decreased insulin secretion by pancreatic beta cells and decreased circulating insulin levels. Metformin acts in hepatocytes by activating the LKB1-AMPK signaling pathway (mentioned above as a pathway activated by high cellular AMP-ATP ratios resulting from nutrient deprivation). It does so by acting on mitochondria, in which it impairs (but does not completely inhibit) oxidative phosphorylation. This impairment reduces ATP production, leading to high AMP-ATP ratios, which activate the LKB1-AMPK pathway.

In a similar way, metformin also activates the LKB1-AMPK pathway (unless it has been rendered inoperative by mutation or other mechanisms) in carcinoma cells (26). Of course, unlike the situation in hepatocytes, this activation has no consequences in terms of gluconeogenesis because neither normal or transformed epithelial cells have important roles in, or the enzymatic machinery for, storing energy or releasing glucose. Rather, AMPK activation in these cells results in inhibition of energy-consuming processes such as protein translation and proliferation, allowing metformin to act as an inhibitor of proliferation (26, 27).

Therefore, metformin has both host effects (lowering insulin) and direct effects (such as reducing protein translation), which may confer antineoplastic activity. With wide use and a favorable safety profile, this and related compounds deserve investigation in the contexts of both cancer prevention and treatment. Early evidence suggests that metformin efficacy may vary according to host metabolic characteristics (28), with benefit confined to hyperinsulinemic subjects.

Retrospective studies show that diabetic patients have a less-than-expected cancer incidence if they are on metformin (29, 30) and that diabetic breast cancer patients had superior rates of pathologic complete response to neoadjuvant chemotherapy if they took metformin (31), intriguing findings that require follow-up. Translational research, including ongoing early-phase clinical trials with biomarker endpoints, may justify larger clinical trials of metformin with efficacy endpoints.

Future research may find that metformin is a useful cancer prevention and/or treatment agent that mimics some of the mechanisms that operate in the reduction of proliferation by dietary restriction. Therefore, comparing the activity of metformin to that of caloric restriction in models such as that used by Rogozina and colleagues (3) as well as the Sabatini lab (17) is an important direction of research in this area.

The ability of dietary restriction to reduce tumor growth was observed early in the twentieth century. Now, nearly 100 years later, our improving mechanistic understanding of this phenomenon is pointing the way to new cancer prevention and treatment strategies.

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