EFFECTS OF SUCROSE, GLUCOSE, AND FRUCTOSE CONSUMPTION ON INTESTINAL TUMORIGENESIS IN APC^{MIN} MICE

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ABSTRACT

EFFECTS OF SUCROSE, GLUCOSE, AND FRUCTOSE CONSUMPTION ON INTESTINAL TUMORIGENESIS IN APC^{MIN} MICE

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Colon cancer incidence is strongly linked to dietary and lifestyle factors such as consumption of Western diets and refined sugars, specifically sucrose. The mechanistic pathways are not clear, yet there is evidence that carbohydrates may act on circulating glucose, insulin levels, and insulin-like growth factors (IGFs). The aim of this study is to determine the effects of feeding APC^{Min} as well as normal C57BL6/J mice diets containing corn starch (control), sucrose, glucose, fructose, or a 1:1 ratio of glucose:fructose (G:F) as the sole carbohydrate source. The G:F treatment is meant to mimic the composition of high fructose corn syrup (HFCS). Overall, mice fed sucrose diets for 10 weeks gained the most weight and body fat. Fructose- and starch-fed mice had the lowest body weights. Percent body fat was the same for mice fed starch, fructose, or G:F. In the small intestine (SI), the fructose-fed mice developed the most tumors. Sucrose-fed mice had fewer tumors in the distal third of the SI, yet were not different than the starch control overall. Sucrose-fed mice also tended to have higher plasma glucose and insulin while starch-fed mice had the lowest, yet these trends were not significant. There was no effect of dietary treatment on colonic tumors or plasma IGF-1 concentrations. Sucrose feeding resulted in higher body weights, body fat, and a shift towards insulin resistance, while fructose feeding resulted in an increased number of SI tumors. These results do not strongly support the hypothesis that intestinal tumorigenesis is driven by circulating glucose or insulin at 10 weeks. Also, G:F-fed mice tended to be more similar to those fed glucose rather than sucrose. This suggests that glucose is mediating some of the effects that are seen with fructose feeding.

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INTRODUCTION

Colon cancer continues to be a significant public health problem in the United States and worldwide. It is currently the third most common cancer diagnosed (excluding skin cancers) in men and women and the second leading cause of cancer deaths in the United States. However, the number of deaths attributed to colon cancer has been declining for at least 20 years, partly because of improved screening and treatment methods as well as the identification of specific risk factors.[1]

Colon cancer incidence is strongly linked to dietary and lifestyle factors. Those factors which are believed to most directly increase colon cancer risk are advancing age, consumption of red meat, processed meat products, alcohol consumption, as well as total body fatness and abdominal fatness.[2] Protective factors include physical activity and consumption of dietary fiber, calcium, milk and garlic.[2]

Several epidemiological studies have shown that typical Western diets (high in fats, sugars, red and processed meats and low in fruits, vegetables, and fiber) increase the risk of certain cancers, including colorectal cancers.[3-7] Furthermore, there is also evidence from epidemiological[8-12] and animal studies[13-17] that consumption of diets containing large quantities of sucrose and refined sugars are correlated to colon cancer risk.

The mechanisms whereby sugar consumption affect colon cancer development are not clearly understood. There is evidence that sucrose may act by stimulating insulin and insulin-like growth factor (IGF) responses. Some epidemiological evidence supports a positive association between high circulating insulin levels and development of colon cancer,[10, 18-20]

and animal studies have demonstrated that increased sucrose intake is associated with increased insulin and IGF concentrations.[16, 21, 22] Increases in circulating insulin and certain IGFs are also associated with increased development of cancer or precancerous lesions.[16, 23, 24] Specifically, IGF-1 is commonly correlated with cancer development in humans and animals.[19, 20, 25]

The purpose of this work is to compare the effects of sucrose to its component monosaccharides (glucose and fructose) on colon cancer development and determine if any effects are mediated through an insulin or IGF response. Also, a 1:1 ratio of glucose:fructose will be used to determine if the metabolic effects and colon cancer outcomes are similar to those of sucrose.

REVIEW OF THE LITERATURE

1. Overall Incidence of Colon Cancer

The overall lifetime risk of developing colon cancer in the US is about 1 in 20, with men having a slightly higher risk than women.[1] The American Cancer Society estimates that there will be approximately 102,000 new cases diagnosed and almost 50,000 colon cancer-related deaths in 2011 in the United States.[1] Worldwide, just over 1 million cases were reported in 2002, with mortality approximately half of the incidence. The USA, Australia, New Zealand and parts of Europe have the highest rates, up to 25-fold higher than those countries with the lowest rates, particularly Africa and Asia. The increase in colon cancer deaths in Japan was noted as far back as the 1980s and has been correlated with increased urbanization and shifts towards more Western-like dietary patterns.[26] Incidence is still increasing rapidly in Japan and other middle to low-income countries, yet seems to be stabilizing in northern and western Europe and the United States.[2]

In the US, the number of deaths attributed to colon cancer has been declining for the last 20 years, partly because of better prevention methods derived from the identification of specific risk factors. Incidence can be linked to certain genetic and lifestyle factors.[1, 2]

2. Risk Factors Associated with Colon Cancer Development

Many risk factors for development of colon cancer have been identified. The major inherited risk factors include known genetic mutations, history or family history of colon cancers or other intestinal diseases, and ethnicity. Diet and lifestyle factors also play a major role in colon cancer development and include many typical "Westernized" characteristics.

A. Inherited Risk Factors

The two recognized genetic conditions that account for 5-15% of colon cancer incidence are hereditary non-polyposis colorectal cancer (HNPCC) and inherited familial adenomatous polyposis (FAP).[2, 27] The more common form of hereditary colon cancer is HNPCC (also referred to as Lynch Syndrome). It is an inherited, autosomal dominant genetic trait which typically leads to onset of colon cancer at an early age (~45 years of age or less). Until the 1990s when germ-line mutations were discovered, HNPCC had been classically diagnosed by family history and other phenotypic distinctions. The underlying genetic causes are not well characterized, but mutations in many genes involved in DNA mismatch repair (including but not limited to hMLH1, hMSH2, hPMS2, hMSH3, and hMSH6) have been identified in 40-60% of classic HNPCC families.[28]

Familial adenomatous polyposis is a rare condition that causes only about 1% of colon cancers worldwide. Classically, FAP is characterized by the onset of hundreds to thousands of colorectal polyps early in life (50% of patients develop adenomas by age 15, 95% by age 35). Polyps are generally benign, but are likely to become cancerous over time. The cause of FAP is an inherited (autosomal dominant) germline mutation in the adenomatous polyposis gene (APC) gene that generally causes truncation of the APC protein product.[27] The APC protein is considered a tumor suppressor due to its role in the Wnt/ β-catenin signaling pathway, wherein it binds to β-catenin and targets it for degradation. When APC is absent (or nonfunctional, in the case of the truncated mutant version) and Wnt is present, β-catenin is not effectively bound and targeted for destruction and can accumulate to high levels. High cellular β-catenin levels can

alter expression of many proteins involved in cell proliferation, differentiation, migration, and apoptosis, including the proto-oncogene c-myc.[27, 29]

A small subset of FAP patients has a recessively inherited mutation in the MYUTH gene that causes clinically similar symptoms to those with APC mutations. The MYUTH gene codes for enzymes involved in DNA base excision repair, which is generally caused by oxidative damage. The MYUTH protein also has binding sites for proliferating cell nuclear antigen (PCNA) and may be involved in proliferation signaling pathways.[27]

Other inherited risk factors for developing colon cancer include a family history of the disease or preexisting inflammatory bowel disease such as ulcerative colitis or Crohn's disease.[2] Certain ethnic factors have also been identified as affecting risk. In the US, African Americans have the highest colon cancer incidence and mortality rates due to reasons which are still unknown. Worldwide, Jews of Eastern European descent (Ashkenazi Jews) have among the highest incidence rates due to a high incidence of gene mutations including, but not limited to, APC mutations.[1, 30]

B. Diet and Lifestyle Risk Factors

Sporadic colorectal cancers (those not linked to a genetic disorder) account for about 85% of cases worldwide.[27] Certain diet and lifestyle factors have been strongly linked to risk of developing sporadic colon cancer. Those factors which are believed to most directly increase colon cancer risk are advancing age, consumption of red meat, processed meat products, and alcohol, as well as total body fatness and abdominal fatness.[2] Protective factors include physical activity and consumption of dietary fiber, calcium, milk and garlic.[2] Limited evidence

for an increase in risk is also available for foods containing iron, animal fat, and sugars. Protective factors (with limited evidence) include fruit and vegetable consumption, fish consumption, and foods containing folate, selenium, or vitamin D.[2] The American Institute for Cancer Research estimates that nearly 45% of colon cancers in the US can be prevented by limiting the intake of red meat, avoiding processed meats, staying as lean as possible, limiting alcohol intake, and engaging in daily physical activity.

3. Diet and Colon Cancer Risk

Although there are many aspects of diet that may be related to colon cancer risk, not all will be addressed within the scope of this research. The two most relevant, the Western dietary pattern and sugar consumption, will be discussed at length.

A. Western Dietary Pattern – Epidemiological Evidence

The typical Western diet pattern exhibits many of the lifestyle risk factors cited above, with an absence of the protective factors. This diet can contribute to the reason why many developed nations have higher incidence of colon cancers. Several epidemiological studies have shown that consumption of typical Western diets (high in fats, sugars, red and processed meats and low in fruits, vegetables, and fiber) is associated with an increased risk of certain cancers, including colorectal cancers.

Using data from a cohort of about 76,000 US women, Fung et al[3] investigated the relationship of overall dietary patterns to colon cancer risk. A Western dietary pattern was defined by "higher intakes of red and processed meats, sweets and desserts, french fries, and refined grains," and a prudent dietary pattern was defined by "higher intakes of fruits, vegetables, legumes, fish, poultry, and whole grains." Out of the approximately 450 colon cancer cases

identified within a 12-year follow-up, persons consuming the Western diet had a higher risk of colon cancer [relative risk (RR) of 1.46 when comparing highest to lowest quintile of Western diet consumption]. The prudent pattern showed a non-significant protective effect for colon cancer risk. No associations were found among the approximately 100 cases of rectal cancer identified.[3]

One prospective study on dietary patterns and colon cancer risk used data from more than 60,000 Swedish women and identified 460 cases of colorectal cancer. No statistically significant associations with colon cancer risk were found in the Western diet consumers (processed and red meats, soda and sweets, refined breads and potatoes, and high-fat dairy products) or Healthy diet consumers (fruits and vegetables, fish and poultry, cereal and whole-grain breads, fruit juice, and low-fat dairy products). Those with dietary patterns low in "healthy" foods had a slight increase in colon cancer risk among women under 50; however, this group had a low incidence of cancer diagnosis.[4]

Through a case control study with approximately 2,000 men and women (and 2,500 controls) from Northern California, Utah, and Minnesota, Slattery et al[5] showed associations between the "Western" dietary pattern and an increased risk of colon cancer in both men and women. The Western diet pattern was described as having "high levels of red and processed meat, fast food, refined grains, and sugar containing foods, and low levels of vegetables (other than potatoes) and fruits, with the predominant fruit being canned fruit." A prudent diet was defined as "one in which all types of fruits and vegetables were consumed and fish and poultry were eaten more often than red meat or processed meat; this dietary pattern was inversely associated with most high sugar foods." For both men and women, the Western diet was a risk

factor in developing colon cancer [odds ratio (OR) of about 2], and the prudent diet was identified as a protective factor (OR of ~0.60). Associations of the Western diet with cancer risk were strongest for distal colon tumors in men, and associations with the Prudent diet were strongest for proximal colon tumors in both men and women.[5]

This study population also was used to study the effects of age at diagnosis, consumption of Western diets and family history of the disease. The Western diet has the most profound effect (OR 14.0) on cancer risk in cases that were diagnosed young (<55 years old) and had a family history of colon cancer (compared to those without a family history and the lowest Western diet consumption levels).[6]

Other studies have shown similar trends in dietary patterns and colon cancer incidence.[7, 11, 31-33]

B. Dietary Sugars – Epidemiological Evidence

Since the Western dietary pattern is associated with colon cancer risk, there also have been attempts to dissect this dietary pattern and identify specific components that contribute to increased risk. One major characteristic of the Western diet that is often examined is the relatively high consumption of refined carbohydrates and sugars, particularly sucrose. Since it can be difficult to accurately separate diet component interactions, there is limited epidemiological evidence that has shown that diets high in refined sugars are correlated with increased colon cancer risk.

Bostick et al[8] observed an association of sucrose consumption with colon cancer risk in a cohort of about 35,000 Iowan women. Four years after initial food intake screening, 212 cases of colon cancer had been diagnosed. Consumption of food and beverages sweetened with

sucrose was associated with an increase in colon cancer risk (RR of 2.0 for highest consumers of sucrose-containing foods compared to the lowest). This relationship was still present when the analysis controlled for milk and milk-containing foods such as ice cream. A similar association was also shown for sucrose consumption with a RR of ~1.8 when comparing the fourth highest quintile of sucrose consumptions to the lowest.[8]

Using participant data from the Women's health study, 174 cases of colon cancer were identified out of about 38,000 participants within an 8 year period. Of these, the highest quintile of sucrose consumers tended to be more likely to develop colon cancer than the lowest consumers (OR 1.51, P=0.06). The highest quintile of fructose consumers also tended to be more likely to develop colon cancer (OR 2.09, P=0.08), although neither of these effects achieved statistical significance at the $P<0.05$ level.[9]

Michaud et al[10] analyzed data from the Nurses' Health Study and the Health Professionals Follow-up Study over a 20-year follow up period and identified approximately 1,800 cases of colorectal cancers. In males, high consumption of sucrose and fructose was associated with an increased risk of colorectal cancers (ORs = 1.30 and 1.37 for sucrose and fructose, respectively). No significant effects were seen among the female population.[10]

In a case control study of a South Indian population, 108 hospitalized colon cancer patients (and 324 controls) were interviewed with food frequency questionnaires about their dietary patterns starting two years prior. The population consisted of a 2:1 ratio of males to females, and when all subjects were considered a strong association of high sugar intake (OR of 2.8 for highest quartile of sugar consumers) to colon cancer development was detected.[11]

One population-based case control study (approximately 2,000 cases and 2,400 control cases) also examined the relationships of sucrose and sugars intake with colon cancer incidence. In younger men (<67 years) the highest quintile of sucrose consumers were significantly more likely to develop colon cancer (odds ratio = 1.59) compared to the lowest quintile. Also, a high sucrose to dietary fiber consumption ratio was associated with an increased colon cancer risk in men (OR = 1.37), and an increase risk of proximal tumors in men (OR = 1.51) and women (OR = 1.38). However, when data for both sexes and all age groups were combined, sucrose consumption was not significantly associated with colon cancer risk.[12]

The relationships of glucose and fructose consumption to colon cancer risk were also investigated in the population mentioned above. Glucose consumption was not associated with colon cancer risk or tumor development in men or women. Women who consumed the highest fructose levels tended to have more colon cancer risk compared to those who consumed the least fructose (OR = 1.26). This fructose effect was more pronounced in older women (≥ 67 years old), but neither association was statistically significant (P=0.08 and P=0.10, respectively).[12]

C. Dietary Sugars – Experimental Evidence

Common approaches to studying colon cancer development in rodent models of human colon cancer utilize either chemical carcinogen-induced tumors or genetic knockout models which are predisposed to tumor development. Carcinogenesis can be induced in rats or mice using azoxymethane (AOM) or dimethylhydrazine (DMH) to test the inhibitory or promoting effects of the compound of interest. More recently, mouse models have been available that carry genetic mutations, such as those in the APC gene, which are analogous to common genetic mutations in human colon cancers.[34] As discussed in a previous section, mutations in the APC

gene greatly increase the risk of colon cancer in humans. The efficacy of both rodent models was recently reviewed and overall these models were found to provide results consistent with human clinical studies on factors influencing colon cancer risk. However, the main drawback of the APC^{Min} model (a mouse strain possessing a mutant allele of the APC gene which leads to intestinal tumorigenesis) is that tumors occur predominately in the small intestine, with a much slower development of tumors in the colon. Because of this, small intestine tumor development is a common endpoint in studies using APC^{Min} mice.[35]

When compared with starch, sucrose administered as boluses has been shown to increase colonic epithelial cell proliferation and aberrant crypt foci (ACF) formation. Increased epithelial cellular proliferation is believed to increase the chances of genetic mutations; therefore increased rates of cell proliferation can be a marker of the uncontrolled growth inherent to cancers.[36, 37] Aberrant crypt foci are generally considered precancerous lesions in rodent and human colons and are often used as a marker of risk in carcinogen induced rodent models.[38] In this study, when a single bolus of sucrose was given one day before AOM was administered, cell proliferation was increased ($P < 0.01$) after 30 days (compared to rodents treated with a starch bolus control). The same effect was seen 40 days after AOM injection when three sucrose boluses were given per week. Continuous feeding of sucrose did not produce an effect different than starch boluses (3/wk for 40 days).[13]

Another study demonstrated a similar effect in Sprague-Dawley rats treated with AOM. Feeding a high sucrose diet and a weekly dose of AOM for 8 weeks produced a higher number of colonic adenomas per rat compared to feeding a corn starch-based control diet (1.06 versus 0.30 adenomas/rat, $P<0.05$). Furthermore, the adenomas in the sucrose-fed mice were significantly

larger than those in the corn starch-fed group (0.99 cm^2 versus 0.56 cm^2) and had more of an invasive potential than those arising in the corn starch-fed group. Dysplasia and differentiation of colonic adenomas were similar among treatments.[14]

Caderni et al also demonstrated a relationship between feeding a high sucrose diet (46% w/w) and cancer development in rats treated with DMH. The number of foci of dysplastic crypts (FDC) was not affected, but after 30 days the percentage of large FDC (formed by 3-4 dysplastic crypts) was greater in sucrose-fed rats than in the starch-fed group (17% large FDC compared to 10%, $P<0.05$). After 105 days, the sucrose-fed group had more dysplastic crypts/ACF than the starch-fed group (2.9 versus 2.6, $P<0.05$). Also, colonic epithelial cell proliferation was significantly greater in the sucrose group (10 versus 4.5%, $P<0.001$).[15]

Wang et al[16] observed that the consumption of diets rich in sucrose (compared to corn starch) increased colon cancer risk in APC^{Min} mice. Each diet was fed for 18 weeks and outcomes measured included colonic tumor incidence and colonic epithelial cell proliferation. The sucrose-fed mice had a higher prevalence of colonic papillary tumors - 60% of mice in the sucrose group being affected compared to 30% in the control (corn starch) group. High sucrose diets were also associated with increased colonic epithelial cell proliferation.[16]

In a study that assessed the effects of different sugar and starch sources on ACF development in F344 rats treated with AOM, it was found that animals fed a high sugar diet (sucrose and dextrin) in the post-initiation period of ACF development had significantly larger numbers of ACF when compared with a control group fed a low sugar diet.[17] This suggests that the observed inconsistencies in studies examining the effects of sucrose on colon cancer

could be caused by differences in treatment exposures relative to the stage of cancer initiation and progression.

The effect of glucose, sucrose, and pasta consumption on intestinal carcinogenesis in rats was examined using a carcinogen induced model (AOM). Rats were fed high carbohydrate diets (44% wt/wt) for 230-254 days after two injections of AOM. At termination of the study, the incidence of intestinal adenomas was similar across all groups.[39]

Glucose, fructose, and sucrose effects on colonic epithelial cellular proliferation were also examined using female Sprague-Dawley rats. Animals were fed for one month and at study termination colonic epithelial cell proliferation was highest in rats fed sucrose. No differences were seen among the glucose and fructose groups compared to starch-fed controls.[40]

Stamp et al[41] used a carcinogen (AOM) induced mouse model to examine the relationship between consumption of simple sugar boluses to changes in colonic epithelial cellular proliferation and the development of ACF. In this study, two strains of mice were given oral gavages of sucrose, fructose, or glucose and compared to a water control. Colonic epithelial cell proliferation in the mice treated with 10 g/kg body weight (bw) fructose was highest compared to control (water). Sucrose administered at 20 g/kg bw showed similar results to the fructose group, while sucrose at 10 g/kg bw had significantly less effect on cell proliferation than either previous treatment. Colonic epithelial cell proliferation was no different in mice administered glucose gavages (each 10 g/kg bw) versus the control mice. Mice receiving sucrose (10 g/kg bw) then AOM had a 3-4 fold ($P < 0.001$) increase of ACF within 28 days compared to control. Fructose gavages also significantly increased ACF development compared

to control; however the effect was significantly lower than what was seen in the sucrose-treated group. Again, glucose gavages did not influence ACF development compared to the control.[41]

4. Glucose, Insulin, and Insulin-Like Growth Factors in Colon Cancer

One hypothesis for the varied effects of sucrose, glucose, and fructose consumption on cancer risk is based on the varied metabolic response to the consumption of each dietary sugar, particularly though blood glucose responses. Increased blood glucose will stimulate insulin secretion as part of normal glucose homeostasis.[42] In turn, elevated insulin levels have been shown to regulate the bioavailability of insulin-like growth factors (IGFs) that may be related to colon cancer risk.[25, 43]

A. High Glycemic Diets - Epidemiological Data

The glycemic index was first proposed in 1981 as a way to describe the metabolism of various carbohydrates and carbohydrate containing foods. Its main purpose is to characterize the changes in blood glucose that are directly attributed to foods consumed, and is also the basis for determining the overall glycemic load of an entire meal or dietary pattern.[42] Overall dietary glycemic load has been linked to colorectal cancer risk in some epidemiological studies. In an Italian case control study with about 2,000 cases and 4,000 controls, an association of consumption of a high glycemic load diet and colon cancer risk was found. Those in the highest quintile of dietary glycemic load compared to the lowest had an increased risk, (OR = 1.7) and notably, being overweight and having a low intake of fiber from fruits and vegetables (typical of western diets) intensified the association.[44]

Approximately 1,800 cases of colorectal cancer were identified in the Nurses' Health Study and the Health Professionals Follow-up Study over a 20 year follow-up period. From these cases, men who had a high glycemic load diet showed an increased risk of colorectal cancer compared to those whose diets had the lowest glycemic load (RR = 1.4 for the upper quintile). However, no associations with colon cancer risk were observed among women consuming high glycemic load diets.[10]

B. Blood Glucose, Insulin, and Colon Cancer Risk – Epidemiological Evidence

Using data from the Cardiovascular Health Study Cohort (observational, population-based, cohort study of risk factors for coronary heart disease and stroke in individuals 65 years old and older) 102 cases of colorectal cancer were identified. It was found that people in the highest quartile of fasting blood glucose had a nearly two-fold increased risk of colorectal cancer (RR = 1.8) compared to those in the lowest quartile. Blood glucose and insulin levels two hours after an oral glucose challenge were also significantly associated with colorectal cancer incidence. The highest quartile of two-hour glucose levels had a RR of 2.4, and the highest quartile of two-hour insulin levels showed a RR of 2.0. Elevated fasting insulin levels also were significantly associated with colorectal cancer, with persons having insulin values above the median being more likely to have colorectal cancer than those below the median (RR = 1.6).[18]

Similar results were observed in a cohort of about 75,000 Norwegian men and women. During 12 years of follow-up, 730 cases of colorectal cancer were reported. A significant increase in colon cancer risk was observed among women who had high non-fasting glucose levels ($\geq$ 8.0 mM compared to those that were low (< 8.0 mM, RR = 1.98). No significant relationships were observed in men.[45]

A review addressing colorectal cancer risk and plasma glucose levels was published in 1994 and highlights many instances where significant relationships between blood glucose and/or insulin with colorectal cancer risk were not observed.[46]

Since elevated blood insulin levels and insulin resistance are characteristics of adult-onset diabetes, colon cancer incidences have been examined in these diabetic populations. A meta-analysis that examined all published studies through July 2005 quantified the risk of colon cancer risk in diabetic populations. Overall, diabetes was identified as a risk factor for colon cancer in men and women (RR = 1.43).[47]

C. Insulin-Like Growth Factors and Colon Cancer Risk - Epidemiological Data

IGFs are circulating peptides that function in almost every organ in the body. The IGF family of proteins consists of IGF-I, IGF-II, their receptors, and six IGF binding proteins (IGFBPs). In general, IGFs have roles in growth, cell proliferation, transformation, and apoptosis – all processes which are affected in cancer development. IGF-1 may also have a role in metabolism. At least 90% of circulating IGFs are bound to IGFPBs, mainly IGFBP-3.[25] It is theorized that high levels of circulating insulin may alter IGFBP concentrations by inhibiting their synthesis. This in turn can increase the availability of IGFs to act on target tissues, which may play a role in colon cancer development. [20, 25]

In a prospective case-control study using data from the Physicians' Health Study, Ma et al[19] examined plasma levels of IGF-1 and IGFBP-3 in relation to colon cancer incidence after a 14 year follow up. Among the almost 15,000 men in the cohort, about 200 were diagnosed with colon cancer and used in the study (approximately 300 controls were also used). Men in the highest quintile for circulating IGF-I had an increased risk of colorectal cancer compared with

men in the lowest quintile (RR = 2.5). Also, men with higher circulating IGFBP-3 concentrations showed a lower risk (RR = 0.28) after controlling for IGF-I and other covariates. IGF-II levels were not associated with an increased or decreased risk of colon cancer.[19]

The relationships between IGFs, IGFBPs and certain cancers were examined in a meta-analysis that included published literature from 1996-2002. Out of the 21 studies that were identified (approximately 3,600 cases of prostate, lung, colorectal, and breast cancers matched with 7,100 controls), five related to colon cancer with almost 700 cases and 1,700 controls. Among the colorectal cancer cases, an association of high circulating IGF-1 levels and colon cancer was present. ORs from the five separate studies (range of 1.23 – 2.51) were estimated to produce an overall OR of 1.58 for the highest IGF-1 levels compared to the lowest.[48]

D. High Glycemic Index Diets, Insulin and IGFs – Experimental Evidence

The effect of a high glycemic index diet on insulin sensitivity was examined in Australian Albino Wistar rats. The high GI diet received amylopectin-based diets and the low GI diet was based on amylose. After seven weeks of feeding, the high GI diet group had an elevated insulin response, as measured over 60 minutes during an intravenous glucose tolerance test. (3783 ± 316 pmol/L compared to 2839 ± 368 pmol/L). The blood glucose response was not different between the groups.[49]

An association of insulin and colonic tumors was observed in 60 Fisher 344 rats treated with AOM. The rats were injected with insulin five times per week for 17 weeks after colon cancer initiation by AOM injection. At the conclusion of the experiment, the percentage of rats having large tumors (diameter ≥ 2 mm) was much greater in the insulin-injected group than the

saline control group (79% compared to 50%, P<0.05). The average number of tumors/rat was also greater in insulin-injected versus control rats (2.00 versus 0.73 tumors/rat; P < 0.001).[23]

In a study using APC^{Min} mice and a high sucrose diet, it was observed that high blood glucose, insulin, and IGF concentrations were associated with an increased incidence of proximal small intestinal after 10 weeks of feeding. The sucrose-fed group (compared to starch control) had higher serum glucose (10 vs 8; P=0.02), insulin (263 vs 174; P=0.08), and IGF concentrations (67 vs 53 P=0.15). Also, increased colonic tumor incidence was associated with hepatic expression of insulin-like growth factor I (IGF-I) mRNA (P=0.05) in a 16 week feeding study of similar design.[16]

Wu et al[24] have attempted to determine the effects of serum IGF-I levels related to new tumor growth and metastasis in a mouse model of colon cancer (adenocarcinoma tissues transplanted). In this study, 74 control mice and 82 liver-specific IGF-I-deficient (LID) mice (serum IGF-I levels are 25% of that in control mice) were used. Control mice showed a higher incidence of larger implanted tumor growth on the cecum than the LID group (56.8% to 31.3%; P < 0.01). Also, when both control and LID mice injected with recombinant human IGF-I, the rate of cecum tumor development significantly increased.[24]

E. Glycemic and Insulinemic Effect of Dietary Sugars

As discussed earlier, the effects of the varied metabolism of glucose, fructose, and sucrose may play a role in colon cancer risk, presumably though their glycemic or insulinemic responses. The differential responses of carbohydrate metabolism have been observed in both animal and human trials.

In a study using a transgenic mouse model, the effects of sucrose on body weight, glucose tolerance, circulating insulin, and cholesterol were examined. Mice were given either sucrose-sweetened water (10% sucrose) or regular water *ad libitum*. After five weeks, the group given sucrose-sweetened water developed glucose intolerance, hyperinsulinemia, and hypercholesterolemia. Fasting insulin levels increased threefold between the control and sucrose groups (~4.5 ng/ml compared to ~1.5 ng/ml). There were no significant differences in plasma triglycerides between the two groups.[22]

A similar study was conducted using fructose-sweetened water (15% fructose) compared to sucrose-sweetened water (10% sucrose), water with a zero calorie sweetener added, and a control of plain water (carbohydrate contents varied in order to mimic popular US and European soft drinks). Three-month-old male NMRI mice were given a standard diet *ad libitum* plus one of the four treatments. The fructose group gained significantly more weight during the 73-day study duration compared to other groups (~21 g compared to ~12 g). The fructose group was the most glucose intolerant, yet the effect was not statistically significant compared to any other treatment group. Plasma insulin concentrations also did not differ for fructose- or sucrose-treated mice, and the only significant increase in plasma insulin concentration was seen in the zero calorie sweetener-treated group, which may have been a direct effect of the artificial sweetener cyclamate.[50]

The comparative effects of consumption of glucose, sucrose, and fructose diets on serum glucose, triglycerides, and insulin were examined in Sprague-Dawley rats. A glucose solution (32%), fructose solution (32%), sucrose solution (32%) or granulated sucrose was given to animals in addition to the standard rodent diet. A control group was given only the standard diet. Fasted blood samples were taken at 50 days, and rats that consumed any of the sugar solutions

had significantly higher weights and consumed more calories than the controls. The granulated sucrose group consumed the same amount as the control group, yet gained more weight per calorie consumed. Also, the animals receiving either fructose or sucrose solutions had higher plasma glucose levels at minute 90 of a glucose tolerance test (~200 mg/dl) than animals receiving granulated sucrose (~185 mg/dl), glucose or the control diet (both ~175 mg/dl). At termination (day 50) fructose-fed mice has the highest triglyceride levels (108 ± 18 mg/100 ml), with granulated sucrose-fed mice having the second highest triglyceride levels (89 ± 16 mg/100 ml). Glucose and insulin concentrations measured at study termination did not vary between groups.[51]

Overall, the metabolism of fructose compared to other sugars has been reviewed and its consumption increases circulating triglycerides, induces *de novo* lipid synthesis and hepatic insulin resistance, yet does not affect blood glucose or insulin concentrations directly.[52-54] Because of this, one study attempted to examine whether the fructose component of sucrose mediates the effects of sucrose on blood glucose and insulin. Male rats were fed either a diet comprised of starch (68% of total calories), sucrose (68% of calories), fructose/glucose (34% of calories from each), or fructose/starch (34% of calories from each) for five weeks. A glucose tolerance test showed the sucrose, fructose/glucose, and fructose/starch group to have blood glucose levels 29% greater over than the starch control but not significantly different from one another. Other tests measuring insulin suppression of glucose appearance and insulin-stimulated glucose disappearance showed similar results (sucrose, fructose/glucose and fructose/starch all lower than starch). These results indicate that fructose mediates some of the metabolic response of sucrose.[55]

In humans, the metabolic responses to glucose, sucrose, and fructose administration are similar to what is observed in animal studies. Crapo et al[56] examined the effects of glucose and sucrose consumption on postprandial glucose and insulin responses in 19 individuals. It was found that when given a drink containing 50 g of either carbohydrate, glucose and fructose have a similar glycemic response, yet sucrose had a 20% higher insulinemic response. Sucrose also produced a 35-65% higher insulin response compared to raw starch.[56]

In another comparison of the effects of sucrose, glucose, and fructose on fasting plasma glucose, eight participants were given either carbohydrate dissolved in a tea or water at 25, 50, or 100 g doses (fructose was not given at 100 g due to malabsorption concerns). The control group received white bread as the carbohydrate. Blood was collected after meal/drink consumption at intervals ranging from 0 – 120 minutes. At all doses, the glucose and insulin responses (averaged over time) followed the same trends. The percent glycemic response (compared to white bread) was 148% for glucose, 87% for sucrose, and 20% for fructose. The insulinemic response was very similar, at 146% for glucose, 83% for sucrose, and 22% for fructose.[57]

In another study, five test beverages were used to identify a relationship between the glycemic response and satiety and food intake. The five beverages consisted of 75 g of either polycose (a commercially available glucose polymer powder), sucrose, glucose, a fructose-glucose mix (80-20%), or sucralose dissolved in 200 ml of water (participants were also given 200 ml of plain water). Twenty minutes after the drink was consumed, blood glucose was measured as change since baseline. The fructose drink evoked the lowest significant response (not considering the sucralose) at 1.9 mM. The other groups were not significantly different than each other, and ranged from 3.1 – 3.5 mM glucose.[58]

5. Summary

It is evident that there is a link between diet and colon cancer risk. The Western dietary pattern, as well as consumption of refined sugars, has been implicated in increasing the risk of colon carcinogenesis. Epidemiological studies have identified correlations between increased sugar consumptions, high glycemic index diets, and high circulating blood insulin and IGF concentrations to colon cancer risk. These interrelationships have been confirmed in animal models as well.

Not all sugars have the same effects on colon cancer risk and the unique metabolism of each carbohydrate likely influences its potential effects on carcinogenesis. Sucrose evokes a higher glycemic and insulinemic response *in vivo*, whereas fructose has little effect on blood glucose levels in the short term. Fructose, however, is known to be rapidly metabolized via a hepatic route and can result in higher circulating triglycerides, increased *de novo* lipid biosynthesis, and increased hepatic insulin resistance. Glucose consumption generally does not have much of an adverse effect on these parameters or colon cancer risk.

RATIONALE AND SPECIFIC AIMS

Colon cancer is one of the most prevalent cancers in Western countries. Certain dietary components and patterns are recognized to increase colon cancer risk, with the Western dietary pattern often identified as a risk factor in human and animal studies. Furthermore, it also has been shown that diets high in sucrose and other refined sugars are correlated to colon cancer risk.[3, 5, 7, 8, 12, 31] This correlation between high sucrose consumption and increased colon cancer risk also has been demonstrated in animal models.[13-17, 40]

Although several animal studies have demonstrated an association between consumption of high sucrose and colon cancer risk, there is little published research on the comparative effects of sucrose and its component monosaccharides, glucose and fructose, and colon cancer risk. Feeding of diets rich in sucrose, glucose, and fructose differentially influence serum glucose and insulin concentrations. Among these carbohydrate sources, fructose consumption typically causes the lowest post-prandial glycemic and insulinemic responses, whereas consumption of glucose or sucrose elicits a greater response.[56, 57, 59] Also, the metabolism of these sugars differs somewhat. Sucrose is first hydrolyzed in the intestine or within epithelial cells before being released and metabolized as its component monosaccharides glucose and fructose.[60] Free fructose is absorbed through the small intestine by facilitated diffusion and is transported to the liver via the portal vein, where the majority is metabolized.[61] Free glucose is readily absorbed by the small intestine by active transport.[61] Fructose absorption is generally slower than that of glucose, but can be enhanced with the presence of glucose.[61, 62] Once absorbed, the presence of glucose can inhibit the phosphyloration of fructose by hexokinase which would slow

fructose utilization in the liver. However, increased fructose consumption can increase fructokinase activity, which phosphorylates fructose and facilitates its utilization.[61] Via the fructokinase phosphorylation pathway, fructose can be converted to glyceraldehydes which can be subsequently phosphorylated to glyceraldehyde-3-phosphate. At this triose phosphate stage, fructose metabolism pathways converge with those of glucose metabolism.[63] Because fructose can enter the glycolytic pathway downstream from the most regulated step in glycolysis (phosphofructokinase conversion of glucose-6-phosphate to glucose 1,6 bisphosphate), fructose metabolism can lead to increased substrates for glycolysis, glycogenesis, gluconeogenesis, lipogenesis, and fatty acid esterification.[52, 53, 64]

Because of this variation of metabolism, fructose has little effect on postprandial glucose concentrations.[52-54, 62] However, these alterations in metabolic pathways are generally thought to play a role in hepatic metabolic insulin resistance and lead to long term consequences such as hyperinsulemia.[52, 54, 65] Consequently, it can be hypothesized that between the differential glycemic and insulinemic responses of fructose, glucose, and sucrose along with the utilization of varied metabolic pathways, each dietary carbohydrate may likewise affect colon cancer development differently.

Because of their differential effects on blood glucose and insulin concentrations, these dietary sugars may differentially influence concentrations of insulin like growth factors (IGFs) that may play a role in the development of cancers. Previous research has demonstrated that IGF administration can directly stimulate tumorigenesis in vivo.[24] Furthermore, studies have identified correlations between dietary carbohydrate administration, increased circulating insulin

and IGF concentrations, and increased cancer risk.[16, 23] Because of the varied insulinemic responses of glucose, sucrose, and fructose, each carbohydrate's relationship to colon cancer risk factors also may be affected.

The increased consumption of refined sugars in the United States and other countries has become a cause of concern in the recent years Between 1978 and 2004, overall daily energy intake among the US population has increased 18% and carbohydrate intake has increased 41%.[66] High fructose corn syrup (HFCS) has become a widely used ingredient in many types of foods and has caused concern among the general public. Produced industrially from corn starch, HFCS is a mixture of fructose and glucose and typically contains either 42% or 55% fructose, and thus is relatively similar to the monosaccharide composition of sucrose. High fructose corn syrup use has also increased from 16% of total sweeteners before used before 1980 to 42% by 1998 and has since stabilized. Increases of naturally occurring fructose consumption over this time frame were no greater than the overall increase of daily energy consumption.[66] Also, between 1998 and 2004, per capita consumption of sugar sweetened beverages (a major source of HFCS in the diet) increased by 46 kcal/day.[67] Increased consumption of HFCS has been under much scrutiny as consumption of HFCS containing foods has been linked to weight gain, obesity, and increased prevalence of obesity-related diseases[68-71] However, there is also evidence the source of dietary sugars (sucrose, fructose, glucose, or HFCS) is less important than amount consumed.[72-75] Furthermore, the consumption of fructose and HFCS has been shown to elicit metabolic responses similar to sucrose[74, 76, 77] and thus HFCS consumption may not present any unique health consequences compared to that of other dietary sugars.

The overall goal of this research is to determine the impact of dietary carbohydrate source on human colon cancer risk using animal models of colon cancer and determine mechanisms of action whereby these dietary factors influence cancer risk. Previous research in our laboratory has demonstrated that starch and sucrose, when administered as the sole dietary carbohydrate source to APC^{Min} mice (C57BL/6J $APC^{Min/+}$), differentially affect intestinal tumorigenesis. In the small intestine, feeding dietary sucrose increased adenoma numbers in the proximal small intestine. However, dietary starch feeding increased the average size of proximal small intestinal adenomas such that overall small intestinal tumor burden was not significantly influenced by dietary carbohydrate source. In the colon, dietary sucrose feeding significantly increased colonic adenoma numbers in a 16-week feeding study wherein APC^{Min} mice that were treated with 100 ppm dietary sulindac to attenuate small intestinal adenoma development. However, dietary carbohydrate source did not significantly influence colonic adenoma formation in APC^{Min} mice subjected to a standard 10-week feeding protocol.

The objective of the current research is to further examine the influence of dietary starch and sucrose, and to determine the impacts of their component monosaccharides, on intestinal tumorigenesis in APC^{Min} mice. The specific aims of this research are to:

1) determine the effects of dietary carbohydrate sources (starch, sucrose, glucose, fructose and a 1:1 mixture of glucose and fructose) on intestinal adenoma development in APC^{Min} mice, and
2) relate changes in intestinal adenoma development caused by dietary carbohydrates to alterations in body weight, body composition, and blood parameters elicited by these diets.

The working hypothesis of specific aim one is that the dietary sucrose will stimulate intestinal adenoma development in APC^{Min} mice compared to mice consuming dietary starch. We further hypothesize that dietary glucose, fructose, and a 1:1 mixture of glucose and fructose also will increase intestinal adenoma development compared to dietary starch. To test this hypothesis, the numbers and sizes of adenomas present in the large and small intestines will be quantified at the end of a feeding study wherein these carbohydrate sources are administered as the sole carbohydrate sources in the diets fed to APC^{Min} mice for ten weeks.

The working hypothesis of specific aim two is that changes in intestinal adenoma development caused by feeding these dietary carbohydrate sources will be related to increases in body weight, body composition (fat percentage), and concentrations of blood glucose, insulin, and IGF. To test this hypothesis, body weight of mice will be measured weekly and total body fat and lean percentages will be measured at study termination. Glucose, insulin and IGF will be measured in blood samples obtained at study termination. It is anticipated that increased body weight, body fat percentage, and blood concentrations of glucose, insulin, and IGF will be associated with increased adenoma numbers and total burdens. The effects of the same diets on these parameters in normal C57BL/6J mice also will be investigated as a secondary goal.

We are particularly interested in studying the effects of the 1:1 mixture of glucose and fructose because this represents the molar contribution of glucose and fructose to sucrose, and also because this diet can be used to mimic the effects of feeding HFCS diets (which typically contain 55% or 42% fructose with the remainder being comprised of glucose).

It is anticipated that intestinal tumor burden will be directly associated with blood glucose and insulin concentrations elicited by feeding these diets, with the effects on tumorigenesis being mediated by IGF and other growth factors which are modulated by these

diets. Therefore, the diet that provokes the largest insulin response should have the highest IGF levels and, consequently, the highest intestinal tumor numbers and burdens. For the diets studied in this experiment, we anticipate that the consumption of glucose will elicit the highest glycemic and insulinemic responses, followed by sucrose which we expect to be similar to 1:1 glucose:fructose in these effects. It is expected that consumption of the high fructose diet and the starch control diet will have the least effects on serum glucose and circulating insulin.

MATERIALS AND METHODS

Breeding Colony and Animals

All animals were housed in research facilities located in the G.M. Trout Food Science Building which are overseen by MSU University Laboratory Animal Resources. Temperature and humidity were maintained at 70-74 °F and 40-60% humidity, with a twelve hour light:dark cycle. All aspects relating to animal use were approved by the MSU All University Committee on Animal Use and Care before the start of this study.

A breeding colony was developed and used as the source of study animals. Because of the high cost of APC^{Min} mice and the large numbers of mice needed for this experiment, this is the most cost effective and timely method to produce mice for these studies. Normal C57BL/6J female mice were mated to males who are known to carry the APC^{Min} defect (C57BL/6J $APC^{Min/+}$). All mice used to start the breeding colony were purchased from The Jackson Laboratory (Bar Harbor, ME). Male APC^{Min} breeders were given sulindac (200 ppm) in their drinking water to attenuate small intestinal adenoma development and thus prevent morbidity due to intestinal adenomas. Normal female breeders were not exposed to the sulindac as it is teratogenic. Due to basic Mendelian inheritance and autosomal dominant inheritance, approximately 50% of the offspring produced were carriers of the Min defect with the other 50% being normal. All offspring were used for the feeding study, and at termination of the study allele-specific PCR was used to determine which mice were carriers.

All healthy pups used as study animals were weaned at three to four weeks of age and then randomly assigned to one of five dietary treatment groups which differed only in their carbohydrate sources: corn starch (control), sucrose, glucose, fructose, or 1:1 glucose:fructose

ratio. Weaning and assigning of treatments was done once a week such that any pup between 20-26 days old was weighed at day 0 and randomly assigned to a treatment group for the subsequent day. Due to the uneven numbers of available pups and a varied sex distribution at any given week, treatment groups had an uneven sample size (n). To improve the power of detecting statistically significant differences and owing to the relatively low incidence and burden of colon tumors in APC^{Min} mice at 13-14 weeks of age, each group was intended to include at least 25 APC^{Min} mice with an equal ratio of males to females. To obtain these numbers of APC^{Min} mice, 50-60 mice were assigned to each treatment. Given the size of the breeding colony we maintained, this constituted all offspring produced from the breeding colony during a period of approximately four months. The final numbers of animals per treatment group appear in Table 1.

Immediately after weaning, mice were fed exclusively one of the study diets *ad libitum* for 10 weeks, unless early termination due to significant morbidity was necessary (this was observed only in one mouse in this experiment). Mouse weights were measured weekly in order to track growth and/or morbidity.

Experimental Diets

All diets were based on a standard AIN-93G rodent diet. The diets were modified by increasing fat content from 7% to 15% in order to reflect a more typical human diet. Because of this increase in fat content and energy density of the diets, the concentrations of essential nutrients were also increased to account for the anticipated decrease in the amount of food consumed. The diets contained 19% crude protein and were adequate in all other nutrients.

The five treatment diets differed only in the composition of the carbohydrate portion of the diet as follows: 1. corn starch (control), 2. sucrose, 3. glucose, 4. fructose, and 5. 1:1 glucose:fructose ratio. All other aspects of the diets were identical across treatment groups. Details of all diet compositions are presented in Table 2. All dietary ingredients were obtained from Dyets, Inc (Bethlehem, PA). All diets were made in 10 kg batches as needed.

Weight and Body Composition

All mice were weighed weekly starting at day 0 in order to track weight gain over time. Body composition analysis was also preformed one day prior to sacrifice (day 69 or 70 of feeding) to obtain whole body fat mass and total lean tissue mass. This was done using an EchoMRI-100 body composition analysis system for live animals (Echo Medical Systems LLC, Houston, TX) according to the manufacturer's instructions. Body composition analyses were conducted in duplicate for each mouse and the average value was used for statistical analyses.

Tissue and Blood Collection

All mice were sacrificed at day 70 or 71 of treatment by CO_2 asphyxiation. Immediately after asphyxiation blood was removed from the heart via cardiac puncture, collected in K_2EDTA coated tubes, and centrifuged for 10 minutes at 2,000 g within 20 minutes to obtain plasma. Plasma was then frozen (-20 °C) for later use to determine glucose, insulin, and IGF-1 levels. Samples of liver and abdominal fat were also saved for future analyses if necessary. Liver tissues were saved to confirm mouse genotypes by analysis of APC gene expression as necessary.

The entire small intestine, cecum, and colon were removed following blood collection. The small intestine was divided into three approximately equal sections: proximal, medial, and

distal. The colon and cecum were separated from the small intestine and all tissues were cut open longitudinally and rinsed with tap water and phosphate buffered saline (PBS, pH adjusted 7.4) to remove contents. All intestinal tissues were then pinned flat on cardboard and fixed in 10% neutral-buffered formalin (NBF, pH adjusted to 7.4) for 24 hours. After fixation, one-centimeter sections of the medial region of the colon from each mouse were removed and saved for later histological analysis. Any adenomas present on this colonic section were noted. The small intestine, cecum, and remaining colon pieces were stained with 0.3% methylene blue in PBS for two minutes to facilitate tumor identification. Fixed, stained tissues were then stored in 1% neutral buffered formalin.

Quantification of Intestinal Tumor Numbers and Burdens

The numbers and sizes of tumors in each segment of the intestine (proximal, medial, distal, and total small intestine; cecum, and colon) were determined by direct counting using a Nikon SMZ stereomicroscope. Stained intestinal sections were placed on a transparent grid (marked in 0.5 mm increments) to aid determination of tumor sizes. All adenomas in the small intestine were flat, 2-dimensional tumors and their sizes were quantified by measuring the width (w) and length (l) of each tumor. Tumor size was calculated using the formula: a (area) = (π * w * l) / 4. Colonic tumors were polypoid (three-dimensional) and their sizes were quantified by measuring the width (w), length (l), and height (h) of each tumor. Tumor size was calculated using the formula: a (area) = (π * w * l * h) / 6. All tumor dimensions were measured in 0.25 mm increments. For the small intestinal sections, tumor multiplicity was expressed as tumors identified per tumor bearing mouse and tumor burden was expressed as average tumor size per tumor bearing mouse as well as the total tumor area per tumor bearing mouse. For colon tumors,

the same multiplicity and burden measurements are reported as well as tumor incidence (% of mice with solid tumors).

Plasma Analyses

Plasma glucose, insulin, and IFG-1 levels were determined on a subset of samples from each treatment (Starch: 7 normal males, 7 APC^{Min} males, 7 normal females, 7 APC^{Min} females; Fructose: 7 normal males, 7 APC^{Min} males, 6 normal females, 6 APC^{Min} females; Glucose: 7 normal males, 7 APC^{Min} males, 7 normal females, 7 APC^{Min} females; 1:1 Glucose:Fructose: 7 normal males, 7 APC^{Min} males, 6 normal females, 7 APC^{Min} females: Sucrose: 7 normal males, 7 APC^{Min} males, 7 normal females, 7 APC^{Min} females). The sample subset was filtered to exclude any mouse whose weight at day 69 was more than one standard deviation different than the mean for its respective treatment and sex. From the remaining samples which fit this criterion, seven samples (in some cases six) from each treatment X sex combination were selected based on samples having sufficient plasma volume to complete the analyses and overall quality of sample (we excluded plasma samples that had significant hemolysis).

Plasma glucose was determined using the Glucose (HK) Assay Kit (Sigma-Aldrich, St. Louis, MO). In this assay, the glucose in the sample reacts with ATP (in the presence of hexokinase) to form glucose-6-phosphate which then reacts with NAD (in the presence of glucose-6-phosphate dehydrogenase) to form 6-phosphogluconate and NADPH. The increase in NAPDH is measurable by change in absorbance at 340 nm and is directly proportional to the original glucose concentration.

Plasma insulin concentrations were determined using the ALPCO Insulin ELISA kit for mice (ALPCO Diagnostics, Salem, NH) according to the manufacturer's instructions. IGF-1 concentrations were measured using the Mouse IGF-1 Duoset Immunoassay kit (R&D Systems, Inc; Minneapolis, MN). This assay is a basic sandwich ELISA in a 96-well plate format.

Statistical Analyses

Data were analyzed using the General Linear Models procedure of SAS (version 9.2). Least-square means procedures were used for all the measurements. Parameters included in the statistical analyses included diet, sex, APC status, and the interactions of these parameters. When significant effects were detected for these parameters or their interactions (F test significant at $P < 0.05$), the least significant difference method was used to compare appropriate means. Differences between means were declared significant at $P < 0.05$, and trends toward significance were declared at $P < 0.10$. Results in tables and figures are presented as least-square means ± SEM (standard error of the mean). The colon adenoma incidence for mice consuming diets containing different carbohydrate sources was analyzed using the GENMOD procedure of SAS (version 9.2). Effects on colon tumor incidence due to treatment or sex were determined using the appropriate Chi Square tests as "estimate" statements within the GENMOD procedure.

Table 1. Final number of C57BL6J (normal) and C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed either starch, glucose, fructose, a G:F (glucose:fructose) mix, and sucrose for ten weeks†

	Sex	APC^{Min}	Normal
Starch	Male	12	14
	Female	17	13
	Total	*29*	*27*
Glucose	Male	13	9
	Female	13	15
	Total	*26*	*24*
Fructose	Male	18	21
	Female	14	13
	Total	*32*	*34*
G:F	Male	14	17
	Female	14	15
	Total	*28*	*32*
Sucrose	Male	11	22
	Female	17	11
	Total	*28*	*33*
Total		143	150

Table 2. Diet compositions. G:F refers to an equal mix of glucose and fructose as the carbohydrate source

	Starch (g/kg)	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	G:F (g/kg)
Casein	221	221	221	221	221
Corn Starch	523	0	0	0	0
Sucrose	0	523	0	0	0
Glucose	0	0	523	0	261.5
Fructose	0	0	0	523	261.5
Soybean Oil	150	150	150	150	150
Cellulose	50	50	50	50	50
AIN-93G-MX	39	39	39	39	39
AIN-93-VX	11	11	11	11	11
L-Cysteine	3	3	3	3	3
Choline Bitartrate	3	3	3	3	3
Tert-Butylhydroquinone	0.03	0.03	0.03	0.03	0.03

RESULTS

Weight and Body Composition

There were no statistically significant differences in mouse weights among treatments or sexes at Day 0 of treatment (average weight 9.5 g, See Figures 1a and 1b). For all weekly weights after Day 0, male mice weighed significantly more than the females, across all treatments ($P < 0.05$). The difference in average body weights of male and female mice continued to expand throughout the feeding period. At the conclusion of the study, male mice outweighed female mice by an average of 6.25 grams. By week three of treatment, the mice carrying the APC^{Min} gene defect began to weigh significantly less than the normal mice. This difference was maintained through the conclusion of the feeding study, at which time the APC^{Min} mice weighed an average of 2.2 grams less than their normal counterparts.

Dietary treatments began having a significant effect on body weight after the first week of feeding. At day 7, the fructose-fed mice were significantly smaller than those on other treatments except for G:F, and fructose-fed mice weighed significantly less that mice on all other treatments at week two of feeding. However, after this time fructose-fed mice gained weight at a pace similar to the control (starch-fed) mice. By week three, the starch controls and fructose-fed mice did not differ in weight, but the fructose-fed mice weighed significantly less than those on the other three treatments. After week three, starch- and fructose-fed mice continued to be similar in body weight through the end of the feeding experiment. The sucrose-, glucose-, and G:F-fed mice had significantly greater body weights that the starch-fed controls beginning on day 28 of feeding. By day 49, the sucrose-fed mice had significantly greater body weight than mice fed G:F, and by day 63 the sucrose-fed mice weighed significantly more than mice on all

other dietary treatments. The final body average weights at conclusion of the feeding study were 26.0 ± 0.3 g, 24.9 ± 0.3 g, 24.6 ± 0.3 g, 23.7 ± 0.3 g, 23.0 ± 0.3 g for mice consuming sucrose, glucose, G:F, fructose and starch, respectively.

Body composition analysis was only preformed on a single occasion for each mouse at either day 69 or 70 of feeding (whichever was one day before sacrifice). The body composition data mimicked overall body weights with normal mice having significantly higher body fat percentages than the APC^{Min} mice and males having significantly higher body fat than females (Table 3; both effects $P < 0.001$). There was a significant treatment effect for body fat percentage ($P < 0.001$), and differences among treatment means followed the same trends as were observed for body weights. Sucrose-fed mice had the highest body fat percentage (18.4 ± 0.7%), whereas mice fed the starch control diet were the leanest (13.0 ± 0.7%). Mice fed glucose diets were significantly fatter than those fed starch, but there were no statistically significant differences in body fat percentage between mice fed diets containing glucose, fructose, or G:F. There also was a significant sex X genotype interaction for body fat percentage ($P < 0.01$). This was due to the observation that normal male mice had much higher body fat percentages than normal females (18.9 ± 0.6% versus 15.0 ± 0.6%, respectively), whereas the body fat percentages for APC^{Min} male and female mice were much more similar (13.6 ± 0.6% versus 13.0 ± 0.6% for male and female APC^{Min} mice, respectively).

Results for average lean body mass (Table 3) were essentially the inverse of results for body fat percentage, with sex, APC gene status, and treatment all significantly influencing lean body mass ($P < 0.001$ for all effects). There also was a significant sex X genotype interaction ($P < 0.01$). Normal male mice had much lower lean body mass than normal females (73.8 ± 0.6%

versus 79.3 ± 0.6%, respectively), whereas the average lean body masses for APC^{Min} male and female mice were much more similar (79.2 ± 0.6% versus 81.5 ± 0.6% for male and female APC^{Min} mice, respectively).

Since diets were provided *ad libitum* and food intake was not measured, there is a possibility that variations in food intake due to palatability may be influencing results. The daily intake of food was not measured due to the difficulty of doing so given the powdered nature of the diets. However, we were able to estimate the total quantity of each diet utilized during the experiment and these quantities were used to calculate a rough estimate of daily diet disappearance for each mouse on the respective dietary treatments. These results are presented in Table 4. From these estimates, it appears that the starch- and fructose-fed mice may have consumed less diet overall compared to those on the other treatments, but it is important to remember that these are only rough estimates meant to show any potentially large deviations.

Tumor Burden

Tumor incidence, numbers, average sizes, and total burdens were assessed in the proximal, medial, and distal small intestine (SI), cecum, and colon. Small intestinal tumor frequency was 100% in APC^{Min} mice and 0% in normal mice. No cecal tumors were observed in any mice in this study. Small intestinal tumor data are presented in Tables 5a-c (proximal, medial and distal thirds) and Table 6 (total SI).

Proximal SI adenoma number was not influenced by sex, but was significantly influenced by diet (Table 5a). Mice consuming starch had the lowest numbers of adenomas in the proximal SI (17.4 ± 1.9) and mice consuming fructose the greatest number (30.8 ± 2.0), with the other treatments being intermediate. The average size of adenomas in the proximal SI was

significantly greater for female (1.19 ± 0.05) versus male mice (0.96 ± 0.06). Diet also significantly influenced average adenoma size in the proximal SI, with starch-fed mice having significantly larger adenomas in this section than mice consuming any of the other dietary treatments. Total adenoma burden in the proximal SI was significantly greater in females (26.9 ± 1.6) than males (18.9 ± 1.7), but was not significantly influenced by dietary treatment. There were no sex X diet interactions detected.

Medial SI adenoma number (Table 5b) tended ($P < 0.10$) to be greater in females (32.7 ± 1.5) versus males (28.8 ± 1.5). Medial SI adenoma number was significantly influenced by diet. Mice consuming sucrose had the lowest numbers of adenomas in the medial SI (25.6 ± 2.4) and mice consuming fructose the greatest number (36.0 ± 2.4), with the other treatments being intermediate. The average size of adenomas in the medial SI was significantly greater for male (1.05 ± 0.04) versus female mice (0.93 ± 0.04). Diet did not significantly influence average adenoma size in the medial SI. Total adenoma burden in the medial SI was not significantly influenced by sex or dietary treatment. There were no sex X diet interactions detected for any of these parameters.

Distal SI adenoma number (Table 5c) was not influenced by sex, but was significantly influenced by diet (Table 5c). Mice consuming sucrose had the lowest numbers of adenomas in the distal SI (22.7 ± 2.3) and mice consuming fructose the greatest number (39.7 ± 3.0), with the other treatments being intermediate. The average size of adenomas in the distal SI was significantly greater for male (0.91 ± 0.04) versus female mice (0.74 ± 0.04). Diet did not significantly influence average adenoma size in the distal SI. Total adenoma burden in the distal SI was not significantly influenced by sex, but was significantly different for mice consuming the different dietary treatments. Mice consuming sucrose had the smallest distal SI adenoma

burdens, whereas mice consuming fructose and starch had the greatest burdens in the distal SI, with mice consuming glucose and G:F being intermediate. There were no sex X diet interactions detected for any of these parameters.

Overall SI adenoma numbers, average sizes and burdens are presented in Table 6. Female mice tended ($P = 0.087$ for total SI) to have higher total SI tumor numbers (89.3 ± 3.9) than males (79.6 ± 4.1). Total SI adenoma number was significantly influenced by diet. Mice consuming sucrose had the lowest numbers of adenomas in the SI (67.7 ± 6.4) and mice consuming fructose the greatest number (106.4 ± 6.5), with the other treatments having intermediate numbers of SI adenomas. The average size of adenomas in the SI was not influenced by sex. Diet significantly influenced average adenoma size in the SI, with starch-fed mice having significantly larger adenomas than mice consuming any of the other dietary treatments. Total adenoma burden in the SI was not significantly influenced by sex, but tended ($P < 0.10$) to be influenced by dietary treatment. Mice consuming sucrose tended to have lower total SI adenoma burdens than mice consuming fructose, starch or glucose, with mice consuming the G:F diet being intermediate. There were no sex X diet interactions detected for any of these parameters.

In the colon, no significant treatment effects on adenoma numbers, average size, total burden or incidence were observed (Table 7). However, males were much more likely to develop colon tumors than were the females. Males also developed larger tumors and had more tumors/mouse than the females.

Plasma Analyses

Glucose, insulin, and IGF-1 were all measured in plasma obtained at the time of sacrifice. Glucose and insulin results are presented in Table 8. There were no significant differences

between the normal and APC^{Min} mice in either plasma glucose or insulin. Overall, males had significantly higher plasma glucose and insulin concentrations than females. Treatment tended to have effects on glucose and insulin concentrations ($P = 0.066$ for glucose; $P = 0.106$ for insulin). Mice fed the starch diet tended to have lower plasma glucose concentrations compared to those consuming fructose, sucrose or G:F. Similarly, mice consuming starch tended to have lower plasma insulin concentrations than those consuming sucrose or fructose.

Neither sex nor dietary treatment influenced plasma IGF-1 concentration (Table 9). Genotype tended ($P < 0.10$) to have an effect on plasma IGF-1, with APC^{Min} mice having higher IGF-1 levels than normal mice.

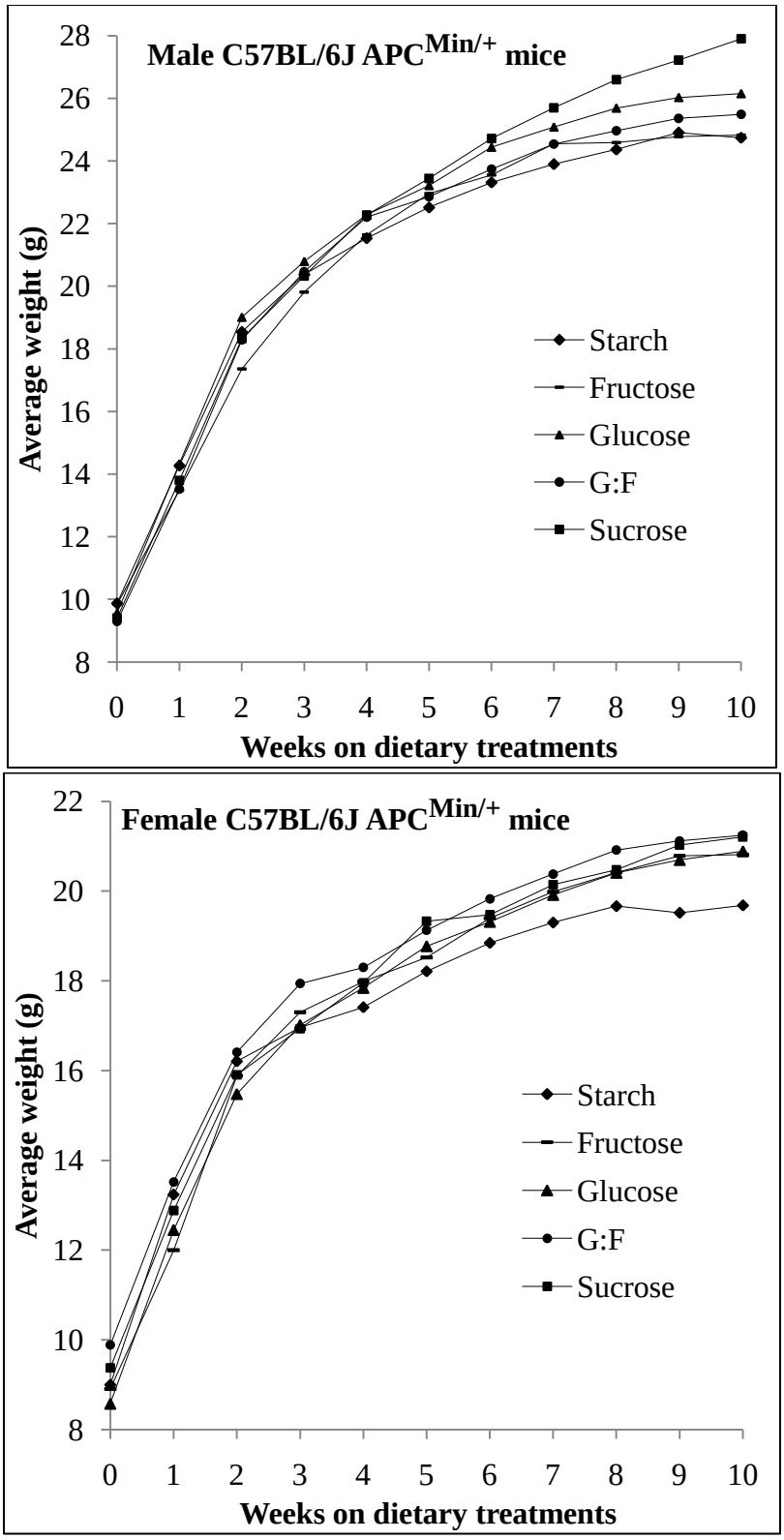

Figure 1a. Weight gain over time among male (top) and female (bottom) carriers of the APC^{Min} defect when fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.

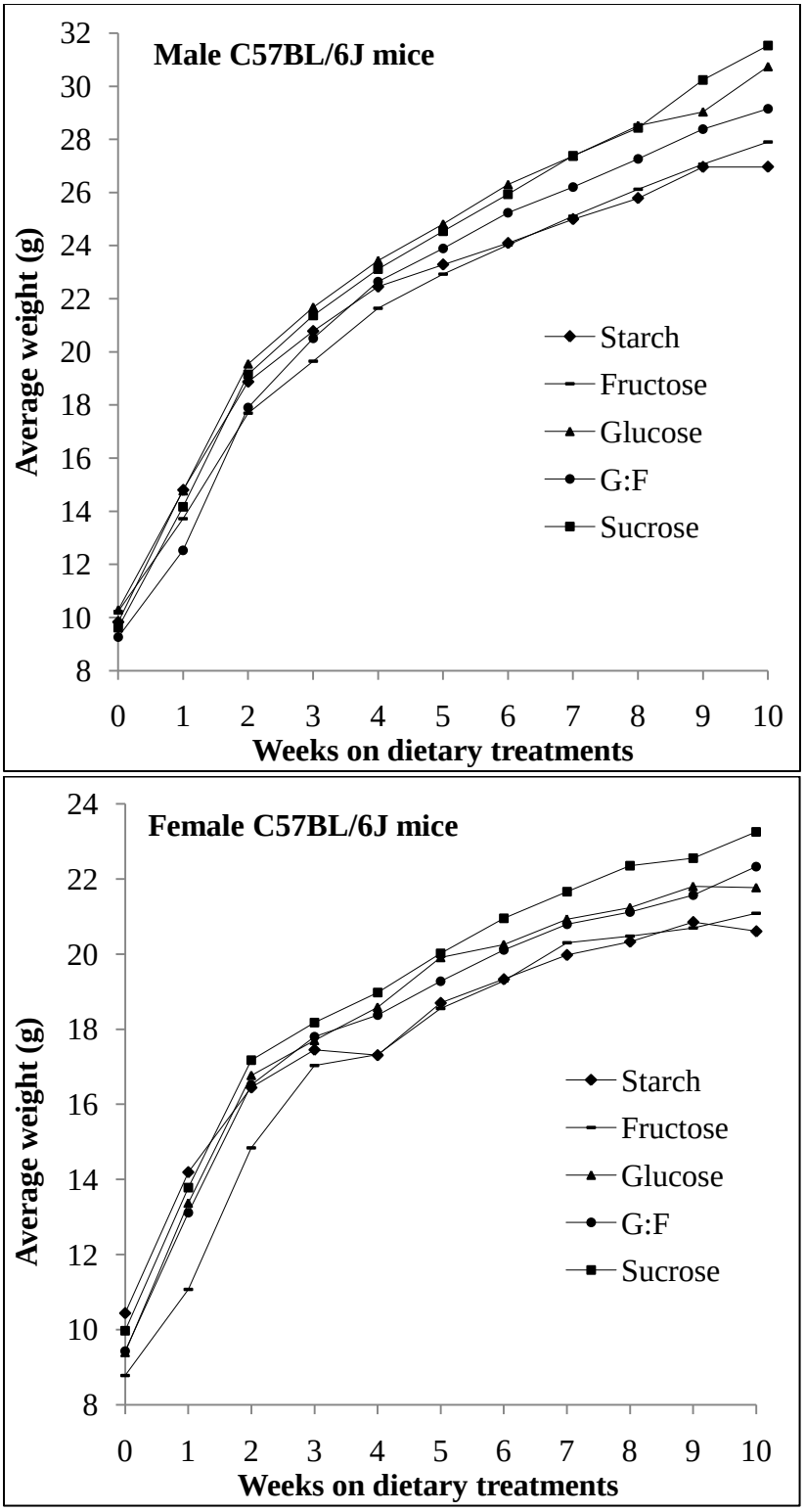

Figure 1b. Weight gain over time among normal male (top) and female (bottom) C57BL/6J mice when fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.

Table 3. Average body fat and lean mass among C57BL/6J (normal) and C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice after feeding starch, glucose, fructose, a G:F (glucose:fructose) mix, and sucrose for ten weeks.†

	Body Fat (%)	Lean Body Mass (%)
Genotype		
Normal	16.9 ± 0.4 [b]	76.5 ± 0.4 [a]
APC^{Min}	13.3 ± 0.4 [a]	80.4 ± 0.4 [b]
Sex		
Male	16.3 ± 0.4 [b]	76.5 ± 0.4 [a]
Female	14.0 ± 0.4 [a]	80.4 ± 0.4 [b]
Treatment		
Starch	13.0 ± 0.7 [a]	80.3 ± 0.7 [c]
Fructose	14.0 ± 0.8 [ab]	79.5 ± 0.7 [bc]
Glucose	15.8 ± 0.6 [b]	77.8 ± 0.6 [b]
G:F	14.4 ± 0.7 [ab]	79.0 ± 0.6 [bc]
Sucrose	18.4 ± 0.7 [c]	75.7 ± 0.7 [a]
All Parameters		
Normal Males		
Starch	15.0 ± 1.3 [a]	77.0 ± 1.3 [c]
Fructose	17.7 ± 2.0 [ab]	74.8 ± 1.9 [bc]
Glucose	20.5 ± 1.1 [b]	72.4 ± 1.0 [b]
G:F	16.9 ± 1.3 [ab]	75.4 ± 1.3 [bc]
Sucrose	24.3 ± 1.1 [c]	69.3 ± 1.0 [a]
Normal Females		
Starch	13.0 ± 1.5 [a]	81.2 ± 1.4 [b]
Fructose	14.1 ± 1.2 [ab]	80.2 ± 1.2 [ab]
Glucose	15.1 ± 1.4 [ab]	79.3 ± 1.3 [ab]
G:F	15.1 ± 1.3 [ab]	79.0 ± 1.3 [ab]
Sucrose	17.7 ± 1.7 [b]	77.0 ± 1.6 [a]

Table 3 (Cont'd)

	Body Fat (%)	Lean Body Mass (%)
APC^{Min} Males		
Starch	12.2 ± 1.4 [a]	80.0 ± 1.3 [b]
Fructose	11.4 ± 1.4 [a]	81.5 ± 1.3 [b]
Glucose	14.2 ± 1.2 [a]	78.6 ± 1.1 [ab]
G:F	12.2 ± 1.5 [a]	80.4 ± 1.4 [b]
Sucrose	18.2 ± 1.5 [b]	75.3 ± 1.4 [a]
APC^{Min} Females		
Starch	11.9 ± 1.2	82.9 ± 1.2
Fructose	12.6 ± 1.4	81.7 ± 1.4
Glucose	13.5 ± 1.5	80.9 ± 1.4
G:F	13.6 ± 1.2	81.1 ± 1.2
Sucrose	13.6 ± 1.4	81.1 ± 1.4

† Data are presented as least square means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$.

Table 4. Estimate of food intake based on remaining diet after study termination.†

	Total Diet Disappearance (kg)	Diet/Mouse/Day (g)*
Starch	24.2	5.5
Fructose	26.8	5.1
Glucose	24.3	6.3
G:F[1]	26.7	6.1
Sucrose	27.8	6.0

* Values are not equal to Total Diet Consumed/n mice due to correction for animals sacrificed before study termination.

† Calculations are based on assumptions of similar amount of food lost as waste across all treatments.

[1] 1:1 glucose:fructose ratio.

Table 5a. Proximal small intestine tumor number, average size, and burden in C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.†

PROXIMAL SMALL INTESTINE	**Tumor Number**	**Average Size (mm^2)**	**Total Burden* (mm^2)**
Sex			
Male	21.3 ± 1.2	0.96 ± 0.06 [a]	18.9 ± 1.7 [a]
Female	23.4 ± 1.2	1.19 ± 0.05 [b]	26.9 ± 1.6 [b]
Treatment			
Starch	17.4 ± 1.9 [a]	1.51 ± 0.09 [b]	24.9 ± 2.6
Fructose	30.8 ± 2.0 [c]	0.92 ± 0.09 [a]	27.3 ± 2.6
Glucose	23.0 ± 1.8 [b]	0.90 ± 0.08 [a]	21.7 ± 2.4
G:F	20.6 ± 1.9 [ab]	1.08 ± 0.09 [a]	20.6 ± 2.5
Sucrose	20.0 ± 2.0 [ab]	0.98 ± 0.09 [a]	20.0 ± 2.6

* Total burden was calculated by summing the total tumor area within the proximal third of the SI.

† Data are presented as Least Square Mean ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$.

Table 5b. Medial small intestine tumor number, average size, and burden in C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.†×

MEDIAL SMALL INTESTINE	**Tumor Number**	**Average Size (mm^2)**	**Total Burden* (mm^2)**
Sex			
Male	28.8 ± 1.5 ^{y}	1.05 ± 0.04 ^{b}	30.8 ± 2.4
Female	32.7 ± 1.5 ^{z}	0.93 ± 0.04 ^{a}	31.7 ± 2.3
Treatment			
Starch	28.6 ± 2.4 ^{ab}	1.09 ± 0.07	32.1 ± 3.7
Fructose	36.0 ± 2.4 ^{c}	0.94 ± 0.07	33.5 ± 3.8
Glucose	31.5 ± 2.2 ^{abc}	1.00 ± 0.06	33.6 ± 3.5
G:F	32.3 ± 2.3 ^{bc}	0.95 ± 0.07	31.8 ± 3.7
Sucrose	25.6 ± 2.4 ^{a}	0.97 ± 0.07	25.3 ± 3.8

* Total burden was calculated by summing the total tumor area within the proximal third of the SI.
† Data are presented as Least Square Means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$, overall $F < 0.05$.
× Lettered superscripts y-z indicate trends at $P \leq 0.10$, overall $F \leq 0.10$.

Table 5c. Distal small intestine tumor number, average size, and burden in C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.┼×

DISTAL SMALL INTESTINE	**Tumor Number**	**Average Size (mm^2)**	**Total Burden* (mm^2)**
Sex			
Male	29.4 ± 1.9	0.91 ± 0.04 ^{b}	29.3 ± 2.7
Female	32.7 ± 1.8	0.74 ± 0.04 ^{a}	27.0 ± 2.6
Treatment			
Starch	31.8 ± 3.0 ^{bc}	0.95 ± 0.07	33.6 ± 4.1 ^{b}
Fructose	39.7 ± 3.0 ^{c}	0.90 ± 0.07	36.6 ± 4.2 ^{b}
Glucose	29.0 ± 2.8 ^{ab}	0.77 ± 0.07	26.6 ± 4.1 ^{ab}
G:F	32.0 ± 2.9 ^{bc}	0.76 ± 0.07	26.0 ± 4.1 ^{ab}
Sucrose	22.7 ± 2.3 ^{a}	0.75 ± 0.07	17.9 ± 4.3 ^{a}

* Total burden was calculated by summing the total tumor area within the proximal third of the SI.

┼ Data are presented as Least Square Means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$, overall $F < 0.05$.

× Lettered superscripts y-z indicate trends at $P \leq 0.10$, overall $F \leq 0.10$.

Table 6. Total small intestine tumor number, average size, and burden in C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.┼×

TOTAL SMALL INTESTINE	**Tumor Number**	**Average Size (mm^2)**	**Total Burden* (mm^2)**
Sex			
Male	79.6 ± 4.1^{z}	0.97 ± 0.03	79.3 ± 5.7
Female	89.3 ± 3.9^{y}	0.92 ± 0.03	86.3 ± 5.5
Treatment			
Starch	77.9 ± 6.4^{ab}	1.12 ± 0.05^{b}	90.6 ± 8.9^{y}
Fructose	106.4 ± 6.5^{c}	0.90 ± 0.05^{a}	97.5 ± 9.0^{y}
Glucose	85.5 ± 6.1^{b}	0.91 ± 0.05^{a}	84.4 ± 8.7^{y}
G:F	84.8 ± 6.3^{ab}	0.90 ± 0.05^{a}	78.4 ± 8.7^{zy}
Sucrose	67.7 ± 6.4^{a}	0.90 ± 0.05^{a}	63.3 ± 9.1^{z}

* Total burden was calculated by summing the total tumor area within the proximal third of the SI.

┼ Data are presented as Least Square Means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$, overall $F < 0.05$.

× Lettered superscripts y-z indicate trends at $P \leq 0.10$, overall $F \leq 0.10$.

Table 7. Colon tumor number, average size, burden and incidence in C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice fed corn starch, fructose, glucose, an equal G:F (glucose:fructose) mix, or sucrose.†

COLON	**Tumor Number**	**Average Size**	**Tumor Burden**	**Incidence (%)**
Sex				
Male	0.91 ± 0.12 [b]	3.87 ± 0.59 [b]	6.77 ± 0.96 [b]	51.4 ± 6.0 [b]
Female	0.40 ± 0.11 [a]	1.58 ± 0.57 [a]	2.32 ± 0.92 [a]	26.7 ± 5.1 [a]
Treatment				
Starch	0.73 ± 0.18	3.26 ± 0.93	5.36 ± 1.49	41.4 ± 9.3
Fructose	0.62 ± 0.18	1.96 ± 0.95	3.78 ± 1.52	33.3 ± 9.3
Glucose	0.69 ± 0.17	3.81 ± 0.88	6.11 ± 1.41	43.8 ± 8.9
G:F	0.87 ± 0.18	2.17 ± 0.92	4.79 ± 1.47	44.8 ± 9.4
Sucrose	0.38 ± 0.18	2.44 ± 0.94	2.68 ± 1.51	28.6 ± 8.7

† Data are presented as Least Square Means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$, overall $F < 0.05$.

Table 8. Average plasma glucose and insulin concentrations among C57BL/6J (normal) and C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice after feeding starch, glucose, fructose, a G:F (glucose:fructose) mix, and sucrose for ten weeks.†×

	Glucose (mM)	Insulin (ng/ml)
Genotype		
Normal	16.4 ± 0.5	1.6 ± 0.1
APC Min	17.3 ± 0.5	1.4 ± 0.1
Sex		
Male	17.5 ± 0.5 ^{b}	2.0 ± 0.1 ^{b}
Female	16.2 ± 0.5 ^{a}	1.0 ± 0.1 ^{a}
Treatment		
Starch	15.2 ± 0.7 ^{z}	1.1 ± 0.2 ^{z}
Fructose	17.8 ± 0.7 ^{y}	1.7± 0.2 ^{zy}
Glucose	16.3 ± 0.7 ^{zy}	1.4 ± 0.2 ^{zy}
G:F	17.5 ± 0.7 ^{y}	1.4 ± 0.2 ^{zy}
Sucrose	17.5 ± 0.7 ^{y}	1.8 ± 0.2 ^{y}

† Data are presented as Least Square Means ± SEM and lettered superscripts a-c indicate significant differences within columns and subheadings at $P < 0.05$, overall $F < 0.05$.
× Lettered superscripts y-z indicate trends at $P \leq 0.10$, overall $F < 0.07$ for glucose results, overall $F < 0.11$ for insulin results.

Table 9. Average Plasma IGF-1 concentrations among C57BL/6J (normal) and C57BL/6J $APC^{Min/+}$ (APC^{Min}) mice after feeding starch, glucose, fructose, a G:F (glucose:fructose) mix, and sucrose for ten weeks. [×]

	IGF-1 (ng/ml)
Genotype	
Normal	66.6 ± 7.0 [z]
APC Min	83.0 ± 6.7 [y]
Sex	
Male	77.3 ± 6.8
Female	72.4 ± 6.9
Treatment	
Starch	62.6 ± 11.3
Fructose	84.7 ± 10.1
Glucose	77.4 ± 9.8
G:F	53.6 ± 12.8
Sucrose	95.7 ± 9.6

× Lettered superscripts y-z indicate trends at $P \leq 0.10$.

DISCUSSION

Weight and Body Composition

It is commonly observed that carriers of the APC^{Min} defect gain less weight than their wild type littermates,[78] and that males gain more weight than females. Because of this, treatment effects on body weight are more easily observed in the normal mice as the potential for weight gain is higher overall. At study termination, the sucrose-fed mice had gained the most weight (Figures 1a,b) and accumulated the most body fat (Table 3). These results confirm the earlier findings in our laboratory published by Wang et al (2009)[16].

Consumption of sucrose as a beverage or in solid form can be linked to weight gain and adiposity in humans and animals.[16, 51, 68, 70, 71, 79, 80] One study has shown that rats eating granulated sucrose gained more weight per calorie consumed than those eating a control diet, [51] which would lead to increased weight gain in the sucrose-fed mice compared to the other sugar based diets. Glucose-fed mice had the second highest body weights, only being lower in weight than sucrose-fed mice at the end of the experiment. The starch- and fructose-fed groups had the lowest body weights at the end of the study, with the weights of the G:F group being similar to the glucose-fed mice. Although there is evidence that links fructose consumption to weight gain and obesity, these studies tend to extrapolate to HFCS without a true comparison. Many reviews in the literature on the effects of dietary carbohydrates on body weight find few differences between HFCS, sucrose, or other sugars' contribution to weight gain and attribute overweight and obesity to consumption of excess sugars, calories, and shifting dietary patterns in general.[72,

[73, 75, 76] The data from the current study suggest that sucrose may be more harmful than HFCS in terms of weight gain and body fat accumulation.

Since food was provided *ad libitum*, there is a possibility that differences in food intake by the mice could have influenced the results. The intent of this study was not to control for food intake but rather to allow free access to food just as people make daily choices in how much they consume. By using an estimate of daily food intake per mouse (Table 4) it can be seen that the starch-fed and fructose-fed mice may have consumed less food overall compared to the mice fed the other dietary treatments. Despite the fact that these food disappearance data are only an indirect measure of food intake by the mice, in general they roughly correlate with the final mean body weights of mice on the respective treatments. The starch group may have consumed less diet due to lower palatability compared to the other treatments, whereas food consumption by the fructose group may have been affected by possible malabsorption which is commonly associated with consuming high levels of fructose. Body weight observations at early time points in the study suggest that mice consuming the fructose diets may have taken one to two weeks to adjust to fructose consumption, as body weights for the fructose-fed mice were lowest at day 7. However, this lag in body weight gain did not appear to confound the overall experiment, as mice consuming fructose maintained body weights similar to those consuming starch throughout the rest of the experiment.

Tumor Numbers and Burden

The development of small intestinal tumors tended ($P < 0.10$) to be higher in females than males. Many previous studies comparing carbohydrate effects on colon cancer use a single sex model,[14, 15, 39-41, 81] or see no sex differences.[16] The differences observed between females

and males in this experiment were modest and there were no diet X sex interactions detected for tumor numbers, average sizes, or total burdens in the small intestine.

Effects of treatment on tumor number can be seen in all intestinal sections as well as the total SI overall. Fructose-fed mice had the largest numbers of tumor numbers overall and in each section, whereas the sucrose-fed mice had the fewest small intestinal tumors overall (although total SI adenoma numbers were not statistically different for sucrose-, starch-, or G:F-fed mice). We anticipated that sucrose-fed mice would have the largest numbers of SI adenomas in this experiment, so the observation that this treatment elicited the smallest numbers of tumors was unexpected. However, other studies have also failed to show a relationship between sucrose consumption and tumorigenesis [35, 39] and the results observed in this experiment may be due to the short term nature of the feeding study. In previous research in our laboratory, the detrimental effects of sucrose on intestinal adenoma development were more pronounced in longer-term feeding studies facilitated by adding relatively low concentrations of sulindac to the diets to delay small intestinal tumorigenesis.

Compared to the effects of other sugars, the impact of fructose consumption on intestinal tumorigenesis has not been widely studied, and published results are inconsistent. Fructose-fed rats have been observed to have higher rates of colonic epithelial cell proliferation[41] in one study while feeding fructose to rats in another study has shown to have almost a protective effect.[39] However, the potential for fructose to stimulate colon cancer risk and possible mechanisms for these effects warrants further study based upon the results of the present experiment as well as human epidemiological data which correlate fructose consumption with increased risk.[9, 10, 12]

Mice fed the glucose and G:F diets had overall SI adenoma numbers that were statistically similar to mice consuming starch-based diets. We were surprised to observe that mice consuming sucrose had significantly fewer SI adenomas than mice consuming glucose, and tended ($P = 0.058$) to have fewer adenomas than mice consuming the G:F mixture. No comparable studies of the effects of these monosaccharides on intestinal tumorigenesis were found in the scientific literature. These observations merit further study to assess if sucrose feeding elicits differential effects on intestinal tumorigenesis compared to feeding its component monosaccharides.

In this experiment, the only dietary treatment that had an effect on average tumor size was the starch control. Starch-fed mice developed significantly larger SI tumors, and this effect was primarily observed in the proximal SI. This relationship is still present when the total SI is considered and is consistent with results that have been previously seen in this lab.[16] The mechanisms driving differences in intestinal adenoma size are not known but clearly are repeatable in this model of human intestinal tumor development. Future research should study potential mechanisms (such as the potential for increased rates of crypt fission) to explain the impact of starch feeding on SI adenoma development in APC^{Min} mice. These studies also should be repeated in other models to determine if this effect is unique to this cancer model.

There were no significant effects of diet on colon tumor development. This is also comparable to what has been observed previously in our laboratory when APC^{Min} mice were fed starch or sucrose based diets for ten weeks.[16] Male mice had a significantly greater incidence of colonic adenomas compared to females. Males also had significantly greater numbers of

tumors, a larger average tumor size, and greater overall colonic tumor burden compared to female mice. Being male is a recognized risk factor for colon cancer in humans.[1, 2]

Plasma Analyses

Plasma glucose and insulin concentrations did not differ between the normal and APC^{Min} mice in this experiment, and there were no genotype X diet interactions detected, indicating that diets equally affected glucose and insulin levels in both normal and APC^{Min} mice. Males had significantly higher plasma insulin and glucose than females, which may be a consequence of generally higher body weights.

Although the effects of diet on blood glucose (overall treatment $F = 0.066$), and insulin were not statistically significant (overall treatment $F = 0.106$), the trends still merit discussion. Sucrose-fed mice gained more weight and had greater body fat percentages compared to all other treatment groups, and it appears the sucrose-fed mice may have been developing insulin resistance at the end of this study based on the higher levels of plasma insulin and glucose compared to mice consuming starch based diets. Insulin resistance describes a metabolic state wherein the effects of insulin on glucose uptake are altered in a way that requires higher insulin concentrations to return blood glucose to homeostasis. Insulin resistance is more common in people with higher body weight.[82]

In this experiment, no significant effects of diet on plasma IGF-1 concentrations were observed after 10 weeks of dietary treatment. Insulin is known to positively influence IGF-1 concentrations,[25] and since dietary treatments did not significantly alter plasma insulin within this study, it is reasonable that IGF-1 concentrations also were not significantly altered. Previous

research in this lab have shown similar results (i.e. relatively weak effects of diet on insulin and IGF-1 concentrations in sucrose compared to starch-fed mice).

Taken together, the results of this study suggest that mice consuming sucrose-based diets were not yet insulin resistant despite their greater average body weights and body fat percentage after ten weeks of dietary treatment. Extension of the duration of dietary treatment, as was done by Wang et al (2009)[16] is probably necessary to more fully induce insulin resistance by sucrose feeding in this model. In the APC^{Min} variant of the C57BL mouse model, extending the feeding duration is impractical because the onset of morbidity caused by small intestinal adenoma development usually occurs at approximately 13-14 weeks of age. Wang et al used a modified treatment protocol where sulindac (100 ppm) was added to diets to delay small intestinal adenoma development. Although this approach allowed more time for colonic tumorigenesis in the model, the use of sulindac to extend colon tumor promotion could have significant negative consequences on interpretation of results related to overall adenoma development throughout the intestine. For that reason, we elected to use the 10-week feeding protocol without added sulindac for this feeding study. Future research should assess the use of other protocols (e.g. such as the use of azoxymethane-induced tumorigenesis in normal C57BL mice) which allow for extended tumor promotion periods, as such models might allow for more thorough assessments of the impact of insulin resistance on adenoma development.

Summary and Conclusions

Overall, the effects of dietary sugars on colon cancer development in APC^{Min} mice are not completely clear. Feeding sucrose as the sole carbohydrate source resulted in an increase (compared to other treatment groups) of body weight, higher body fat accumulation, and tended

to stimulate plasma glucose and insulin concentrations at 10 weeks. These observations largely fit our original hypothesis and agree with much published literature.

Feeding fructose as the sole carbohydrate source increased overall SI adenoma development and also tended to increase plasma glucose and insulin similar to that observed for sucrose. This result was somewhat unexpected and the inherent differences in fructose metabolism compared to other sugars may be contributing to the observed effects. Fructose consumption is generally found to increase circulating triglycerides, induce *de novo* lipid synthesis and hepatic insulin resistance and not affect blood glucose or insulin concentrations directly.[52-54, 83] High serum triglyceride levels have been correlated to colon cancer risk in humans[46, 84] and the metabolic syndrome (which is characterized by increased body mass index/waist circumference, blood pressure, plasma glucose, and triglycerides, as well as decreased high-density lipoprotein cholesterol) has been determined to be a high risk state for cancers, including colorectal cancer.[85, 86] Based on these observations, it would be useful to assess serum triglycerides and/or lipoprotein levels for mice consuming the different dietary treatments in order to better characterize the short term metabolic effects that may influence intestinal tumorigenesis in APC^{Min} mice.

In this study, mice consuming the diets based on glucose and 1:1 glucose:fructose generally had very similar body weights, body composition, adenoma numbers, sizes and burdens, and blood parameters. Conversely, fructose-fed mice had lower average body weight, less body fat, greater adenoma numbers, and tended to have higher blood glucose and insulin compared to those consuming glucose or G:F. Based on these observations, we conclude that feeding combinations of glucose and fructose (as in the G:F diet) significantly reduces the

adverse effects of feeding a diet containing fructose as its sole carbohydrate source. Further study is warranted to assess the minimum quantity of dietary glucose necessary to reduce the adverse effects of fructose feeding.

Mice consuming sucrose based diets had several differences compared to mice consuming diets containing a mixture of glucose and fructose. Sucrose-fed mice had greater final body weights, greater body fat, and less lean body mass, yet tended to have fewer small intestinal adenomas than mice consuming G:F. Further study is needed to more fully assess mechanisms whereby these diets elicit different effects on these parameters. We hypothesize that diet palatability and food intake may explain some of these observations, although other effects including potential influence on metabolic pathways and cell signaling cannot be discounted.

In conclusion, this research demonstrated that dietary carbohydrates, when fed as the sole carbohydrate source in the diet, differentially affect growth and body composition in C57BL mice and intestinal adenoma development in C57BL mice carrying the APC^{Min} defect. The effects of diet on intestinal adenoma development were restricted to the small intestine in this study, and were not dependant on blood glucose, insulin, or IGF-1 concentrations. It is also important to note that our original hypothesis was based on colonic tumor development, whereas in this study dietary effects were only seen in the small intestine. The hypothesized mechanisms of action we proposed for feeding the different dietary carbohydrates may be more relevant for colonic tumors than small intestinal tumors and should not be discounted based on these results. We further hypothesize that these diets would significantly impact colonic tumorigenesis in models which allow examination of the potential longer-term effects of increased plasma glucose, insulin and IGF-1, which are all commonly associated with insulin resistance and

metabolic syndrome. Additional studies are warranted to more fully assess these dietary effects and potential mechanisms whereby dietary carbohydrate sources influence colon cancer risk.

REFERENCES

REFERENCES

1. American Cancer Society. **Cancer Facts and Figures 2011**. Atlanta, GA:American Cancer Society, 2011.

2. American Institute for Cancer Research/World Cancer Research Fund (AICR/WCR). **Colon and rectum cancers. In: AICR/WCR. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective.** Second Report. 2007. Washington DC: AICR.280-288.

3. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC: **Major dietary patterns and the risk of colorectal cancer in women.** *Arch Intern Med* 2003, **163:**309-314.

4. Terry P, Hu FB, Hansen H, Wolk A: **Prospective study of major dietary patterns and colorectal cancer risk in women.** *Am J Epidemiol* 2001, **154:**1143-1149.

5. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN: **Eating patterns and risk of colon cancer.** *Am J Epidemiol* 1998, **148:**4-16.

6. Slattery ML, Potter JD, Ma KN, Caan BJ, Leppert M, Samowitz W: **Western diet, family history of colorectal cancer, NAT2, GSTM-1 and risk of colon cancer.** *Cancer Causes Control* 2000, **11:**1-8.

7. Stefani ED, Deneo-Pellegrini H, Ronco AL, Correa P, Boffetta P, Aune D, Acosta G, Mendilaharsu M, Luaces ME, Lando G, Silva C: **Dietary patterns and risk of colorectal cancer: a factor analysis in uruguay.** *Asian Pac J Cancer Prev* 2011, **12:**753-759.

8. Bostick RM, Potter JD, Kushi LH, Sellers TA, Steinmetz KA, McKenzie DR, Gapstur SM, Folsom AR: **Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States).** *Cancer Causes Control* 1994, **5:**38-52.

9. Higginbotham S, Zhang ZF, Lee IM, Cook NR, Giovannucci E, Buring JE, Liu S: **Dietary glycemic load and risk of colorectal cancer in the Women's Health Study.** *J Natl Cancer Inst* 2004, **96:**229-233.

10. Michaud DS, Fuchs CS, Liu S, Willett WC, Colditz GA, Giovannucci E: **Dietary glycemic load, carbohydrate, sugar, and colorectal cancer risk in men and women.** *Cancer Epidemiol Biomarkers Prev* 2005, **14:**138-147.

11. Nayak SP, Sasi MP, Sreejayan MP, Mandal S: **A case-control study of roles of diet in colorectal carcinoma in a South Indian Population.** *Asian Pac J Cancer Prev* 2009, **10:**565-568.

12. Slattery ML, Benson J, Berry TD, Duncan D, Edwards SL, Caan BJ, Potter JD: **Dietary sugar and colon cancer.** *Cancer Epidemiol Biomarkers Prev* 1997, **6:**677-685.

13. Luceri C, Caderni G, Lancioni L, Aiolli S, Dolara P, Mastrandrea V, Scardazza F, Morozzi G: **Effects of repeated boluses of sucrose on proliferation and on AOM-induced aberrant crypt foci in rat colon.** *Nutr Cancer* 1996, **25:**187-196.

14. Caderni G, Luceri C, Spagnesi MT, Giannini A, Biggeri A, Dolara P: **Dietary carbohydrates modify azoxymethane-induced intestinal carcinogenesis in rats.** *J Nutr* 1994, **124:**517-523.

15. Caderni G, Bianchini F, Mancina A, Spagnesi MT, Dolara P: **Effect of dietary carbohydrates on the growth of dysplastic crypt foci in the colon of rats treated with 1,2-dimethylhydrazine.** *Cancer Res* 1991, **51:**3721-3725.

16. Wang B, Bobe G, LaPres JJ, Bourquin LD: **High sucrose diets promote intestinal epithelial cell proliferation and tumorigenesis in APC(Min) mice by increasing insulin and IGF-I levels.** *Nutr Cancer* 2009, **61:**81-93.

17. Poulsen M, Molck AM, Thorup I, Breinholt V, Meyer O: **The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon.** *Cancer Lett* 2001, **167:**135-143.

18. Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, Tracy RP, Dobs A, Savage PJ: **Increased blood glucose and insulin, body size, and incident colorectal cancer.** *J Natl Cancer Inst* 1999, **91:**1147-1154.

19. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ: **Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3.** *J Natl Cancer Inst* 1999, **91:**620-625.

20. Kaaks R, Toniolo P, Akhmedkhanov A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, Zeleniuch-Jacquotte A, Shore RE, Riboli E: **Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women.** *J Natl Cancer Inst* 2000, **92:**1592-1600.

21. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Scrive M: **Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice.** *Metabolism* 1995, **44:**645-651.

22. Cao D, Lu H, Lewis TL, Li L: **Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease.** *J Biol Chem* 2007, **282:**36275-36282.

23. Tran TT, Medline A, Bruce WR: **Insulin promotion of colon tumors in rats.** *Cancer Epidemiol Biomarkers Prev* 1996, **5:**1013-1015.

24. Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D: **Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis.** *Cancer Res* 2002, **62:**1030-1035.

25. Sandhu MS, Dunger DB, Giovannucci EL: **Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer.** *J Natl Cancer Inst* 2002, **94:**972-980.

26. Tajima K, Hirose K, Nakagawa N, Kuroishi T, Tominaga S: **Urban-rural difference in the trend of colo-rectal cancer mortality with special reference to the subsites of colon cancer in Japan.** *Jpn J Cancer Res* 1985, **76:**717-728.

27. Half E, Bercovich D, Rozen P: **Familial adenomatous polyposis.** *Orphanet J Rare Dis* 2009, **4:**22.

28. Lynch HT, Lynch JF: **Hereditary nonpolyposis colorectal cancer.** *Semin Surg Oncol* 2000, **18:**305-313.

29. Galiatsatos P, Foulkes WD: **Familial adenomatous polyposis.** *Am J Gastroenterol* 2006, **101:**385-398.

30. de la Chapelle A: **Genetic predisposition to colorectal cancer.** *Nat Rev Cancer* 2004, **4:**769-780.

31. Giovannucci E, Willett WC: **Dietary factors and risk of colon cancer.** *Ann Med* 1994, **26:**443-452.

32. Giovannucci E, Stampfer MJ, Colditz G, Rimm EB, Willett WC: **Relationship of diet to risk of colorectal adenoma in men.** *J Natl Cancer Inst* 1992, **84:**91-98.

33. Skraastad O: **[Dietary factors and risk of colon cancer].** *Tidsskr Nor Laegeforen* 1990, **110:**32-34.

34. Su LK, Kinzler KW, Vogelstein B, Preisinger AC, Moser AR, Luongo C, Gould KA, Dove WF: **Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene.** *Science* 1992, **256:**668-670.

35. Corpet DE, Pierre F: **Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system.** *Cancer Epidemiol Biomarkers Prev* 2003, **12:**391-400.

36. Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE: **Increased cell division as a cause of human cancer.** *Cancer Res* 1990, **50:**7415-7421.

37. Hanahan D, Weinberg RA: **The hallmarks of cancer.** *Cell* 2000, **100:**57-70.

38. Bird RP: **Role of aberrant crypt foci in understanding the pathogenesis of colon cancer.** *Cancer Lett* 1995, **93:**55-71.

39. Caderni G, Luceri C, Lancioni L, Biggeri A, Giannini A, Fazi M, Brighenti F, Cresci A, Orpianesi C, Dolara P: **Modification of azoxymethane intestinal carcinogenesis in rats by feeding sucrose boluses, pasta, and glucose.** *Nutr Cancer* 1997, **28:**146-152.

40. Caderni G, Luceri C, Lancioni L, Dolara P: **Dietary sucrose, glucose, fructose, and starches affect colonic functions in rats.** *Nutr Cancer* 1996, **25:**179-186.

41. Stamp D, Zhang XM, Medline A, Bruce WR, Archer MC: **Sucrose enhancement of the early steps of colon carcinogenesis in mice.** *Carcinogenesis* 1993, **14:**777-779.

42. Ludwig DS, Eckel RH: **The glycemic index at 20 y.** *Am J Clin Nutr* 2002, **76:**264S-265S.

43. Giovannucci E: **Insulin, insulin-like growth factors and colon cancer: a review of the evidence.** *J Nutr* 2001, **131:**3109S-3120S.

44. Franceschi S, Dal Maso L, Augustin L, Negri E, Parpinel M, Boyle P, Jenkins DJ, La Vecchia C: **Dietary glycemic load and colorectal cancer risk.** *Ann Oncol* 2001, **12:**173-178.

45. Nilsen TI, Vatten LJ: **Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyperinsulinaemia hypothesis.** *Br J Cancer* 2001, **84:**417-422.

46. McKeown-Eyssen G: **Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk?** *Cancer Epidemiol Biomarkers Prev* 1994, **3:**687-695.

47. Larsson SC, Orsini N, Wolk A: **Diabetes mellitus and risk of colorectal cancer: a meta-analysis.** *J Natl Cancer Inst* 2005, **97:**1679-1687.

48. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M: **Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis.** *Lancet* 2004, **363:**1346-1353.

49. Pawlak DB, Bryson JM, Denyer GS, Brand-Miller JC: **High glycemic index starch promotes hypersecretion of insulin and higher body fat in rats without affecting insulin sensitivity.** *J Nutr* 2001, **131:**99-104.

50. Jurgens H, Haass W, Castaneda TR, Schurmann A, Koebnick C, Dombrowski F, Otto B, Nawrocki AR, Scherer PE, Spranger J, et al: **Consuming fructose-sweetened beverages increases body adiposity in mice.** *Obes Res* 2005, **13:**1146-1156.

51. Kanarek RB, Orthen-Gambill N: **Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats.** *J Nutr* 1982, **112:**1546-1554.

52. Havel PJ: **Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism.** *Nutr Rev* 2005, **63:**133-157.

53. Le KA, Tappy L: **Metabolic effects of fructose.** *Curr Opin Clin Nutr Metab Care* 2006, **9:**469-475.

54. Basciano H, Federico L, Adeli K: **Fructose, insulin resistance, and metabolic dyslipidemia.** *Nutr Metab (Lond)* 2005, **2:**5.

55. Thresher JS, Podolin DA, Wei Y, Mazzeo RS, Pagliassotti MJ: **Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance.** *Am J Physiol Regul Integr Comp Physiol* 2000, **279:**R1334-1340.

56. Crapo PA, Reaven G, Olefsky J: **Plasma glucose and insulin responses to orally administered simple and complex carbohydrates.** *Diabetes* 1976, **25:**741-747.

57. Lee BM, Wolever TM: **Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread.** *Eur J Clin Nutr* 1998, **52:**924-928.

58. Anderson GH, Catherine NL, Woodend DM, Wolever TM: **Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men.** *Am J Clin Nutr* 2002, **76:**1023-1030.

59. Bantle JP, Laine DC, Castle GW, Thomas JW, Hoogwerf BJ, Goetz FC: **Postprandial glucose and insulin responses to meals containing different carbohydrates in normal and diabetic subjects.** *N Engl J Med* 1983, **309:**7-12.

60. Gray GM, Ingelfinger FJ: **Intestinal absorption of sucrose in man: interrelation of hydrolysis and monosaccharide product absorption.** *J Clin Invest* 1966, **45:**388-398.

61. Hallfrisch J: **Metabolic effects of dietary fructose.** *FASEB J* 1990, **4:**2652-2660.

62. Henry RR, Crapo PA, Thorburn AW: **Current issues in fructose metabolism.** *Annu Rev Nutr* 1991, **11:**21-39.

63. Mayes PA, Laker ME: **Effects of acute and long-term fructose administration on liver lipid metabolism.** *Prog Biochem Pharmacol* 1986, **21:**33-58.

64. Mayes PA: **Intermediary metabolism of fructose.** *Am J Clin Nutr* 1993, **58:**754S-765S.

65. Wei Y, Wang D, Topczewski F, Pagliassotti MJ: **Fructose-mediated stress signaling in the liver: implications for hepatic insulin resistance.** *J Nutr Biochem* 2007, **18:**1-9.

66. Marriott BP, Cole N, Lee E: **National estimates of dietary fructose intake increased from 1977 to 2004 in the United States.** *J Nutr* 2009, **139:**1228S-1235S.

67. Bleich SN, Wang YC, Wang Y, Gortmaker SL: **Increasing consumption of sugar-sweetened beverages among US adults: 1988-1994 to 1999-2004.** *Am J Clin Nutr* 2009, **89:**372-381.

68. Malik VS, Schulze MB, Hu FB: **Intake of sugar-sweetened beverages and weight gain: a systematic review.** *Am J Clin Nutr* 2006, **84:**274-288.

69. Bray GA, Nielsen SJ, Popkin BM: **Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity.** *Am J Clin Nutr* 2004, **79:**537-543.

70. Malik VS, Popkin BM, Bray GA, Despres JP, Hu FB: **Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk.** *Circulation* 2010, **121:**1356-1364.

71. Hu FB, Malik VS: **Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence.** *Physiol Behav* 2010, **100:**47-54.

72. Forshee RA, Storey ML, Allison DB, Glinsmann WH, Hein GL, Lineback DR, Miller SA, Nicklas TA, Weaver GA, White JS: **A critical examination of the evidence**

relating high fructose corn syrup and weight gain. *Crit Rev Food Sci Nutr* 2007, **47:**561-582.

73. White JS: **Misconceptions about high-fructose corn syrup: is it uniquely responsible for obesity, reactive dicarbonyl compounds, and advanced glycation endproducts?** *J Nutr* 2009, **139:**1219S-1227S.

74. White JS: **Straight talk about high-fructose corn syrup: what it is and what it ain't.** *Am J Clin Nutr* 2008, **88:**1716S-1721S.

75. Jenkins DJ, Kendall CW, Marchie A, Augustin LS: **Too much sugar, too much carbohydrate, or just too much?** *Am J Clin Nutr* 2004, **79:**711-712.

76. Melanson KJ, Zukley L, Lowndes J, Nguyen V, Angelopoulos TJ, Rippe JM: **Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women.** *Nutrition* 2007, **23:**103-112.

77. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ: **Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals.** *Am J Clin Nutr* 2008, **87:**1194-1203.

78. The Jackson Laboratory. **C57BL/6J-ApcMin/J**. *JAX Mice Database.* 2011. Bar Harbor, ME.

79. Kanarek RB, Aprille JR, Hirsch E, Gualtiere L, Brown CA: **Sucrose-induced obesity: effect of diet on obesity and brown adipose tissue.** *Am J Physiol* 1987, **253:**R158-166.

80. Fortino MA, Lombardo YB, Chicco A: **The reduction of dietary sucrose improves dyslipidemia, adiposity, and insulin secretion in an insulin-resistant rat model.** *Nutrition* 2007, **23:**489-497.

81. Bianchini F, Caderni G, Magno C, Testolin G, Dolara P: **Profile of short-chain fatty acids and rectal proliferation in rats fed sucrose or cornstarch diets.** *J Nutr* 1992, **122:**254-261.

82. Kahn BB, Flier JS: **Obesity and insulin resistance.** *J Clin Invest* 2000, **106:**473-481.

83. Thorburn AW, Storlien LH, Jenkins AB, Khouri S, Kraegen EW: **Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats.** *Am J Clin Nutr* 1989, **49:**1155-1163.

84. Bruce WR, Wolever TM, Giacca A: **Mechanisms linking diet and colorectal cancer: the possible role of insulin resistance.** *Nutr Cancer* 2000, **37:**19-26.

85. Cowey S, Hardy RW: **The metabolic syndrome: A high-risk state for cancer?** *Am J Pathol* 2006, **169:**1505-1522.

86. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR: **The metabolic syndrome and risk of incident colorectal cancer.** *Cancer* 2006, **107:**28-36.