High Sucrose Diets Promote Intestinal Epithelial Cell Proliferation and Tumorigenesis in APC^{Min} Mice by Increasing Insulin and IGF-I Levels

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Epidemiological studies report that high sucrose consumption is associated with increased risk of colon cancer. One hypothesis is that this association is mediated by elevated circulatory insulin and IGF levels promoting intestinal proliferation. To test this hypothesis, APC^{Min} mice and their wild type littermates were fed, starting at 4 wk of age, sucrose or cornstarch as the sole carbohydrate source in the absence or presence of low levels of dietary sulindac for 10 or 16 wk, respectively. APC^{Min} mice fed sucrose had an increased tumor number in the proximal third of the small intestine in both studies and a higher incidence of papillary colon tumors in the 16-wk feeding study (P ≤ 0.05). Mice fed sucrose (relative to cornstarch) had higher body weights and greater Ki67-labeling indexes in colonic epithelium than mice fed cornstarch in both feeding studies (P ≤ 0.05). Furthermore, mice fed sucrose had higher serum glucose and liver IGF-I mRNA concentrations (P ≤ 0.05) and tended to have higher serum insulin levels (P = 0.08). These results support the hypothesis that high dietary sucrose intake promotes intestinal proliferation and tumorigenesis by increasing circulating levels of insulin and IGF-I.

INTRODUCTION

Colon cancer is the second most common cause of cancer mortality in the United States and claims more than 50,000 lives every year (1). Epidemiological studies suggest that high dietary intakes of sucrose are associated with greater colon cancer risk (2,3). Most studies using carcinogen-induced colon cancer models have reported that feeding high-sucrose diets to rats promoted development of aberrant crypt foci (ACF) and increased epithelial cell proliferation in colon crypts compared with feeding high-cornstarch diets (4–7). Colon adenocarcinomas in carcinogen-treated rats consuming high-sucrose diets were significantly larger and had more invasive potential compared with those in rats consuming cornstarch (8).

The underlying biological mechanism whereby high dietary sucrose may increase colon cancer risk is unclear. One hypothesis is that high dietary sucrose intake promotes intestinal proliferation and tumorigenesis by increasing circulating levels of insulin and IGF-I (9–11). High intakes of sucrose (vs. cornstarch) were found to decrease insulin sensitivity in the liver and later in peripheral tissues as assessed by euglycemic hyperinsulinemic clamps (12–14). High circulating insulin and IGF-I levels are associated with increased colon cancer risk (15–17).

To our knowledge, the effect of dietary carbohydrate source on intestinal proliferation and tumorigenesis has not been studied in APC^{Min} mice, which carry an inherited mutation in one allele of the APC gene and develop numerous adenomas in the intestine. APC gene mutation is an early event in colon carcinogenesis (18). Thus, APC^{Min} mice represent a useful model to study diet and gene interactions during colon carcinogenesis. One drawback of APC^{Min} mice is that tumorigenesis in the small intestine progresses more quickly than in the large intestine. The nonsteroidal anti-inflammatory drug sulindac reduces small intestinal tumor development but not that of colon tumors in APC^{Min} mice (19,20). We have previously administered a low dietary dose of sulindac to attenuate small intestinal...
tumorigenesis and extend the life span of APCMin mice, thereby increasing the likelihood of detecting dietary effects on colon tumorigenesis (21).

The objectives of the current study were to determine the effect of high dietary sucrose (vs. cornstarch) consumption on intestinal tumorigenesis and epithelial cell proliferation in APCMin mice and the influence of these diets on plasma concentrations of glucose, insulin, insulin-like growth factors (IGF), and insulin-like growth factor binding proteins (IGFBP).

MATERIALS AND METHODS

Animals and Diets

APCMin mice used for these studies were generated by mating C57BL/6J APCMin/+ males with C57BL/6J APC+/+ females in a breeding colony maintained at Michigan State University. Progeny were weaned at 4 wk of age and randomly assigned to dietary treatments. Mice were tested for APCMin carrier status (22) only after sacrifice. Therefore, the number of APCMin/+ mice per treatment differed among diets. Mice were housed in a facility with controlled conditions (temperature: 21–24°C; humidity: 40–70%; and light/dark cycle: 12 h light and 12 h dark). Animal care and experimental procedures were conducted with approval of the Michigan State University All-University Committee on Animal Use and Care.

Two feeding experiments were conducted with different durations of dietary treatment. Diets used in both feeding studies were modified from standard AIN-93G diets and contained either sucrose or cornstarch as the sole carbohydrate sources (Table 1). These modified diets also contained a greater proportion of fat (15% by weight) compared to standard AIN-93G diets (23), so essential nutrient concentrations in the modified diets were increased to account for the increased energy density of the diets used in this study. In the first experiment, 114 mice were fed diets containing either sucrose (APCMin/+: 12 males, 15 females; APC+/+: 17 males, 14 females) or cornstarch (APCMin/+: 13 males, 8 females; APC+/+: 13 males, 22 females) for 10 wk. The second experiment was designed using a 2 × 2 factorial arrangement of treatments, with two different dietary carbohydrate sources (sucrose vs. cornstarch) and two different dietary protein sources (casein vs. soy). A total of 218 mice were fed diets containing either sucrose and casein (APCMin/+: 12 males, 10 females; APC+/+: 12 males, 17 females), sucrose and soy (APCMin/+: 17 males, 15 females; APC+/+: 10 males, 12 females), cornstarch and casein (APCMin/+: 12 males, 16 females; APC+/+: 16 males, 11 females), or cornstarch and soy (APCMin/+: 15 males, 20 females; APC+/+: 14 males, 9 females). Furthermore, in the second experiment (16-wk study), a low dose of sulindac (Sigma Chemical Co., St. Louis, MO; purity certified by company) was added to all diets (100 mg/kg diet) to retard small intestinal tumorigenesis and allow more time for colon tumor development.

In doing so, we were able to sustain APCMin mice for 16 wk on dietary treatments in the second feeding experiment.

We observed no interactions between dietary carbohydrate and protein sources for any parameters observed in Experiment 2. Therefore, we are presenting only main effect means for dietary carbohydrate source in this article. Effects of dietary protein source on intestinal tumorigenesis will be presented in a separate article.

Sample Collection

Mouse body weights were measured weekly during both feeding experiments. Each experiment was terminated when we observed that 10% of mice had lost more than 10% of their maximum body weight or were moribund. Mice were sacrificed by CO2 asphyxiation. One lobe of the liver was sampled from each mouse and used for genotyping by an allele-specific PCR procedure to identify APCMin carriers (22). Serum samples were also collected from each mouse at the end of Experiment 1 by cardiac puncture during euthanasia for later analyses.

For each mouse, the entire small intestine, cecum, and colon was removed, opened longitudinally, rinsed with water, pinned on cardboard, and fixed with 10% (vol/vol) neutral buffered formalin overnight. Following fixation, a 1-cm section was excised from the middle of the colon and processed and paraffin-embedded for immunohistochemistry analyses. The fixed small intestine, cecum, and colon tissues were stained with 0.3% (wt/vol) methylene blue for 3 min to facilitate visualization and measurement of tumors.

Intestinal Tumor Analyses

Numbers of adenomas in each intestinal section (proximal, medial, and distal thirds of small intestine; cecum; colon) were determined by using a stereo microscope. The diameters of adenomas also were measured using a transparent grid placed under the specimen. All adenomas in small intestine were flat and their diameters were measured in two dimensions (d1, d2). The areas of adenomas were calculated using the formula area = π × d1 × d2. In the colon, only papillary adenomas were determined. The diameters of these tumors were measured in 3 dimensions (d1, d2, and d3), and the spherical volumes of each adenoma were calculated by using the formula volume = (π × d1 × d2 × d3)/6. No cecal papillary adenomas were detected in these experiments. All tumor measurements were performed by one person who was blinded to the treatments. In this study, H & E staining of a representative sample of papillary adenoma sections was performed. No signs of adenoma invasiveness or dietary differences in colon adenoma morphology were observed.

Cell Proliferation and Apoptosis in Colon Crypts

Intestinal cell proliferation and apoptosis was assessed in the colon and proximal third of the small intestine. Results are presented only for the colonic crypts because we were not
Table 1: Composition of diets (in g/kg diet) used in Experiment 1 (10-wk study without sulindac) and Experiment 2 (16-wk study with 100 mg sulindac per kg of diet)\(^a\)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>10-Wk Study</th>
<th>16-Wk Study</th>
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<tr>
<td>Soybean oil</td>
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<td>Cellulose</td>
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</tr>
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<td>AIN-93-VX(^b)</td>
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<td>11</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sulindac</td>
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</tr>
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</table>

\(^a\)Not all columns sum to 1,000 g/kg due to rounding.

\(^b\)Composition of AIN 93-MX mineral mix and AIN 93-VX vitamin as described in Reeves et al. (23).
FIG. 1. Influence of dietary carbohydrate source (sucrose vs. cornstarch) on small intestinal A: adenoma number, B: average adenoma size, C: total adenoma burden in the small intestine and on colonic D: adenoma prevalence, E: average adenoma size per colon adenoma-bearing mice, and F: total adenoma burden per colon adenoma-bearing mice (means ± standard errors) of APCMin mice (10-wk study without sulindac: 27 mice fed sucrose and 21 mice fed cornstarch; 16-wk study with 100 mg sulindac per kg diet: 54 mice fed sucrose and 63 mice fed cornstarch).

2 μg/ml in 10 mM Tris–HCl) for 15 min at room temperature. Slides were then rinsed twice with PBS, incubated with 3% H₂O₂ (Sigma-Aldrich) for 10 min at room temperature, and again rinsed twice with PBS. Slides were then covered with a slide cover slip and mounted on Shandon IHC staining racks (Thermo Electron Corporation, Waltham, MA). Slides were exposed to 2% bovine serum albumin (Sigma-Aldrich) for 20 min to mask nonspecific antigens. TUNEL reaction mixture (50 μl) consisting of terminal deoxynucleotidyl transferase and the nucleotide were added (the original enzyme solution in vial 1 was diluted with 2 times volume of TUNEL dilution buffer and then 5 μl of the diluted enzyme were mixed with 45 μl of the label solution to obtain the 50 μl working TUNEL reaction mixture). The slides were incubated for 60 min at room temperature in the dark. Slides were then rinsed 3 times with PBS, incubated for 30 min at 37°C with peroxidase-conjugated antibody (Converter-POD; Roche), and rinsed 3 times with PBS. Slides were then incubated for 10 min at room temperature
with 100 µl of 3-amino-9-ethylcarbozol (AEC) chromogen substrate solution (Dako), counterstained with hematoxylin for 15 s, rinsed in distilled water, blued with 0.3% ammonia water, and cover slipped using Paramount aqueous mounting media (Dako). Cells having red stained nuclei were identified as TUNEL positive. Ten intact intestinal crypts were scored for each animal, and the apoptotic index was calculated as the number of stained cells in each crypt divided by the total number of epithelial cells in each crypt.

We observed no interactions between dietary carbohydrate source and APC<sup>Min</sup> genotype for markers of proliferation and apoptosis. Therefore, we are presenting only main effect means for dietary carbohydrate source in this article. Effects of APC<sup>Min</sup> genotype on markers of proliferation and apoptosis will be presented in a separate article.

Serum Analysis

Blood glucose, insulin, and IGF-I was measured in a subset (n = 39) of all mice from Experiment 1 [sucrose: APC<sup>Min/+</sup> (5 males, 5 females), APC<sup>+/+</sup> (7 males, 3 females); cornstarch: APC<sup>Min/+</sup> (4 males, 5 females), APC<sup>+/+</sup> (5 males, 5 females)]. Differences in numbers of mice per group were a consequence of our ability to obtain serum volumes sufficient for completing these tests. Serum glucose concentrations were measured using the QuantiChrom Glucose Assay Kit (DIGL-200; BioAssay System, Hayward, CA) by following the manufacturer’s instructions. Briefly, 5 µl glucose standards and samples were transferred to appropriately labeled tubes. Glucose assay reagent (500 µl) was added to each tube and then mixed thoroughly. Samples were incubated in a boiling water bath for 8 min and cooled in a cold-water bath for 4 min. Duplicates (200 µl) from each serum sample were transferred into a clear bottom, 96-well plate. Optical density was measured at 570 nm using a microplate reader (Model 550; Bio-Rad Laboratories, Hercules, CA), and the absorbance data were analyzed using SOFTmax PRO (Molecular Devices Corporation, Sunnyvale, CA).

Serum insulin concentrations were measured using the Mercodia Mouse Insulin ELISA kit (Mercodia AB, Uppsala, Sweden) by following the manufacturer’s instructions. Briefly, 25 µl each of the standards and samples were added to a 96-well plate coated with anti-insulin antibodies. Enzyme conjugate (50 µl) was added to each well, and plates were incubated on a shaker for 2 h at room temperature. Plates were then washed 6 times with wash buffer solution. The chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (TMB; 200 µl) was added to each well, and plates were incubated for 15 min, at which time 50 µl stop solution was added. Optical density of each well was measured at 450 nm using a microplate reader, and the absorbance data was analyzed using SOFTmax PRO.

In addition, serum IGF-I concentrations were measured using the Quantikine Mouse IGF-I Immunoassay kit from R&D Systems, Inc. (Minneapolis, MN) and following the manufacturer’s instructions. An unknown serum compound (we assume elevated lipid levels) interfered with the analysis of most serum samples; therefore, IGF-I levels can be presented only for 6 mice fed sucrose (APC<sup>Min/+</sup>: 1 male, 0 females; APC<sup>+/+</sup>: 4 males, 1 females) and 9 mice fed cornstarch (APC<sup>Min/+</sup>: 1 male, 3 females; APC<sup>+/+</sup>: 2 males, 3 females).

Quantitative Real-Time-PCR Analyses of Liver Tissue

Hepatic mRNA levels were measured in a subset (n = 25) of all mice from Experiment 1 [sucrose: APC<sup>Min/+</sup> (4 males, 3 females), APC<sup>+/+</sup> (3 males, 2 females); cornstarch: APC<sup>Min/+</sup> (2 males, 3 females), APC<sup>+/+</sup> (4 males, 4 females)]. Total RNA from liver samples was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). The RNA concentrations were determined by spectrophotometry, and RNA integrity was monitored by agarose gel electrophoresis. Consensus sequences for the genes of interest were obtained from the NCBI Web site. PCR primers were designed using the web-based Primer3 program (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The specificity of the primer sequences was confirmed by BLAST. Primer sequences used for each of the genes of interest were (from 5′ to 3′) IGF-I: forward CTACCAAAATGACCGCACCT, reverse CACGAACCTGAAGACATC; IGF-II: forward CCCTCAGCAAGTGCTAAGAGG, reverse TTAGGTTGCCTCAGAGTT; IGFBP1: forward AGCCAGAGATGACAGAGGA, reverse GTTGCGCTGACGTAATCCT; IGFBP3: forward TGTTTTCCTGTGCACCCCT, reverse CAAGCCACTCCTCTTGTCT; β-Actin: forward GCTACAGCTTACCACCCA, reverse TCTCCAGGGAGGAAGAG.

cDNA was synthesized from total RNA (1 µg) using the Superscript II system (Invitrogen) following manufacturer’s instructions. The primer and Mg<sup>2+</sup> concentrations were optimized, and the PCR was performed using 5% of the reverse transcription product described above. The assays for standards, unknowns, and internal controls were performed on an ABI PRISM 7700 Real Time PCR System (PE Applied Biosystems, Foster City, CA) under standard thermal cycling parameters: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The mRNA expression for each gene was determined by comparing it with a standard curve of known quantities of the specific target. This measurement was controlled for RNA quality, quantity, and reverse transcription efficiency by normalizing it to the expression level of β-actin, whose expression was not influenced by the dietary treatments.

Statistical Analyses

Data were analyzed using the General Linear Models procedure of SAS (version 8.2). Least square means procedures were used for all the measurements. When significant dietary effects were detected (F test significant at P < 0.05), the least significant difference method was used to compare appropriate means. Differences between means were declared significant when the data were analyzed using the General Linear Models procedure of SAS (version 8.2).
at $P < 0.05$, and trends toward significance were declared at $P < 0.10$. Least-squares means ± SEM (standard error of the mean) are presented throughout the article. The colon adenoma incidence of mice consuming diets containing sucrose or cornstarch was analyzed with Fisher’s exact test using the frequency procedure of SAS.

RESULTS

Influence of Dietary Carbohydrate Source on Intestinal Tumorigenesis

Small and large intestinal tumor number (Figs. 1A, 1D), but not average tumor size (Figs. 1B, 1E) or total tumor burden
(Figs. 1C, 1F), tended to be greater in mice consuming the high-sucrose diet compared to mice consuming the high-cornstarch diet. In the 10-wk study without sulindac, APC<sub>Min</sub> mice fed high-sucrose diets (72.2 ± 4.4 tumors/mouse) tended to have greater tumor numbers in the small intestine compared to APC<sub>Min</sub> mice fed high-cornstarch diets (59.7 ± 5.1 tumors/mouse; Fig. 1A). A similar trend, although not statistically significant, was observed in the 16-wk study (Fig. 1A). In the 16-wk study with sulindac, the prevalence of colonic papillary tumors was greater in APC<sub>Min</sub> mice fed sucrose compared to APC<sub>Min</sub> mice fed cornstarch (32 of 54 vs. 19 of 63; P = 0.003; Fig. 1D). Although the prevalence of colonic papillary tumors in the 10-wk study without sulindac followed a similar trend, the treatment effect did not reach statistical significance (15 of 27 for sucrose vs. 9 of 21 for cornstarch; P = 0.38; Fig. 1D). No differences were observed in average adenoma size (Fig. 1E) or adenoma burden (Fig. 1F) in colon papillary adenoma-bearing mice fed diets containing the different carbohydrate sources in either experiment.

The effect of dietary carbohydrate source on small intestinal tumorigenesis was limited to the proximal third of the small intestine. In both experiments, APC<sub>Min</sub> mice fed high-sucrose diets had a significantly greater number of adenomas in the proximal third of the small intestine compared to APC<sub>Min</sub> mice fed high-cornstarch diets (Figs. 2A, 2D). The average size of adenomas in the proximal small intestine was significantly smaller in mice fed sucrose than in those fed cornstarch in both experiments (Figs. 2B, 2E), resulting in similar mean tumor burden for both treatment groups in both experiments (Figs. 2C, 2F). These differences in tumor number and average size of proximal intestinal tumors often were accompanied by differences in tumor morphology of the tumors. Sucrose-fed mice often had larger, more protruding tumor cells having a more opaque color (with methylene blue staining) than cornstarch-fed mice, which typically had larger necrotic centers in the tumors (representative photomicrographs not shown). In the proximal small intestine, APC<sub>Min</sub> mice fed a high-sucrose diet and no sulindac (Experiment 1) had on average 21.9 ± 1.4 tumors/mouse with an average size of 1.07 ± 0.22 mm²/tumor in comparison to 13.1 ± 1.6 tumors/mouse with an average size of 2.10 ± 0.25 mm²/tumor in APC<sub>Min</sub> mice fed a high-cornstarch diet and no sulindac (both P < 0.01; Figs. 2A, 2B). In the presence of sulindac (Experiment 2), APC<sub>Min</sub> mice fed a high-sucrose diet had on average 18.1 ± 1.4 tumors/mouse with an average size of 1.26 ± 0.17 mm²/tumor in comparison to 13.3 ± 1.3 tumors/mouse with an average size of 1.96 ± 0.16 mm²/tumor in APC<sub>Min</sub> mice fed a high-cornstarch diet (both P ≤ 0.05; Figs. 2D and 2E). In the medial and distal thirds of the small intestine, similar tumor numbers, average tumor sizes, and total tumor burdens were observed for the two treatment groups in both experiments (Fig. 2).

Although sulindac-treated (100 mg/kg diet) mice were 6 wk older at sacrifice, their total small intestinal tumor number was lower (35% reduction in sucrose-fed mice and 27% reduction in cornstarch-fed mice) compared APC<sub>Min</sub> mice receiving no sulindac (Figs. 1A, 1D). Average tumor size was less than 10% larger in mice receiving suboptimal levels of dietary sulindac when compared to those not receiving sulindac (Figs. 1B, 1E). The effect of dietary sulindac on tumor number was primarily limited to the distal two-thirds of the small intestine with reductions of 17%, 43%, and 44% in sucrose-fed mice and reductions of –1%, 36%, and 34% in cornstarch-fed mice for the proximal, medial, and distal thirds of the small intestine, respectively (Figs. 2A, 2D).

### Influence of Dietary Carbohydrate Source on Body Weight

For both feeding studies, APC<sub>Min</sub> mice consuming the sucrose-based diets gained significantly more weight than mice consuming the cornstarch diets (Fig. 3). In the 10-wk study without sulindac (Experiment 1), the weight gain began at 9 wk of dietary treatment, and in the 16-wk study with sulindac (Experiment 2), the weight gain began at 1 wk of dietary treatment.
Table 2

Influence of dietary carbohydrate source (sucrose vs. cornstarch) on proliferation (Ki67-antigen and proliferating cellular nuclear antigen [PCNA] expression) and apoptosis (TUNEL assay) markers (means ± SE) in colon mucosa of mice fed diets containing no sulindac (10-wk study) or 100 mg sulindac per kg diet (16-wk study)ab

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10-Wk Study</th>
<th>16-Wk Study</th>
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<tr>
<td></td>
<td>Sucrose</td>
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<tr>
<td><strong>Proliferation</strong></td>
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<tr>
<td>Ki67-antigen expression</td>
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<td>n = 17</td>
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<td>Crypt height (cells)</td>
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<td>19.9 ± 0.28</td>
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<td>Total labeling index</td>
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<td>0.320 ± 0.016b</td>
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<td>Proliferative zone</td>
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<tr>
<td>Bottom 1/3 labeling index</td>
<td>0.746 ± 0.021a</td>
<td>0.664 ± 0.020b</td>
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<td>Top 1/3 labeling index</td>
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<td>Top 1/3 labeling index</td>
<td>0.023 ± 0.005</td>
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*aAbbreviation is as follows: TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling.

*bMeans within a study not sharing a common subscript are different (P < 0.05).

Fed the cornstarch-based diets during the nutritional intervention, regardless of whether they received dietary sulindac or not (both Ps < 0.05; Fig. 3). These diet effects on body weight gain also were observed in mice that did not carry an APCMin mutation (results not shown). In the 10-wk feeding study (without sulindac; Experiment 1), APCMin mice fed sucrose as the sole carbohydrate source gained an average of 12.40 ± 0.44 g during the 10-wk nutritional intervention in comparison to 10.93 ± 0.55 g for APCMin mice fed cornstarch. Statistically significant differences in average weight gain were detected after 9 wk of dietary intervention. In the 16-wk study (with sulindac; Experiment 2), APCMin mice fed sucrose-based diets gained an average of 14.64 ± 0.50 g during the nutritional intervention, whereas APCMin mice fed cornstarch-based diets gained an average of 11.79 ± 0.44 g. In the 16-wk feeding study, differences in mean weight gain first became statistically significant after 1 wk of dietary intervention. No differences in food disappearance were observed between diets. Feed intakes for individual mice were not measured in either study. Based on visual observations during necropsy, much of the observed difference in body weight for the two dietary treatments could be attributed to increased abdominal fat deposition in mice fed high-sucrose diets (data not shown). Addition of 100 mg of sulindac per kg of diet extended the approximate life span of mice by 43% as estimated from the age at which at least 10% of the mice showed at least 10% weight loss from the highest body weight (20 vs. 14 wk).

Influence of Dietary Carbohydrates on Colonic Epithelial Cell Proliferation and Apoptosis

For assessing cell proliferation in the colon, the expression of both Ki67-antigen and PCNA in colonic epithelium was used. Mice fed a high-sucrose diet had greater total crypt Ki67-labeling indexes than mice fed a high-cornstarch diet, regardless whether they received dietary sulindac or not (both Ps < 0.05; Table 2). In the 10-wk feeding study without sulindac (Experiment 1), mice fed a high-sucrose diet had significantly greater Ki67-labeling indexes in the bottom third of colonic crypts when compared to mice fed a high-cornstarch diet irrespective of their APCMin genotype (0.746 ± 0.021 vs. 0.664 ± 0.020; P = 0.01; Table 2). In the 16-wk study with sulindac (Experiment 2), APCMin mice (no IHC was done for wild type littermates) fed a high-sucrose diet had significantly greater Ki67-labeling indexes in the middle and top (apical) thirds of
colonic crypts when compared to APC\textsuperscript{Min} mice fed a high-
cornstarch diet (0.485 ± 0.024 vs. 0.350 ± 0.026 in the middle
third and 0.079 ± 0.014 vs. 0.022 ± 0.015 in the top third,
respectively; both \( P_s = 0.01 \)). Similarly, mice consuming su-
crose as the sole carbohydrate source in the 16-wk study had
a significantly expanded proliferative zone compared to mice
consuming cornstarch (0.608 ± 0.018 vs. 0.535 ± 0.020; \( P =
0.02 \); Table 2).

Expression of PCNA in colonic mucosa was affected some-
what less by dietary carbohydrate source than Ki67-antigen ex-
pression (Table 2). In the 16-wk study with sulindac, APC\textsuperscript{Min}
mice consuming sucrose had significantly greater PCNA-
labeling indexes in the top third of colonic crypts (0.198 ±
0.023 vs. 0.114 ± 0.020; \( P = 0.01 \)) and tended to have a sig-
ificantly expanded proliferative zone (0.708 ± 0.019 vs. 0.660
± 0.017; \( P = 0.07 \)) compared to mice consuming cornstarch as
the sole carbohydrate source (Table 2).

Results of TUNEL assays to determine apoptosis of colonic
epithelial cells are presented in Table 2. No differences in
apoptosis indexes were observed in the 10-wk feeding study
without sulindac. In the 16-wk feeding study with sulindac,
APC\textsuperscript{Min} mice consuming sucrose as sole carbohydrate source
had a significantly lower total crypt apoptosis index compared to
mice consuming cornstarch (0.038 ± 0.006 vs. 0.056 ± 0.006;
\( P = 0.03 \)). This treatment difference was primarily due to dif-
f erences in apoptosis that were observed in the top third of
colonic crypts (0.111 ± 0.019 vs. 0.167 ± 0.018; \( P = 0.02 \)).

Influence of Dietary Carbohydrate Source on Serum
and Liver Constituents

Concentrations of serum and liver constituents were mea-
sured only in the 10-wk feeding study without sulindac (Ex-
periment 1). Mice fed the high-sucrose diet had significantly
greater serum glucose concentrations (10.09 ± 0.57 vs. 8.08
± 0.57 mM; \( P = 0.02 \); Fig. 4A) and tended to have higher in-
sulin levels (263 ± 35 vs. 174 ± 34 pmol/l; \( P = 0.08 \); Fig. 4B)
when compared to mice consuming the high-cornstarch diet. We
also observed numerically greater serum IGF-I concentrations
in mice fed the high-sucrose diet (67.2 ± 8.3 vs. 53.0 ± 5.8 ng/ml;
\( P = 0.15 \)).

Liver expression of mRNA for IGF-I, IGF-II, IGFBP1,
and IGFBP3 are presented in Fig. 5. Relative expression of
IGF-I mRNA in APC\textsuperscript{Min} mice fed sucrose as the sole car-
bohydrate source was significantly greater than that observed
in APC\textsuperscript{Min} mice fed cornstarch. No significant dietary treat-
ment effects were observed in relative mRNA expressions
of IGF-II, IGFBP1, and IGFBP3 (Fig. 5). We did not ob-
serve significant effects of APC genotype or an interaction be-
tween APC genotype and dietary carbohydrate source on any
of the serum or liver analytes that were measured (data not
shown).

DISCUSSION

Previous studies examining the effects of high-sucrose
diets on colon cancer development have predominantly

![FIG. 4](image-url) Influence of dietary carbohydrate source (sucrose vs. cornstarch) on
A: serum glucose and B: insulin concentrations (means ± standard errors) of
APC\textsuperscript{Min} mice in Experiment 1 (10-wk study without sulindac).

![FIG. 5](image-url) Influence of dietary carbohydrate source (sucrose vs. cornstarch) on
mRNA expressions (relative units) of IGF-I, IGF-II, IGFBP1, and IGFBP3
(means ± standard errors) in liver tissue of APC\textsuperscript{Min} mice in Experiment 1
(10-wk study without sulindac).
utilized carcinogen-induced colon cancer models (4–7). To our knowledge, the effects of high-sucrose diets on intestinal tumorigenesis and epithelial cell proliferation in APCMin mice have not been previously reported. In the current study, the influence of high-sucrose diets (vs. cornstarch) on intestinal tumorigenesis and epithelial cell proliferation was investigated in 2 feeding studies using APCMin mice and their wild type littermates. We also determined the influence of these high-sucrose diets on serum glucose and insulin and on hepatic IGF and IGFBP expression.

APCMin mice develop adenomas both in the small intestine and the colon (22). In the first feeding experiment (in the absence of dietary sulindac), APCMin mice displayed signs of morbidity after 10 wk of dietary treatment. We found that APCMin mice consuming high-sucrose diets had significantly greater numbers of adenomas in the proximal small intestine and tended to have a greater number of adenomas in the whole small intestine compared to mice consuming cornstarch (Figs. 1 and 2). The primary marker for evaluating the effect of dietary interventions on colon cancer prevention using the APCMin mouse model is small intestinal tumor number rather than total tumor burden or average tumor size (24,25). One of the reasons is that at the age when significant morbidity occurs, the tumor number in the small intestine is so high that individual tumors retard each others growth as they compete for blood and nutrient supply, which leads to an upper limit on adenoma burden in the small intestine of these mice. Colon papillary adenoma incidence also was numerically greater in APCMin mice consuming sucrose vs. those consuming cornstarch (56% vs. 43%) in the 10-wk study. However, this difference was not statistically significant ($P = 0.38$; Fig. 1).

In both feeding studies, the influence of high dietary sucrose on small intestinal tumorigenesis only occurred in the proximal small intestine (Fig. 2), which corresponds to the predominant site of digestion and absorption of dietary sucrose. Consumption of high-sucrose diets has been suggested to increase oxidative stress and also cause genotoxic effects in the intestine (26,27). We propose that high-sucrose diets might promote tumor initiation by increasing mutation rates in the proximal small intestine.

APCMin mice fed cornstarch had a greater average size of proximal small intestinal adenomas compared with mice fed sucrose (Figs. 2B and 2E). The total adenoma burden did not differ between the 2 diet groups in either the proximal third of the small intestine or the entire small intestine (Figs. 2C and 2F). One potential explanation for this observation is that the large tumor number in sucrose-fed mice restricts the growth of individual tumors, as overall space in the small intestine for tumor growth becomes limited. Another potential explanation is that differences in diet-associated tumor morphology (larger, more protruding tumor cells with smaller necrotic centers in sucrose-fed mice) results in differences in tumor growth pattern, which might be related to a greater nutrient supply to tumors in sucrose-fed mice. We consider these observations preliminary, which need to be followed up and confirmed in further studies.

Because the colonic adenomas in APCMin mice typically develop at a slower pace compared to those in the small intestine, we speculated that the 10-wk dietary treatment duration utilized in the first experiment may not be sufficient for diets to exert their full impact on colonic tumor development. To confirm our speculation, we conducted the second, longer term feeding study wherein a low dosage of sulindac (100 mg/kg diet) was added to each of the diets to delay the development of small intestinal adenomas. Sulindac has been widely studied as a potential chemopreventive agent for colon cancer in animal models (19,20,28–30) and showed chemopreventive effects in clinical trials in humans, particularly when coadministered with difluoromethylornithine (31–33). Sulindac is metabolized in the intestine into two principal active species, sulindac sulfide and sulindac sulfone, which have multiple distinct mechanisms of action including inhibition of cyclooxygenase (COX) and prostaglandin synthesis (28–30). Previous studies in APCMin mice have demonstrated that sulindac significantly reduces the number and size of small intestinal adenomas but does not significantly influence colon tumor development (19,20,29,30). Adding sulindac to the diets in the second study allowed us to extend the feeding duration from 10 wk to 16 wk, which was a sufficiently long duration to allow the detection of a significant diet effect on colonic adenoma incidence. Consistent with previous studies, suboptimal dosages of dietary sulindac (100 ppm) resulted in partial reduction of small number tumor number (with the primary effect on the distal two-thirds of the small intestine) without decreasing colonic adenoma incidence (19–21, 29,30).

In the second study, feeding high-sucrose diets significantly increased colonic adenoma incidence compared with feeding diets based on cornstarch (Fig. 1D). It has been previously reported that rats fed high-sucrose diets had significantly greater numbers of colonic adenomas per rat compared with those fed cornstarch-based diets using the DMH-induced rat colon cancer model (8). To our knowledge, our study is the first to demonstrate that high-sucrose diets increase colon tumor incidence in APCMin mice.

The exact mechanism(s) whereby high-sucrose diets increase risk for colon cancer is unclear. Depending upon the source, dietary carbohydrates may differentially influence colonic luminal environment due to fermentation of undigested starch and production of short chain fatty acids (34). Previous research has demonstrated that colonic epithelial cell proliferation is significantly increased in rats fed sucrose compared to cornstarch (4–7). Effects of carbohydrate source on colon proliferation were not associated with difference in luminal concentrations of short-chain fatty acids or cecal pH (34). Typically, both sucrose and cornstarch are well digested in the small intestine and thus would presumably contribute little to cecal or colonic fermentation in rodents. Therefore, we consider it more likely that dietary sucrose influences colon..
cancer risk via changes in the concentrations of factors in the circulation rather than via changes in the colonic luminal environment.

Ki67 antigen is widely used as a surrogate biomarker for cell proliferation (35–38). In both feeding experiments, overall Ki67 labeling indexes in colonic epithelial cells were significantly greater in APCMin mice and their wild type littermates consuming sucrose (Table 2), which indicated that diets containing high levels of sucrose increased epithelial cell proliferation in colon crypts. Increased cell proliferation in colon crypts is associated with increased risk for colon cancer (39–42). This observation also is consistent with other studies wherein high-sucrose diets were found to be associated with increased cell proliferation (5,6). We also observed in the 16-wk feeding study (with sulindac) that sucrose-based diets significantly increased the proliferative zone compared to APCMin mice consuming cornstarch (Table 2). The Ki67-antigen proliferative zone and total labeling index also was considerably larger for both dietary treatments in the 16-wk study compared to the 10-wk study, which may have been a consequence of the increased age of mice at the end of the 16-wk experiment and the fact that only APCMin mice were used in the 16-wk study. In the 10-wk study, APCMin mice had a greater Ki67 labeling index (0.370 ± 0.016 vs. 0.321 ± 0.018; \( P = 0.04 \)) and proliferative zone (0.491 ± 0.08 vs. 0.440 ± 0.020; \( P = 0.04 \)) than their wild type littermates.

Expression of PCNA, another commonly used cell proliferation marker, in colonic epithelial cells was influenced less by dietary carbohydrate source in either experiment (Table 2) than was Ki67-antigen expression. Dietary sucrose tended to expand the proliferative zone and significantly increased the proportion of PCNA labeling in the upper third of colonic crypts in the 16-wk study with sulindac. These observations likely were associated with the increased age of mice at the end of this experiment, as overall PCNA labeling indexes also were elevated in the second experiment (Table 2). The stronger influence of dietary carbohydrate source on expression of Ki67-antigen compared to PCNA can be explained partly by the greater specificity of Ki67-antigen as an intestinal proliferation marker (35,37,38). The gold standard for proliferation is BrdU incorporation into intestinal cells (38); however, BrdU incorporation was inadequate in this study for unknown reasons, thereby precluding its use as another proliferation marker. Apoptosis indexes in colonic epithelium, as assessed by TUNEL assays, were not influenced by dietary carbohydrate source in the first experiment but were significantly greater in mice consuming the cornstarch diets in the 16-wk study (Table 2). As expected, the vast majority of apoptotic cells were detected in the apical one-third of colonic crypts (Table 2). Collectively, these indexes of cell proliferation and death support the hypothesis that increased colonic cell proliferation and reduced apoptosis are associated with increased risk of tumor development. Further studies that include other markers of proliferation (such as BrdU and cyclin D1) and apoptosis (such as caspase activation) are warranted to confirm our results before more definite conclusion about the effect of carbohydrate source on cell proliferation and apoptosis can be drawn.

It has been proposed that hyperinsulinemia promotes colorectal carcinogenesis and that the role of insulin in colorectal cancer is mediated through IGF-I (9–11). To help understand possible underlying mechanisms, we assessed the effect of high-sucrose diets (vs. cornstarch) on body weight gain, serum glucose, insulin, and IGF-I concentrations and hepatic expression of IGF and IGFBP mRNA levels at the end of the 10-wk feeding study. In both feeding experiments, mice consuming the high-sucrose diets gained significantly more weight compared with mice consuming cornstarch (Fig. 3). Serum glucose concentrations were significantly greater in APCMin and wild type APC mice consuming high-sucrose diets compared to that observed in mice consuming diets containing cornstarch (Fig. 4A). Similarly, the circulating levels of insulin also tended to be greater in mice consuming the high-sucrose diet (\( P = 0.08 \); Fig. 4B). Serum IGF-I levels also tended to be higher in sucrose-fed mice; however, the difference was not statistically significant due to the small sample size. These results suggest that compared to cornstarch-based diets, consumption of high-sucrose diets compromises insulin action in mice. These observations are consistent with results of other studies in which high intakes of sucrose (vs. cornstarch) were found to first reduce insulin sensitivity in the liver and later in peripheral tissues as assessed by euglycemic hyperinsulinemic clamps (12–14). Longer term feeding of sucrose-rich diets will eventually lead to compensatory hyperinsulinemia (43). The lack of an interaction between APCMin genotype and dietary carbohydrate source suggest this is a general diet effect and is not limited only to APCMin mice. Further studies that conduct glucose tolerance tests of APCMin mice and their wild type littermates fed high-sucrose diets are needed to confirm our hypothesis that high-sucrose diets promotes insulin resistance and Type II diabetes.

Another potential mechanism, which was not the objective of this study, was the effect of high-sucrose diets on lipid metabolism in APCMin mice. Previous studies showed hyperlipidemia and hepatic lipidosis in APCMin mice close to the end of their life span but not in their wild type (44). Decreasing hyperlipidemia by improving hepatic lipid metabolism decreased small intestinal tumor number (45,46). In this study, we did not prepare liver sections to evaluate hepatic lipidosis and did not measure serum lipids. However, we did observe a greater number of samples from sucrose-fed APCMin mice that had interference problems for serum IGF-I determination in comparison to the other 3 diets by APCMin genotype groups (9 of 10 vs. 14 of 29), which might be related to elevated serum lipid levels. Furthermore, we report in a companion paper that high-sucrose diets (compared to high cornstarch diets) alter the expression of genes involved in lipid metabolism (increase in stearoyl-coenzyme A desaturase 2 and decrease in carnitine palmitoyltransferase 1a and phospholipid scramblase 3), which are consistent with an increased risk of hyperlipidemia, obesity, and cancer (47–50).
Sucrose is comprised of two monosaccharides—glucose and fructose. Unlike glucose, fructose does not stimulate insulin secretion nor enhance leptin production (51). Insulin and leptin act as key afferent signals regulating food intake and body weight. Therefore, dietary fructose may contribute to increased energy intake and weight gain (43). It also has been suggested that the increased insulin sensitivity caused by high-sucrose diets likely is related to the fructose component of sucrose (14,52). Further studies on the role of dietary fructose in colon carcinogenesis are warranted.

The IGF-system includes two insulin-like growth factors (IGF-I and IGF-II), which exert their actions on cell growth, differentiation, and apoptosis by interacting with IGF-I receptors on the cell membrane. IGF-I and IGF-II are further regulated by a group of specific binding proteins [i.e. IGFBP-1 through IGFBP-6; (53,54)]. IGF-I in circulation is primarily produced in the liver. We assessed hepatic mRNA expressions of IGF-I, IGF-II, IGFBP1, and IGFBP3 at the end of the 10-wk feeding study and found that the relative expression of IGF-I mRNA in the liver of mice fed sucrose was significantly greater compared with that in mice fed cornstarch (Fig. 5). We speculate this increase in hepatic IGF-I mRNA expression with sucrose feeding could be a consequence of high plasma insulin concentrations resulting in increased numbers of growth hormone receptors on hepatic cells, thereby resulting in increased IGF-I transcription in response to growth hormone binding to its hepatic receptors (55,56). IGF-II mRNA expression in liver did not differ between mice fed sucrose or cornstarch (Fig. 5) but was higher in the small intestine of mice fed sucrose, as reported in our companion article (47). The major IGF binding proteins in the circulation are IGFBP3 and IGFBP1. These proteins limit access of both IGF-I and IGF-II to the peripheral tissues. In the current study, neither IGFBP3 nor IGFBP1 mRNA concentrations in liver were influenced by diet (Fig. 5); however, IGFBP3 mRNA concentrations in the proximal small intestine were decreased by sucrose feeding (47). Based on all these observations, we hypothesize that high-sucrose diets increase plasma IGF-I concentrations via increased production of IGF-I in liver. Western analysis of IGF and IGF binding proteins in liver and colon tissues of sucrose-fed (vs. cornstarch-fed) mice are warranted to draw more definitive conclusions.

High levels of circulating IGF-I are associated with increased colon cancer risk in human epidemiological studies (15,16). Wu et al. (17) demonstrated that high levels of IGF-I in circulation caused by injections of IGF-I significantly increased rates of growth of transplanted colon tumors on the cecum and metastasis to the liver in mice. In the current study, increased intestinal tumor incidence in APCMin mice consuming high-sucrose diets was associated with increased IGF-I expression in liver (Fig. 5). In summary, results of this study demonstrated that high-sucrose diets increase colon tumor incidence in APCMin mice and that this is associated with elevated levels of insulin in circulation and increased expression of hepatic IGF-I.

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