REVIEW

Mechanisms Linking Diet and Colorectal Cancer: The Possible Role of Insulin Resistance

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Abstract: Diet is clearly implicated in the origin of colorectal cancer, with risk factors for the disease including reduced consumption of vegetables, fiber, and starch and increased consumption of red meat and animal fat. Several hypotheses have been developed to explain these associations. Most recently, McKeown-Eyssen and Giovannucci noted the similarity of the risk factors for colorectal cancer and those for insulin resistance and suggested that insulin resistance leads to colorectal cancer through the growth-promoting effect of elevated levels of insulin, glucose, or triglycerides. We briefly review the evidence from observational, epidemiological, and experimental animal studies linking diet with insulin resistance and colorectal cancer. The evidence suggests that diets high in energy and saturated fat and with high glycemic index carbohydrate and low levels of fiber and n–3 fatty acids lead to insulin resistance with hyperinsulinemia, hyperglycemia, and hypertriglyceridemia. We then consider how insulin, the related insulin-like growth factors, triglycerides, and nonesterified fatty acids could lead to increased growth of colon cancer precursor lesions and the development of colorectal cancer. Finally, we consider the implications of this scheme on possible future research directions, including studies of satiety and clinical tests of the importance of insulin resistance in the colon carcinogenesis process.

Introduction

Population, case-control, and cohort studies are generally consistent in demonstrating that colon cancer is associated with a sedentary lifestyle and a diet high in meat and fat and low in grain, fruit, vegetables, and fiber (1–4). These risk factors are usually thought to act by luminal mechanisms, by directly interacting with colon cells. Thus the apparent protection by fiber has been attributed to its action on fecal fermentation and to the formation of short-chain fatty acids and apparent *N*-nitroso compounds in the fecal stream (5,6); the protective effect of fruits and vegetables has been attributed to their content of antioxidants or inhibitory compounds such as allyl sulfides (7,8). The apparent risk of fat and meat has been attributed to increased levels of toxic bile acids and to increased exposure to carcinogenic heterocyclic amines formed from heating proteins (9,10). Support for each of these hypotheses can certainly be cited. However, the similarity in the risk factors for many cancers (2), and perhaps other common chronic diseases in developed countries, suggests that there is a more general problem with our lifestyle and diet.

This review is concerned with developing a deeper understanding of the mechanisms linking dietary risk and cancer that are involved in the metabolic pathogenesis of colon cancer. It is based on a hypothesis developed to explain epidemiological associations and on preliminary animal studies that support this hypothesis. The hypothesis is that metabolic and signaling pathways involved in insulin resistance are also involved in promotion, progression, and possibly initiation of colon cancer.

Insulin Resistance Hypothesis

McKeown-Eyssen (11) and Giovannucci (12) noted several years ago that the risk factors for colon cancer were remarkably similar to those for insulin resistance. Insulin resistance is a condition in which higher levels of insulin are needed to normalize plasma glucose and is associated with an increased risk of type 2 diabetes. It was suggested that lifestyle and dietary factors first lead to insulin resistance and that some factors associated with insulin resistance lead to colon cancer promotion. The metabolic consequences of insulin resistance include hyperinsulinemia, hyperglycemia, and/or glucose intolerance, hypertriglyceridemia, increased plasma nonesterified fatty acids (NEFA), and increased abdominal fat. McKeown-Eyssen and Giovannucci suggested that

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hyperinsulinemia could act as a growth factor and tumor promoter. McKeown-Eyssen suggested further that hyperinsulinemia and hypertriglyceridemia could act to increase cellular energy and the growth of cancer cells. Because excess fuel availability has also been proposed to characterize a diet associated with insulin resistance (13), it is also possible to envisage a situation where diet would lead to insulin resistance and colon cancer through some common mechanism related to energy excess.

Accumulating epidemiological evidence continues to support the "insulin resistance" hypothesis. First, studies continued to show that colon cancer patients frequently have evidence of glucose intolerance and insulin resistance (14), although it is possible that this is secondary to the cancer itself. Second, cohorts of type 2 diabetes patients have been found to have an excess mortality due to colon cancer (reviewed in Reference 15). Third, prospective case-control and cohort studies have shown a clear association of early colon cancer, colon cancer, and colonic polyps with increased levels of fasting insulin, triglycerides, very-low-density lipoproteins, and abdominal obesity (G. E. M. Eyssen and others, unpublished data; 17–19). Fourth, a case-control study of the diet records of subjects who developed colonic polyps has shown that the subjects consumed carbohydrate with a higher glycemic index than controls (G. E. M. Eyssen and others, unpublished data). Diets with a high glycemic index are thought to be associated with insulin resistance (21). Recent case-control studies have also shown an association between plasma insulin-like growth factor I (IGF-I), which is often increased in insulin-resistant individuals, and IGF-binding protein (IGF-BP) levels with risk for colon cancer (22,23).

Experimental Evidence Supporting a Role of Insulin Resistance in Colon Carcinogenesis

Much of our understanding of the role of diet in insulin resistance and colon carcinogenesis has come from animal studies (24,25). The literature relating to insulin resistance and that relating to carcinogenesis have largely been separate, with few studies exploring the interrelationships of the two diet-related phenomena. Our studies over the past five years have sought to develop an understanding of the interrelation of the two fields. We used azoxymethane-induced colon cancer in the Fischer 344 rat as our model system. We used the rat because this species is widely used in physiological studies of insulin resistance, the Fischer 344 strain, because it has been studied in detail in many carcinogenesis studies, and azoxymethane as the carcinogen because it produces a consistent tumor yield in a process that closely approximates morphologically that observed in humans.
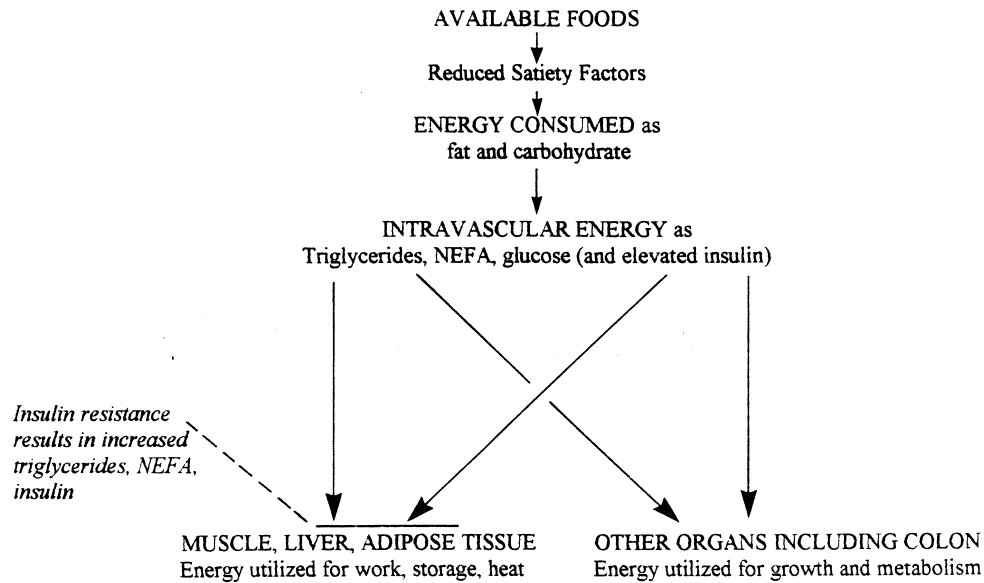
The first studies of the proposed insulin-resistance mechanism examined the effect of exogenous insulin. We noted a nearly twofold increase in the number of colon tumors in carcinogen-treated rats given insulin compared with control animals given only vehicle (26). Corpet and others (27)

noted a significant promotion of the growth of aberrant crypt foci (ACF), a widely used putative precursor of colon cancer (28,29), in carcinogen-treated rats given insulin in a short-term study of 100 days. We have extended these observations further (T. T. Tran and others, unpublished data) by examining other metabolic consequences of insulin injection. We noted, as expected, that insulin injections (repeated 5 days/wk) increased plasma insulin concentration to >10 times normal levels, reduced the concentrations of plasma glucose, led to significantly increased food consumption over the five injection days, and led to a significant increase in serum triglyceride. These complex changes make it difficult to ascribe the promotional effects directly to only insulin.

We then used an approach based on evidence from human (2,30) and animal studies (31,32) showing that increased dietary saturated fat leads to insulin resistance and to promotion of colon cancer (33). We argued that if insulin resistance is indeed the cause of promotion, animals fed a high saturated fat diet should show evidence of insulin resistance before they show evidence of promotion. We tested this supposition by using impaired glucose tolerance as an indirect measure of insulin resistance and ACF growth as the measure of tumor promotion. We found that the high-fat diet led to decreased glucose tolerance by 35 days, a time that was much earlier than 100 days, when promotion of cancer was evident. The result was consistent with the hypothesis but, of course, did not establish a causal relationship between insulin resistance and tumor promotion.

We next examined the effect of several other dietary factors on the association of glucose intolerance and ACF promotion (34). ACF promotion is known to be affected not only by fat, but also by dietary energy, the content of n–3 fatty acids in the fat, and the glycemic index of the carbohydrate portion of the diet. Similar effects were also known to occur with insulin resistance. We argued that if insulin resistance leads to tumor promotion, diets differing in these factors should produce similar effects on ACF growth and on glucose intolerance. This expectation was tested with diets that differed in quantity of fat, content of n–3 fatty acids, and dietary energy. The results of this study showed that glucose intolerance and ACF promotion were closely related over a wide range of diets. Furthermore, both were also related to fasting insulin and triglyceride levels as well as energy consumed (34).

Fasting levels may not be as representative of the metabolic effects of insulin resistance as postprandial levels. We therefore also examined the effect of the dietary factors on metabolic factors in studies in which blood samples were collected over 24 hours (Chia and associates, unpublished observations). After four weeks on a high-fat diet, the postprandial (9 PM–5 AM) insulin, triglyceride, and NEFA levels were elevated, nearly threefold for the NEFAs, whereas glucose was little affected. The high-fat diet was also, as expected, associated with increased intracellular triglyceride in muscle and liver (35). Interestingly, triglycerides were also increased in purified colonic epithelial cells and even in the spleen.

AVAILABLE FOODS
↓
Reduced Satiety Factors
↓
ENERGY CONSUMED as
fat and carbohydrate
↓
INTRAVASCULAR ENERGY as
Triglycerides, NEFA, glucose (and elevated insulin)

Insulin resistance results in increased triglycerides, NEFA, insulin

MUSCLE, LIVER, ADIPOSE TISSUE
Energy utilized for work, storage, heat

OTHER ORGANS INCLUDING COLON
Energy utilized for growth and metabolism

Figure 1. Proposed model linking diet, insulin resistance, and colon carcinogenesis. Insulin resistance, with hypertriglyceridemia, elevated nonsterified fatty acids (NEFA), and hyperinsulinemia, occurs when energy is consumed in an amount in excess of that utilized in muscle, liver, and adipose cells for mechanical work, storage, and conversion to heat. Other cell populations, such as colonic epithelial cells, are exposed to elevated levels of insulin (with normal or elevated levels of glucose) and lipids. Through mechanisms considered in text, this can result in initiation, promotion, and progression of colon cancer.

Mechanisms Involved in the Development of Insulin Resistance

An abbreviated summary of the pathways involved in energy utilization and of the effect of insulin resistance is shown in Figure 1. Satiety factors affect the amount of food consumed and energy intake. The energy substrates that are absorbed enter the vascular system largely as triglycerides and glucose. A large fraction of these substrates is captured by the liver, muscle, and adipose tissue, where they are stored, as triglycerides in adipocytes and as glycogen in liver and muscle cells, or are converted to mechanical energy in muscle or to heat in adipocytes. The intravascular levels of triglycerides normally fluctuate considerably after meals, but the levels of glucose show less change because of the modulating effect of pancreatic insulin production.

Insulin resistance is seen when there is a continued excess in energy intake compared with energy utilization. With excess availability of energy substrates, liver and muscle cells become less responsive to insulin, and glucose concentration tends to rise. The result is that there is a higher level of insulin production and higher intravascular levels of insulin, triglyceride, NEFA (from increased lipolysis with the expanded fat stores), and possibly glucose.

The mechanism(s) by which high fat and energy result in a reduced response to insulin is not completely clear, although it is possible to demonstrate insulin resistance in a relatively short period of time with experimental animal and clinical models (36,37). Typically, a triglyceride emulsion is administered intravenously with heparin to activate lipoprotein lipase and increase intravascular NEFA. This rapid increase in intravascular energy is usually found to reduce the effectiveness of insulin on glucose utilization within a period of a few hours. That is, insulin resistance is observed af-

ter administration of the intravenous NEFA. Less glucose needs to be infused to maintain plasma glucose "clamped" at its normal concentration when exogenous insulin is given at a constant, high rate (38).

For many years, it was thought that increased NEFA oxidation decreased the use and oxidation of glucose through substrate competition (also referred to as glucose-fatty acid competition or the Randle cycle) (39). Substrate competition, however, does not explain the phenomenon completely. For instance, increased levels of circulating NEFA reduce not only oxidative glucose uptake but also nonoxidative glucose uptake into glycogen (36). Furthermore, substrate competition does not explain why increased NEFA reduce insulin-stimulated glucose uptake but do not reduce basal glucose uptake (40).

Insulin resistance could also be explained by increased intracellular energy availability. In a state of energy excess, intracellular long-chain fatty acyl-CoA (LC-CoA) and triglycerides accumulate (41). This is because high glucose availability results in the activation of acetyl-CoA carboxylase and an increase in the formation of malonyl-CoA. Elevated malonyl-CoA inhibits carnitine palmitoyltransferase I, which is necessary for the transfer of fatty acids as LC-CoA through the mitochondrial membrane for oxidation and is thought to act as an intracellular signal of fuel availability. LC-CoA may inhibit glycogen synthase to reduce glycogen synthesis (42), or it may have allosteric effects on enzymes involved in glucose uptake (43). An accumulation of LC-CoA could also increase the concentration of diacylglycerol (DAG), an activator of protein kinase C (PKC) (44). This would result in a reduced responsiveness to insulin, because PKC is a serine/threonine kinase that phosphorylates the insulin receptor, leading to a decreased tyrosine phosphorylation by insulin. High-fat diets, with insulin resistance,

can upregulate muscle PKC (isoforms ε and θ) (45). Similar effects on intracellular energy might be expected with elevated levels of NEFA and glucose. Glucose, through glucolysis, can also increase DAG and activate PKC (46).

The development of insulin resistance could involve intracellular calcium. Intracellular calcium levels are normally reduced by insulin (47). With insulin resistance and with increased intracellular LC-CoA, this effect is impaired (48,49). The development of insulin resistance can also involve reactive oxygen intermediates (ROI), which are increased by NEFA and glucose (50). ROI may also contribute to the induction of insulin resistance through increased tumor necrosis factor-α signaling and gene expression (51).

Finally, insulin resistance could develop through effects of glucose and NEFA on transcription factors involved in the gene expression of metabolic enzymes. NEFA can affect gene expression by activating peroxisome proliferator agonist receptors (PPARs) (52–54). PPAR-α, -β, and -γ are nuclear receptors of the steroid-thyroid family that dimerize with retinoid X receptors to act as transcription factors for genes involved in fat metabolism. PPAR activation decreases insulin resistance. However, the mechanism by which insulin resistance is decreased by PPAR agonists is still unclear. It may involve the uncoupling proteins and an increase in energy dissipation (55,56). Interestingly, n–3 fatty acids, which increase insulin sensitivity, are better activators of PPARs than saturated NEFA, and conjugated linoleic acid is a potent and naturally occurring ligand and activator of PPAR-α (57). Transcription factors that bind carbohydrate-response elements have also been described (58).

The multiple and complex effects of diets high in energy content on intravascular energy substrates and intracellular signaling pathways make it difficult to define "a path" from diet to insulin resistance. Certainly, diets that result in insulin resistance increase intravascular insulin, triglycerides, NEFA, and possibly glucose, increase intracellular levels of LC-CoA and DAG, and may increase intracellular calcium and ROI and the activation of PKC. There is no reason to believe that these intracellular effects will be limited to only adipocytes and cells of the liver and muscle. Presumably, all cells exposed to the excess vascular levels of triglyceride, NEFA, and insulin could be subject to these effects, including cells of the colon.

Possible Mechanisms Linking Insulin Resistance and Colon Carcinogenesis

In animals and individuals with insulin resistance, colonic cells are exposed to elevated levels of intravascular insulin, triglyceride, NEFA, and possibly IGF-I and glucose. These elevated levels of hormones and energy substrates could have effects on cell cycle control, cell survival, and cell mutations in processes that could affect colon carcinogenesis.

Elevated levels of insulin can have growth as well as metabolic effects. The hyperinsulinemia associated with insulin resistance presumably leads to increased proliferation through the mitogen-activated protein (MAP) kinase pathway (59–61) and to reduced apoptosis (62) through the phosphatidylinostol 3-kinase and PTEN pathways (63). The metabolic effects of hyperinsulinemia associated with insulin resistance are attenuated in liver, muscle, and adipose tissue, but this may not be the case in other organs.

The importance of hyperinsulinemia for the association between type 2 diabetes and colon cancer is underscored by a recent report (64) that shows a stronger association between diabetes and colon cancer risk with shorter times since the diagnosis of diabetes. This probably would be due to increasing β-cell failure over the course of diabetes.

Elevated levels of insulin and increased nutrient availability can also have growth effects through an increase in IGF-I levels. Obesity and insulin resistance have long been associated with changes in IGFs and IGF-BPs. Insulin inhibits the production of IGF-BP1 in the liver. This can occur in the hyperinsulinemic insulin-resistant state. IGF-BP1 levels decrease and the corresponding free IGF-I levels may be elevated (65). Insulin and nutrients can also increase IGF-I production by the liver. However, in obese insulin-resistant individuals, total levels of IGF-I, bound and unbound, may be quite variable, although the levels described are usually normal or slightly elevated (66). Furthermore, the levels of IGF-I in tissues and their action there may not parallel the levels of IGF-I in plasma (67).

Normal and transformed colonic epithelium have receptors for IGF-I as well as insulin (68). Diet, in particular fat, can affect local IGF-I and IGF-I receptor expression in the colon (69) through luminal or generalized nutritional factors.

The increased proliferation and reduced apoptosis that result from the elevated insulin and IGF could act as a selective growth stimulus, leading to the expansion of clones of epithelial cells with defective growth control and promoting carcinogenesis. Such processes might be expected to select for cells with an overexpression of insulin and IGF-I receptors, and, indeed, tumors may have an excess of these receptors compared with normal colonic epithelium (70).

Elevated intravascular levels of the energy substrates, triglycerides and NEFA, together with the metabolic effects of insulin in the presence of normal or elevated glucose, increase intracellular energy substrates and affect important cell-signaling pathways (71). Increased intracellular triglyceride, LC-CoA, and DAG could activate PKC and MAP kinase and also stimulate cell growth (72). This pathway has been implicated in carcinogenesis (73,74). Although PKC activators do not stimulate normal colonic epithelial cells to proliferate, they do stimulate the proliferation of transformed cells that may lack functional tumor-suppressor proteins or may have a mutant hyperactive Ras (75,76).

Intracellular energy substrates may have effects on tumorigenesis through their effects on PPARs and gene expression, although these effects differ in different organs and may be consequently complex. PPAR-γ₁, for instance, is found in colonic epithelial cells (76,77) and colonic tumors (77–79) at levels only slightly below that found in adipose tissue. Thus, although PPAR-γ activation by thiazolidine-

dione inhibits the growth of breast (80) and prostate cancer (81), its effects on colon cancer are not consistent (76–78). It inhibits the growth and increases the differentiation of colon cancer cells (73,76) but increases the yield of colonic tumors in APC-*min* mice (77,78). Other PPAR activators, such as conjugated linoleic acid, appear to protect against colon cancer (82).

The effect of intracellular energy substrates on intracellular calcium may contribute to cell proliferation (83), but again the pathways are not clear. In colonic epithelia, proliferation is stimulated by low calcium concentrations and is inhibited by high calcium concentrations (84), although colon tumor cells can proliferate even further in the presence of low-to-intermediate calcium concentrations (84) and are insensitive to the growth arrest induced by high calcium (85).

Increased energy availability could also affect colon carcinogenesis through the formation of ROI. These can activate MAP kinase and increase the expression of oncogenes such as c-*fos* and c-*jun* (86). They can also lead to the formation of DNA oxidation products and increase mutation and the possibility for initiation (87). There is evidence that high levels of insulin, which lead to the accumulation of excess cellular energy, can result in the generation of lipid oxidation products (88) and the increased utilization of antioxidants (89). Hyperglycemia can also increase oxidative stress (50). Several studies have suggested an association of oxidative damage to DNA with diabetes and carcinogenesis (90,91). Diets low in fat are known to decrease the formation of oxidation products in human peripheral nucleated blood cells (92).

The putative pathways from diet to promotion assume that increased energy leads to epithelial cell proliferation and promotion. Certainly, the reverse is the case. Dietary restriction reduces cell proliferation (93), the rate of *in vivo* somatic mutations (94), and carcinogenesis, but an increased steady consumption of fat and energy does not always affect colonic proliferation (95). The crypts of animals fed such diets can be longer, and the pattern of labeling in the colon and crypt can be affected without increasing proliferation (96). Presumably, some as yet unidentified cell-signaling pathways balance the long-term production of cells from colon stem cells against the loss of cells into the lumen (97). However, the proliferative effect of dietary energy can be revealed by sudden increases in food consumption. Boluses of fat or sugar increase proliferation of cells in the colonic crypt a short time later (98,99), even in the human colon (100). This intermittent growth stimulus could again lead to the expansion of clones of epithelial cells with defective growth control.

Questions and Implications for Cancer Prevention

Perhaps the major question we now face is the relative importance of mechanisms related to insulin resistance to other mechanisms in human colon carcinogenesis. There are certainly other important mechanisms. The mechanisms responsible for the protective effect of nonsteroidal anti-inflamatory agents or for increased risk associated with the consumption of meat, with exposure to bile acids or with heritable risk factors, act through quite different mechanisms. Nevertheless, the mechanisms associated with insulin resistance appear to be major, modifiable determinants of cancer rates that could be exploited in cancer prevention.

The mechanisms described above and illustrated in Figure 1 appear to be consistent with the results of many experimental studies and may be a useful reference in considering ways in which cancer rates may be reduced.

First, it implies that the factor leading to promotion, increased exposure of colonic cells to the growth-enhancing effects of intravascular factors, is a consequence of an inappropriate excess energy intake for a given utilization rate. This implies a defect in the satiety mechanism with the current diet of populations at increased risk for colon cancer. Substantial efforts are being made to identify satiety factors in foods and pathways that influence food consumption rates and obesity. An identification of such factors could have important applications in the inhibition of carcinogenesis.

Second, excess energy intake could increase risk but may not itself be associated with obesity. This is because intermittent excess energy consumption and intermittent intravascular energy substrate excess could result in increased risk, even in the absence of excess average energy intake and in the absence of obesity. This consideration would suggest that attention should be given to the temporal pattern of energy consumption as well as the mean rate.

Third, proliferation and cancer risk might be decreased by increasing the disposition of intravascular energy substrates. This could be achieved by increasing mechanical activity as work, by increasing dissipation of energy as heat, and also, paradoxically, by increasing storage as fat. Increased energy storage may not appear to be an appropriate goal, although this could be one of the results of the use of agonists to the various PPAR.

Finally, it is clear from the epidemiological studies that there is a close association between insulin resistance, as assessed by increased plasma insulin, IGF-I, and triglyceride, and risk of colon carcinogenesis. There is also a biological basis for this association. To show that insulin resistance and the associated elevation of triglycerides, NEFA, and insulin are involved in the causative pathway, it is now necessary to demonstrate that reduction of insulin resistance results in reduced cancer risk in humans. Dietary and exercise interventions for insulin resistance have been investigated extensively (101). The putative risk factor markers, the intravascular insulin, IGF-I, and energy substrates, are readily measured, and surrogate end-point biomarkers for colon cancer, such as the ACF in the human colon, can now be quantitated (102). Thus it may now be possible to demonstrate a clear causative association of insulin resistance and colon carcinogenesis. This could lead to a more rational approach to the prevention of colon cancer.

Acknowledgments and Notes

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