

## Glycemic Index, Nutrient Density, and Promotion of Aberrant Crypt Foci in Rat Colon

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**Abstract:** We speculated that a diet with a high glycemic index (GI), or a diet with a low nutrient density (nutrient-to-calorie ratio), would enhance colon carcinogenesis, presumably via increased insulin resistance. Forty-eight female Sprague-Dawley rats (SD) received an azoxymethane injection (20mg/kg) and were randomized to 5 groups given an AIN76 diet containing (1) 65% starch by wt. (2) 65% glucose, GI=100, (3) 65% fructose, GI=23, (4) 82% starch, or (5) 39% oil and 39% sucrose. The nutrient density was halved in 4-5 diets compared to 1-3 diets. Promotion was assessed by the multiplicity (number of crypts) of aberrant crypt foci (ACF), an early marker of colon carcinogenesis. Insulin resistance was estimated by (blood insulin  $\times$  blood glucose), by plasma triglycerides, and by visceral fat. To confirm the results in another rat strain, the whole experiment was duplicated in 48 female Fischer F344 rats. Results show that: (i) ACF multiplicity was not different in glucose- and fructose-fed rats ( $p>0.7$ ): diets with contrasting GI had the same effect on ACF growth. (ii) Diets of low nutrient density increased visceral fat ( $p<0.05$ ), but reduced the ACF size in F344 ( $p<0.001$ , no reduction in SD). (iii) Indirect insulin resistance markers (FIRI index, blood triglycerides, visceral fat) did not correlate with ACF multiplicity. These results do not support the hypothesis that diets with a high glycemic index, or of low nutrient density, or diets that increase some indirect insulin resistance markers, can promote colon carcinogenesis in female rats.

### Introduction

Diets high in energy, fat, and sucrose, and low in fibers and n-3 polyunsaturated fatty acids, and a sedentary lifestyle, are associated with high risks of colorectal cancer (1). The same risk factors are also related to visceral obesity and insulin resistance, a state where the biological effect for a given insulin concentration is reduced. In this condition the response to insulin is slowly reduced in muscle and fat cells and therefore glucose tolerance is impaired. This leads to increased fasting blood insulin, glucose, and triglycerides, and visceral obesity. Diets high in fat lead to a state of insulin resistance, as assessed by an elevated fasting insulin, and impaired glucose tolerance (2). Insulin resistance is also induced by dietary carbohydrates with a high glycemic index (GI) (3, 4), but it can be improved by dietary n-3 fatty acids or by increased exercise (2). In affluent countries, a large fraction of the population displays insulin resistance, impaired glucose tolerance, high blood triglycerides and visceral obesity (5).

Thus, high levels of blood insulin, glucose, triglycerides, and insulin resistance have been hypothesized to increase the risk of colorectal cancer (5-7). A significant positive association was found between colorectal cancer and diabetes mellitus in a Swedish cohort study (8). Also, cancer risk was associated with high GI diets in a population-based case-control study (9). In rats, injections of exogenous insulin can promote the growth of tumors and of pre-neoplastic lesions in the colon (10, 11). The high fat diets that promote colon carcinogenesis also induce insulin resistance, and a study in rats supports that insulin resistance precedes colon cancer promotion (12).

For the present study, we designed five different experimental diets, in an attempt to increase blood glucose, triglycerides, and endogenous insulin, and to induce insulin resistance in rats. The five diets contained by weight 1) 65% starch, 2) 65% glucose, 3) 65% fructose, 4) 39% oil and 39% sucrose, or 5) 82% starch. We speculated that glucose-fed rats would have their blood glucose and insulin elevated compared to starch-fed controls (see main hypotheses in Table 1). We speculated that fructose-fed rats would have their triglycerides tripled (13-15) but blood insulin and glucose unchanged compared to starch-fed controls (16, 17). Glucose and fructose are both monosaccharides, but their GI is 100 and 23, respectively (18, 19). We thought that the GI of diet might influence the tumor outcome. We also speculated that rats given a diet high in oil and sucrose would develop insulin resistance. Indeed, the long term feeding on a diet containing 38% fat and 38% sucrose, doubles the values of obesity, of blood insulin and of blood triglycerides, compared to controls given a diet containing 56-68% starch and 5% fat both in F344 rats (20) and in C57BL/6J mice (21). A 32% safflower oil diet induces defective glycemic control, hyperglycemia and obesity in mice (22), and safflower oil is the strongest inducer of glucose intolerance among seven dietary oils (23). We therefore decided to induce insulin resistance and obesity with a diet high in sucrose and safflower oil, which contains 75% n-6 polyunsaturated fatty acids, and can promote colon cancer in rodents (24). Oil and sucrose bear lots of calories, but few essential nutrients (except n-6 fatty acids). This oily diet was therefore of low nutrient density (nutrient-to-calorie ratio), since it contained little of the essential materials per calorie of diet (25). Many micronutrients are supposed to protect against carcinogenesis (e.g., n-3 fatty acids, some vitamins, methionine, calcium, cellulose). Therefore the oily diet was compared to a specific control diet of matching nutrient density, containing 82% starch (Table 1).

**Table 1.** Study Main Hypotheses and Main Results.

Hypotheses: Factor Supposed to Promote ACF	Matched Diets: Experimental /Control		Results: Blood Values	p Values	
				SD rats	F344 rats
High blood glucose & insulin	glucose	/starch	no increase	0.6	0.8
High blood triglycerides	fructose	/starch	no increase	0.08	0.5
High insulin resistance	oil-sucrose	/high-starch	decrease	0.09	0.03
<b>ACF Multiplicity</b>					
High glycemic index	glucose	/fructose	no effect	0.7	0.9
Low nutrient density	high-starch	/starch	decrease /F344	0.6	<0.0001
Low nutrient density (bis)	LND <sup>a</sup>	/ HND <sup>b</sup>	decrease /F344	0.5	<0.0002
Insulin resistance markers	All rats fed various diets		no correlation	1	1

*a:* LND, low nutrient density diets, based on starch (82%) or oil and sucrose (39% of each).

*b:* HND, high nutrient density diets based on starch (65%), glucose (65%), or fructose (65%).

Promotion of cancer was assessed with the aberrant crypt focus (ACF) assay (26). ACF are putative precursors of colon cancer (27), specifically induced by colon carcinogens (28), promoted by promoting diets (29), inhibited by inhibitors of carcinogenesis (30). In rodents and humans, ACF display mutations and histologic changes observed in colonic tumors (31, 32), and the growth of ACF correlates with the adenocarcinoma yield (33-35). The number of ACF per animal is an assay for initiators of colon cancer, although the number of crypts per focus (multiplicity) is a measure of promotion effect (34-36).

A first study was done in SD rats, but the diets did not modify insulin parameters as expected. We thus duplicated the whole study in F344 rats. This second study mostly confirmed the first one: Diets with a high GI or of low nutrient density, or diets that increased some insulin resistance markers, did not enhance the growth of ACF in rats.

### Materials and Methods.

#### Animals.

Forty-eight four-week old female Sprague-Dawley rats (SD) and 48 four-week old female Fischer 344 rats (F344) were obtained from Iffa-Credo (Lyon, France). They were acclimatized to the colony for one week, housed two rats per stainless steel wire drop-bottom cage, at 22°C with light-dark cycle 12h-12h, and given a laboratory chow (6% fat, obtained from UAR, Villemoisson, France) and water ad libitum. The rats were initiated between 9 and 10 A.M. with a single intra-peritoneal injection of azoxymethane (Sigma Chemical, St. Quentin, France) at a dose of 20 mg/kg in NaCl 9 g/l. SD and the F344 rats were each randomly allocated a week later to five groups, and given the experimental diets. Body weight was measured weekly. Food and water intakes, and fecal excretion, were measured per cage, during three days, three times (weeks 3, 8 and 13). Fat was measured in freeze-dried feces (day 69), by weighing the methanol-chloroform extract. Fecal pellets were collected directly at the anus of each rat and pH was measured as soon with a needle pH probe (week 9). The animals were sacrificed, 106 d after the carcinogen injection, 98-100 d after the start of

experimental diets, by cervical dislocation between 8:30 and 11:20 A.M. after a fasting night. Blood was taken from the heart, the abdominal fat was excised and weighed, and the colon was kept in formalin for ACF scoring.

#### Diets.

Forty-eight SD rats were randomized to five diet groups after initiation (Table 2). The control group contained twelve rats given an AIN76 diet (37), containing 65% by weight raw wheat starch (GI: 70). Two groups of 9 rats were given an AIN76 diet in which starch was replaced by 65% glucose (GI: 100) or 65% fructose (GI: 23). Two groups of 9 rats were given a modified AIN76 diet in which the nutrient density was halved, either with starch (82% of the diet) or with 39% sucrose and 39% oil (35% safflower and 4% corn oil). The whole experiment was duplicated in 48 F344 rats, also randomized to five diet groups after initiation. Diets were identical to those given to SD rats, except the oil and sucrose diet. Safflower oil was replaced by grape-seed oil, of similar gross composition (linoleic and oleic acids, 75 and 13% in safflower, 77 and 12% in grape-seed). Fat source was changed because, surprisingly, safflower oil did not promote the carcinogenesis in the first study. We wanted to avoid possible protective micronutrients in the second study, by turning to an oil with similar fatty acids but different botanical origin. All dietary ingredients were obtained from UAR, except the safflower oil (Bjorg, St. Genis, France) and the grape-seed oil (Carrefour, Toulouse, France).

#### Assay of Blood Insulin, Glucose and Triglycerides.

Blood samples (1.5 ml) were collected by cardiac puncture in a syringe containing 5 µl (25U) heparin within 1 min after death. After centrifugation for 10 min at 1300 g the plasma was divided in coded microtubes kept at -80°C for further analyses. Insulin was measured by radioimmunoassay, using a kit designed for human plasma, giving a 100% cross-reaction with rat insulin (Insik-5, Sorin Biomedica, Antony France). Glucose and triglycerides were measured by

**Table 2.** Composition of Experimental Diets with Contrasting Glycemic Index or Nutrient Density.

Diet group:	Starch	Glucose	Fructose	High Starch	Oil & Sucrose
Carbohydrate <sup>a</sup>	65	65	65	82	39
Casein	20	20	20	10.3	14.9
Oil <sup>b</sup>	5	5	5	2.6	3.7 + 35
Cellulose		5	5	5	2.6
AIN76 mineral mix (37)	3.5	3.5	3.5	1.8	2.6
AIN76 vitamin mix (37)	1	1	1	0.5	0.74
DL-methionine	0.3	0.3	0.3	0.15	0.22
Choline bitartrate	0.2	0.2	0.2	0.1	0.15
Nutrient density <sup>d</sup>	91	91	91	46	46

a. Percentage (g/100g) of starch, glucose, fructose or sucrose in each diet. AIN76 diet contains 50% sucrose and 15% corn starch.

b. All diets contained some corn oil (2.6, 3.7 or 5%). In addition, oil & sucrose diet contained either 35% safflower oil (for SD rats) or 35% grape-seed oil (for F344 rats).

d: Nutrient density is expressed in g of (casein + corn oil + cellulose + mineral mix + vitamin mix + methionine + choline) per 1,000 cal

enzymatic-spectrophotometric methods (kits BioMérieux, Marcy France). Insulin resistance was estimated by the FIRI index (blood insulin x blood glucose/25) (38), and by the visceral fat weight. In mice, Ikemoto showed that the wet white adipose tissue weight is highly correlated with the glucose level after challenge (23).

#### Assay of Aberrant Crypt Foci.

Promotion was assessed by the multiplicity (number of crypts) of aberrant crypt foci (ACF) after 100 d of experimental diet. ACF were scored 106 d after the carcinogen injection using Bird's procedure (27). Immediately after sacrifice, colons were flushed with Krebs's Ringer solution (Sigma Chemical), then opened longitudinally and fixed flat between coded filter paper in 10% buffered formalin (Sigma Chemical). The colons were stained with methylene blue (0.05%) for 10-15 min, then the mucosal side was observed at 32 x magnification. ACF were distinguished by their slit-like opening, increased staining, size and pericryptal zone. The multiplicity (number of crypts per ACF) was recorded for each ACF in each colon. Those were scored blindly by a single observer.

#### Statistics.

Data are given as mean  $\pm$  standard deviations, with two-sided p values. The statistical analysis of ACF assay for colon cancer promotion was based on ACF multiplicity as defined by the mean number of aberrant crypts per focus (35). Set of data were examined by ANOVA. When the ANOVA showed a significant difference between groups ( $p < 0.10$ ), comparisons with the control group of rats (65% starch diet) were done by Dunnett's test. Student's t-test, or Welch's test when variances were unequal, was used for specific pairwise comparison of a priori selected pairs, matching initial hypotheses (Table 1). Pearson correlations between measures were computed separately for SD and F344 strains, at the individual rat level ( $N=48$ ) and significance was assessed by Bonferroni probabilities with Systat software.

## Results

#### Weights and food intake.

Sprague-Dawley rats gained more body weight than F344 rats (Figure 1, 1.6 and 0.9 g/d respectively,  $p < 0.0001$ ). They also ate more food (16.5 and 9.2 g/d respectively,  $p < 0.0001$ ). The composition of diet did not affect the final body weights ( $p > 0.33$  in SD and F344). However, soon after the start of diets, rats fed on the oily diet gained less weight than the rats fed on the low fat diets (significant at day 7 in SD rats, and days 7, 14, 21, 29, 35 and 42 in F344 rats). The weight of rats fed on the oily diet was in the low range up to day 50, but increased more than in the other groups later (Figure 1).

The food intakes were significantly reduced in rats given the oily diet, high in calories, compared to low fat diets (9.4 and 16.5 g/d respectively in SD rats; 6.1 and 9.2 g/d in F344 respectively; both  $p < 0.0001$ ). The caloric intakes were similar in all groups of F344 rats (33-37 cal/d,  $p > 0.55$  at weeks 3, 8 and 13). By contrast, the caloric intakes were not the same in groups of SD rats: The high-starch diet increased the caloric intake (+20%,  $p < 0.05$ ), and the oil and sucrose diet decreased the caloric intake (-20%,  $p = 0.01$  on third week 3, not significant later), compared to the 65% starch-fed SD controls.

#### Fecal values

In SD and in F344 rats, the fecal excretion was much reduced in both groups given a diet of low nutrient density ( $p < 0.02$  in SD rats,  $p < 0.001$  in F344 rats, Table 3). This seems normal since these diets contained less cellulose per calorie than the other diets. No effect of the nature of carbohydrate on fecal weight was seen in SD rats. The fecal weight was higher in starch-fed rats than in glucose- and fructose-fed F344 rats ( $p < 0.01$ ), which may suggest that a part of the starch escaped digestion in the small bowel of F344 rats. The fecal weight, relative to body weight, was similar in F344 rats and SD rats (e.g., 0.57% in starch fed controls).

**Table 3.** Fecal Weight and pH, in SD and F344 Rats on Diets with Contrasting Glycemic Index or Nutrient Density, for 100 d, After a Single Azoxymethane Injection (20 mg/kg).

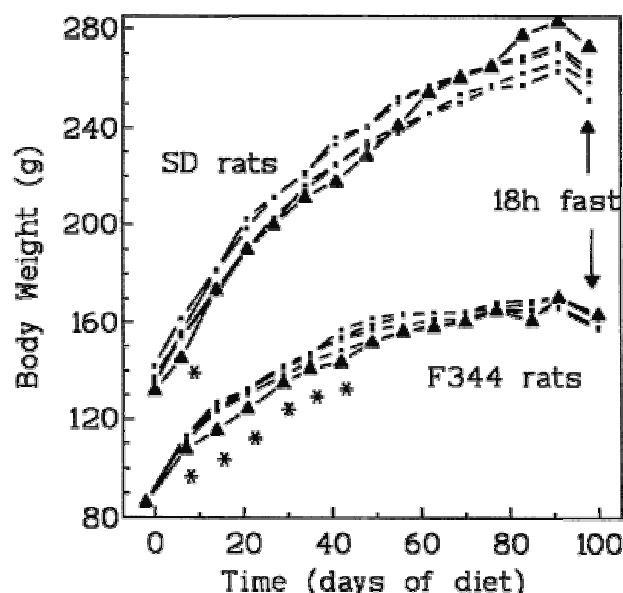
Rats strain	----- SD rats -----		-----F344 rats -----	
	Fecal weight <sup>a</sup>	Fecal pH	Fecal weight	Fecal pH
<i>sampling day</i>	<i>46-49</i>	<i>63</i>	<i>69-71</i>	<i>75</i>
Starch <sup>b</sup>	1.37 ±0.15	7.2 ±0.7	0.93 ±0.09	6.6 ±0.2
Glucose	1.51 ±0.10	7.2 ±0.5	0.63 ±0.09 *	6.5 ±0.2
Fructose	1.55 ±0.24	8.1 ±0.4 *	0.66 ±0.08 *	7.1 ±0.3 *
High starch	1.06 ±0.19 +	6.6 ±0.3 +	0.39 ±0.03 *	6.6 ±0.3
Oil & suc.	0.61 ±0.20 *	7.3 ±0.4	0.37 ±0.03 *	6.8 ±0.4
ANOVA <i>p</i>	0.0001	0.0001	0.0001	0.0001

*a:* Fecal weights (g) were measured per cage, fecal pH per rat. Values are mean ± standard deviation.

*b:* Composition of diet is given in table 2.

\*  $p < 0.01$ , +  $p < 0.05$ , different from the starch-fed control group of rats (first row) by Dunnet's test.

The fecal pH was the same in all groups (Table 3), except a higher fecal pH in rats given the fructose diet (true in both SD and F344 rats,  $p < 0.01$ ). In SD rats only, the fecal pH was lower in rats given 82% starch than in controls ( $p = 0.01$ ). Feces from rats given the oily diet contained more fat than feces of starch-fed rats (fat% of dry feces were  $6.9 \pm 1$  and  $3.3 \pm 1$  respectively,  $p = 0.001$  in SD rats;  $23 \pm 3$  and  $3 \pm 1$  respectively,  $p = 0.0002$  in F344 rats). The fecal fat was lower in SD than in F344 rats, showing a better absorption of fats by SD rats compared to F344. This observation agrees with the low caloric intake of SD rats fed on the oily diet.



**Figure 1.** Mean body weight of SD and F344 female rats, on Diets with Contrasting Glycemic Index or Nutrient Density, for 100 d, After a Single AOM Injection (20 mg/kg). Dots: Mean weight of 9-12 rats given AIN76 diets based on starch (65 or 82%), glucose (65%), or fructose (65%). Identity of groups are not shown, because no difference was significant. Error bars are not given because there are too many curves. Triangles: Diet based on oil and sucrose (39% of each). A star indicates a significant difference with starch-fed controls.

### Visceral fat and Blood values

The weight of visceral fat depended on the diet (Table 4). Taken together, rats given the two diets of low nutrient density had more visceral fat than the rats given the three diets of high density (11.5 and 8.1 g/SD rat respectively,  $p = 0.01$ ; 9.6 and 7.8 g/F344 rat  $p = 0.0005$ ). The absolute weight of visceral fat was similar in both strains of rats (Table 4). The relative weight of visceral fat, percent of body weight, was smaller in SD than in F344 rats ( $3.6\% \pm 0.8$  and  $5.3\% \pm 0.6$  respectively,  $p = 0.0003$ ).

On the day of sacrifice, after a fasting night, the blood glucose was not significantly different in rats given different diets (Table 4). In contrast, the values of blood triglycerides were reduced in rats eating the oily diet (Table 4,  $p < 0.005$  compared to SD and F344 starch-fed controls). The insulin blood concentrations also were reduced in rats fed on the oily diet ( - 64% in SD rats,  $p = 0.05$ ; - 49% in F344 rats,  $p = 0.005$  ). The insulin resistance FIRI index was 3-5 times lower in rats given the diet high in oil and sucrose than in rats given the high starch diet (Student's  $p = 0.05$  in SD,  $p = 0.01$  in F344 rats). Blood triglycerides and insulin were not affected by the other diets (Table 4,  $p = 0.7$ ). Taken together, data show that the diets did not modify insulin parameters as expected (Table 1).

### Promotion of ACF.

A total of 10953 and 9057 ACF was detected in the 48 SD and 48 F344 rats colons respectively (Table 5). The number of ACF per colon, which is not associated with tumor promotion, was not affected by the rat strain ( $p = 0.98$ ) nor by the diet ( $p = 0.7$  in SD and  $p = 0.2$  in F344 rats). By contrast, the crypt multiplicity (or number of crypts/ACF), a predictor of the tumor incidence, was larger in SD than in F344 rats ( $p = 0.005$ ). Crypt multiplicity was affected by the nature of diet in F344 rats, but not in SD rats (Fig. 2). The number of large ACF, another measure for promotion, was affected similarly by the diet (Table 5). Since this study intended to test two main hypotheses, two subsets of data were analyzed separately. On the one hand we compared the effect of diets with contrasting GI, containing glucose or fructose. On the other hand we compared diets with contrasting nutrient density and fat content, containing 65% or 82% starch, or oil

**Table 4.** Visceral Fat and Fasting Blood Values, in SD and F344 Rats, on Diets with Contrasting Glycemic Index or Nutrient Density, for 100 d, After a Single Azoxymethane Injection (20 mg/kg).

Diet group <sup>a</sup> :		Visceral fat	Blood glucose	Blood triglycer.	Blood insulin	FIRI index <sup>b</sup>
SD rats	N	g/rat	mM	μM	μU/ml	
Starch	12	8.9 ±3.4	7.3 ±2.1	710 ±130	14 ±13	4.7 ±6.0
Glucose	9	8.7 ±4.6	7.6 ±1.5	890 ±370	12 ±07	3.7 ±2.2
Fructose	9	6.5 ±2.0	7.1 ±1.4	860 ±240	08 ±05	2.4 ±1.9
High starch	9	9.9 ±4.0	7.5 ±1.8	830 ±210	25 ±30	8.3 ±10
Oil & sucrose	9	13. ±5.9*	6.6 ±1.7	530 ± 70*	05 ±06+	1.8 ±2.1
ANOVA p		0.03	0.77	0.008	0.09	0.13
<b>F344 rats</b>						
Starch	12	7.9 ±2.4	8.3 ±1.5	580 ±120	43 ±25	15 ±10
Glucose	9	8.3 ±1.8	8.4 ±1.2	570 ± 90	44 ±26	16 ±11
Fructose	9	7.3 ±1.8	9.3 ±1.4	620 ±120	41 ±22	16 ±10
High starch	9	9.4 ±1.3*	8.4 ±1.6	570 ± 90	48 ±25	17 ±12
Oil & sucrose	9	9.9 ±1.4*	7.3 ±1.1	260 ± 80*	22 ±08*	06 ±03*
ANOVA p		0.02	0.07	<0.0001	0.09	0.10).

a: Composition of diet is given in table 2.

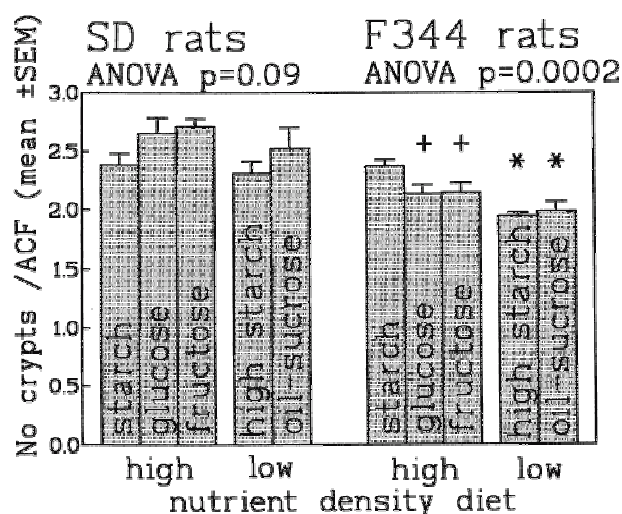
b: The FIRI index is (blood glucose x blood insulin) / 25 (38)

\* p<0.01, + p<0.05, different from the starch-fed control group of rats (first row) by Dunnet's test.

Glycemic index: The ACF multiplicity, the ACF number, and the number of large ACF, were the same in rats given 65% glucose or 65% fructose during 100 d (Fig. 2 and Table 5, p=0.7 in SD, p=0.9 in F344 rats). Thus, two diets with contrasting GI (100 & 23), had the same effect on ACF growth in SD and F344 rats (Table 1). A high GI of diet was not a promoting factor in this model.

Nutrient density: The effect of nutrient density was not significant in SD rats (p=0.48). F344 rats fed on diets of low nutrient density (82% starch, or oil and sucrose), had smaller ACF than F344 rats given the 65% starch control diet (Fig. 2). Both multiplicity and number of large ACF were reduced compared to control (Fig.2 and Table 5, p<0.0001 in F344). Thus, diets of low nutrient density, which increased the visceral obesity, were not promoting factors in this study, and may even be protective factors in F344 rats (Table 1).

The correlations of visceral fat, blood glucose, insulin and triglycerides, with ACF multiplicity were small and not significant, in both strains of rats (Table 6). Moreover, indirect insulin resistance markers (FIRI index, plasma triglycerides) were higher in rats given a high-starch diet than in rats given an oil and sucrose diet (Table 4, p<0.05), but the growth of ACF was the same in both groups (2.31 and 2.52 crypts/ACF, p=0.26 in SD rats, 1.94 and 1.98 p=0.65 in F344 rats, Fig. 2). Thus, some indirect markers of insulin resistance were not associated with the promotion of ACF growth. In addition, as shown in table 6, the ACF multiplicity surprisingly correlated to fecal weight output (taken at the group level in F344 rats, r=0.99, p=0.001, N=5).



**Figure 2.** Multiplicity of aberrant crypt foci (No of crypts/ACF) in the colon of SD and F344 rats given diets with contrasting glycemic index or nutrient density, for 100 d, after a single dose of azoxymethane (20 mg/kg). Rats were given AIN76 diets of high nutrient density, based on starch (65%), glucose (65%), or fructose (65%), or diets of low nutrient density based on starch (82%) or oil and sucrose (39% of each). Means 9 or 12 rats (starch-fed controls), error bars are SEM. Groups that differ from the starch-fed control (first bar) are labelled with a + (p<0.05) or a \* (p<0.01). See high definition figure on last page

**Table 5.** Number of Aberrant Crypt Foci (ACF) and Distribution of ACF Multiplicity in SD and F344 Rats, on Diets with Contrasting Glycemic Index or Nutrient Density, for 100 d, After a Single Azoxymethane Injection (20 mg/kg).

Diet group	Rats	Total No	1 crypt	2 crypt	3 crypt	4 crypt	5-6 crypt	>6 crypt
SD rats	N	ACF /rat	ACF	ACF	ACF	ACF	ACF	ACF
Starch	12	240 ±58	86 ±34	77 ±25	36 ±10	22 ± 6	15 ±6	5.3 ±3.6
Glucose	9	208 ±42	64 ±46	58 ±13	37 ±11	21 ± 6	18 ±8	9.4 ±9.0
Fructose	9	223 ±50	64 ±21	70 ±22	38 ±12	22 ± 5	18 ±6	10.8 ±3.3
High starch	9	234 ±64	89 ±35	73 ±24	33 ± 8	20 ± 4	13 ±5	5.0 ±2.2
Oil & sucrose	9	232 ±51	73 ±19	72 ±25	39 ±11	24 ±12	15 ±9	8.8 ±7.5
ANOVA <i>p</i>		0.72	0.13	0.46	0.78	0.82	0.52	0.10
<b>F344 rats</b>								
Starch	12	205 ±41	68 ±21	64 ±11	34 ±9	22 ±8	14 ±7	3.7 ±1.4
Glucose	9	171 ±33	68 ±19	56 ±11	25 ±8	12 ±5 *	8 ±3 +	2.4 ±2.3
Fructose	9	183 ±37	71 ±23	60 ±12	29 ±6	14 ±4 +	7 ±2 +	1.7 ±1.2 +
High starch	9	207 ±53	95 ±28	61 ±11	28 ±11	15 ±6 +	7 ±3 +	0.7 ±1.3 *
Oil & sucrose	9	172 ±49	73 ±22	57 ±14	23 ±10+	12 ±7 *	6 ±6 *	1.1 ±1.8 *
ANOVA <i>p</i>		0.20	0.07	0.53	0.08	0.003	0.008	0.001

\*  $p < 0.01$ , +  $p < 0.05$ , different from the starch-fed control group of rats (first row) by Dunnet's test.

### Discussion.

Although diets did not modify insulin parameters as expected, the present study yielded three major findings: (i) the glycemic index of diet was not a determinant of colon ACF growth; (ii) diets of low nutrient density did not promote colon ACF growth; (iii) indirect insulin resistance markers (FIRI index, visceral fat, blood triglycerides) did not correlate with colon ACF growth. These three findings are discussed below:

The glycemic index is a classification of foods based on their glucose-raising potential, and is also related to the insulin peak that follows a meal (39). According to McKeown's hypothesis, high blood glucose or insulin might be risk factor for colon cancer. Thus, foods with a high GI may be associated with increased carcinogenesis. In the present study, in two rat strains, we could not show any difference in the ACF growth rate between two diets containing 65% glucose or 65% fructose (Fig. 2 and table 5). These sugars have contrasting GI (100 and 23 respectively) (18). The present study was not designed to measure the GI, since blood values were not measured after meal, but after a fasting night. Fasting blood values were not affected by glucose or fructose diets (Table 4). This contrasts with our opening speculations, and may confuse our finding. However, our data agree with Caderni's ones (40), who concludes that carbohydrates with a high GI do not stimulate colon carcinogenesis.

Diets of low nutrient density, and thus high in "empty" calories, did not promote the growth of ACF, compared to a control diet containing twice more vitamins, minerals, cellulose, and essential amino-acids per calorie. The low nutrient density diets did not affect the ACF growth in one strain of rats (SD), and decreased the ACF growth in the other strain (F344) (Fig. 2). This finding fits with the study of Newberne showing that a high fat diet does not

promote colon cancer in rats when the nutrient-to-calorie ratio is not balanced. By contrast, a high fat diet increases the tumor incidence in rats when the nutrient-to-calorie ratio is balanced by vitamins and minerals supplements (41). It is possible that the diets of low nutrient density lack some nutrients that tumors need to grow. However, these results disagree with many studies showing that some micronutrients and cellulose reduces proliferation endpoints in animals or human (42).

Indirect insulin resistance markers (FIRI index, plasma triglycerides, and visceral fat) did not correlate with the ACF growth in the present study (Table 6). The FIRI index was 3-5 times lower, and the plasma triglycerides twice lower, in oil-and-sucrose fed rats than in high starch-fed rats (Table 4), but the multiplicity of ACF was the same with both diets (Fig. 2). Also, diets of low nutrient density increased the visceral obesity (Table 4), but did not increase the ACF multiplicity (Fig. 2). However, this finding was confused by the fact that rats fed a diet high in oil and sucrose did not develop insulin resistance as assessed by the FIRI index, in contrast with our opening speculation (20-23). We used safflower oil in the diet for SD rats, like Ikemoto (22). For F344 rats, we turned to grape-seed oil. Both oils contain 75% linoleic acid. Surprisingly, both oil-and-sucrose diets decreased fasting blood triglycerides and blood insulin compared to the 82% starch control. This might be due to the rats' gender (female). Indeed, female Wistar rats do not develop sucrose-induced insulin resistance, in contrast with male rats (43). Last, some results do not agree in SD and F344 rats (e.g., effect of nutrient density, Table 1). Similarly, a high fat diet enhances colon carcinogenesis in F344 rats, but not in SD rats (44).

**Table 6.** Correlations Between Aberrant Crypt Foci Multiplicity and Insulin Resistance Markers in SD and F344 Rats on Diets with Contrasting Glycemic Index or Nutrient Density, for 100 d, After a Single Azoxymethane Injection (20 mg/kg).

Correlation of ACF Multiplicity with	in 48 SD rats		in 48 F344 rats		in all 96 rats	
	r <sup>a</sup>	p	r	p	r	p
Blood glucose	- 0.28	1	+ 0.05	1	- 0.30	0.5
Blood insulin	- 0.16	1	+ 0.12	1	- 0.30	0.6
Blood triglyc.	+ 0.01	1	+ 0.14	1	+ 0.33	0.2
Visceral fat	- 0.22	1	- 0.25	1	- 0.13	1
Fecal weight	+ 0.16	1	+ 0.58	0.003	+ 0.53	0.000

**a:** Pearson correlation (r) matrix was calculated between 20 parameters, and probability values (p) were computed with the Bonferroni correction.

Diets with a high glycemic index, and high in calories, fat, sucrose, fructose, amylopectin starch, and low in fiber, resistant starch and n-3 unsaturated fats, should favor the insulin resistance syndrome in rodents (45). These diets should increase blood insulin, and lead to abdominal fat store, obesity, and non-insulinodependant diabetes in many animal models (4, 22, 46), and probably in humans too (47, 48). These same diets can promote experimental carcinogenesis in rodents (45), and are considered risk factors for colorectal cancer in human populations (6). We previously have shown that insulin injections promote the growth of ACF in rats (10). This supports the idea that in humans, hyperinsulinemia and/or insulin resistance may explain the high risk of colorectal cancer associated with sedentary lifestyle, overfeeding, and nutriment cited above (5, 45). However the present results do not support the hypotheses that a diet with a high glycemic index, or a diet of low nutrient density, or a diet that increases some indirect insulin resistance markers, can enhance ACF growth. Since most studies support that ACF are precancerous lesions (27-36), although some disagree (49), these dietary factors may not be true risk factors for colon cancer.

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#### References

- Potter, JD: "Risk factors for colon neoplasia - epidemiology and boil." *Europ J Cancer* **31A**, 1033-8, 1995.
- Storlien, LH, Baur, LA, Kriketos, AD, Pan, DA,

Cooney, GJ, et al.: "Dietary fats and insulin action" *Diabetologia* **39**, 621-631, 1996.

3. Byrnes, SE, Miller, JC, and Denyer, GS: "Amylopectin starch promotes the development of insulin resistance in rats" *J Nutr* **125**, 1430-1437, 1995.

4. Shafir, E: "Development and consequences of insulin resistance: lessons from animals with hyperinsulinaemia" *Diabetes Metab* **22**, 122-131, 1996.

5. Bruce, WR, and Corpet, DE: "The colonic protein fermentation and insulin resistance hypotheses for colon cancer etiology: experimental tests using precursor lesions." *Europ J Cancer Prev* **5** Sup.2, 41-47, 1996.

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

7. Giovannucci, E: "Insulin and colon cancer." *Cancer Causes Control* **6**, 164-179, 1995.

8. Weiderpass, E, Gridley, G, Nyren, O, Ekblom, A, Persson, I, et al.: "Diabetes mellitus and risk of large bowel cancer" *JNCI* **89**, 660-661, 1997.

9. Slattery, ML, Benson, J, Berry, TD, Duncan, D, Edwards, SL, et al.: "Dietary sugar and colon cancer" *Cancer Epidemiol Biom Prev* **6**, 677-685, 1997.

10. Corpet, DE, Jacquinet, C, Peiffer, G, and Tache, S: "Insulin injections promote the growth of aberrant crypt foci in the colon of rats" *Nutr Cancer* **27**, 316-320, 1997.

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

12. Koohestani, N, Tran, TT, Lee, W, Wolever, TM S., and Bruce, WR: "Insulin resistance and promotion of aberrant crypt foci in the colons of rats on a high-fat diet" *Nutr Cancer* **29**, 69-76, 1997.

13. Dai, S, and McNeill, JH: "Fructose-induced hypertension in rats is concentration- and duration-dependent" *J Pharmacol Toxicol Meth* **33**, 101-107, 1995.

14. Kazumi, T, Hirano, T, Odaka, H, Ebara, T, Amano, N, et al.: "VLDL triglyceride kinetics in Wistar fatty rats, an animal model of NIDDM: effects of dietary fructose alone or with pioglitazone" *Diabetes* **45**, 806-811, 1996.

15. Scelles, V, Alessi, MC, Raccach, D, Juhanvague, I, and Vague, P: "Plasma plasminogen activator inhibitor activity in rats with nutritionally induced insulin resistance"

*Thrombosis Haemostasis* **74**, 806-807, 1995.

16. Shafir, E: "Metabolism of disaccharides and monosaccharides with emphasis on sucrose and fructose and their lipogenic potential" *Sugars in Nutrition*, Gracey, M, Kretchmer, N, Rossi, E, eds., Nestlé Nutrition Workshop Series. Nestec Ltd. Vevey/Raven press, Ltd. NY **25**, 1991, 131-152.

17. Kazumi, T, Odaka, H, Hozumi, T, Ishida, Y, Amano, N, et al.: "Effects of dietary fructose or glucose on triglyceride production and lipogenic enzyme activities in the liver of Wistar fatty rats, an animal model of NIDDM" *Endocrine J* **44**, 239-245, 1997.

18. FosterPowell, K, and BrandMiller, J: "International tables of glycemic index" *Am J Clin Nutr* **62**, S871-S890, 1995.

19. Wolever, TMS., Bantum WA, and Jenkins, DJA: "Physiological modulation of plasma free fatty acid concentrations by diet: metabolic implications in nondiabetic subjects" *Diabetes Care* **18**, 962-970, 1995.

20. Barnard, RJ, Faria, DJ, Menges, JE, and Martin, DA: "Effects of a high-fat, sucrose diet on serum insulin and related atherosclerotic risk factors in rats" *Atherosclerosis* **100**, 229-236, 1993.

21. Surwit, RS, Kuhn, CM, Cochrane, C, McCubbin, JA, and Feinglos, MN: "Diet-induced type II diabetes in C57BL/6j mice" *Diabetes* **37**, 1163-1167, 1988.

22. Ikemoto, S, Thompson, KS, Takahashi, M, Itakura, H, Lane, MD, et al.: "High fat diet induced hyperglycemia: prevention by low level expression of glucose transporter (GLU4) minigene in transgenic mice" *Proc Natl Acad Sci USA* **92**, 3096-3099, 1995.

23. Ikemoto, S, Takahashi, M, Tsunoda, N, Maruyama, K, Itakura, H, et al.: "High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils" *Metabolism* **45**, 1539-1546, 1996.

24. Takeshita, M, Ueda, H, Shirabe, K, Higuchi, Y, and Yoshida, S: "Lack of promotion of colon carcinogenesis by high-oleic safflower oil" *Cancer* **79**, 1487-1493, 1997.

25. Newmark, HL: "Nutrient density: an important and useful tool for laboratory and animal studies" *Carcinogenesis* **8**, 871-873, 1987.

26. Bruce, WR, Archer, MC, Corpet, DE, Medline, A, Minkin, S, et al.: "Diet, aberrant crypt foci and colorectal cancer" *Mutation Res* **290**, 111-118, 1993.

27. Bird, RP: "Observation and quantification of aberrant crypts in murine colon treated with a colon carcinogen: preliminary findings" *Cancer Lett* **37**, 147-151, 1987.

28. McLellan, EA, Medline, A, and Bird, RP: "Dose response and proliferative characteristics of aberrant crypt foci - putative preneoplastic lesions in rat colon" *Carcinogenesis* **12**, 2093-2098, 1991.

29. Tang, ZC, Shivapurkar, N, Frost, A, and Alabaster, O: "The effect of dietary fat on the promotion of mammary and colon cancer in a dual-organ rat carcinogenesis model." *Nutr Cancer* **25**, 151-159, 1996.

30. Wargovich, MJ, Harris, C, Chen, CD, Palmer, C, Steele, VE, et al.: "Growth kinetics and chemoprevention of aberrant crypts in the rat colon" *J Cell Biochem S* **16G**, 51-54, 1992.

31. Stopera, SA, Murphy, LC, and Bird, RP: "Evidence for a ras gene mutation in azoxymethane-induced colonic aberrant crypts in Sprague-Dawley rats - earliest recognizable precursor lesions of experimental colon cancer" *Carcinogenesis* **13**, 2081-2085, 1992.

32. Pretlow, TP, Brasitus, TA, Fulton, NC, Cheyer, C, and Kaplan, EL: "K-ras mutations in putative preneoplastic lesions in human colon" *JNCI* **85**, 2004-2007, 1993.

33. Pretlow, TP, O'Riordan, MA, Somich, GA, Amini, SB, and Pretlow, TG: "Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate" *Carcinogenesis* **13**, 1509-1512, 1992.

34. Zhang, XM, Stamp, D, Minkin, S, Medline, A, Corpet, DE, et al.: "Promotion of aberrant crypt foci and cancer in rat colon by thermolyzed protein" *JNCI* **84**, 1026-1030, 1992.

35. Magnuson, BA, Carr, I, and Bird, RP: "Ability of aberrant crypt foci characteristics to predict colonic tumor incidence in rats fed cholic acid" *Cancer Res* **53**, 4499-4504, 1993.

36. Corpet, DE, Stamp, D, Medline, A, Minkin, S, Archer, MC, et al.: "Promotion of colonic microadenoma growth in mice and rats fed cooked sugar or cooked casein and fat" *Cancer Res* **50**, 6955-6958, 1990.

37. American Institute of Nutrition: "Report of the American Institute of Nutrition Ad Hoc committee on standards for nutritional studies" *J Nutr* **107**, 1340-1348, 1977.

38. Duncan, MH, Singh, BM, Wise, PH, Carter, G, and Alaghbandzadeh, J: "A simple measure of insulin resistance" *Lancet* **346**, 120-121, 1995.

39. Jenkins, DJ A.: "Lente carbohydrate: a newer approach to the dietary management of diabetes" *Diabetes Care* **5**, 634-641, 1982.

40. Caderni, G, Luceri, C, Lancioni, L, Biggeri, A, Giannini, A, et al.: "Modification of azoxymethane intestinal carcinogenesis in rats by feeding sucrose boluses, pasta, and glucose" *Nutr Cancer* **28**, 146-152, 1997.

41. Newberne, PM, and Sahaphong, S: "Dietary fat and colorectal cancer: experimental evidence for tumor promotion" *Colorectal Cancer: from Pathogenesis to Prevention*. Seitz, HK, Simanowski, UA, Wright, NA, Eds. Springer Verlag, NY 89-111, 1989.

42. Newmark, HL, Lipkin, M, and Maheshwari, N: "Colonic hyperplasia and hyperproliferation induced by a nutritional stress diet with four components of western style diet" *JNCI* **82**, 491-496, 1990.

43. Horton, TJ, Gayles, EC, Prach, PA, Koppenhafer, TA, and Pagliassotti, MJ: "Female rats do not develop sucrose-induced insulin resistance" *Amer J Physiol* **41**, R1571-R1576, 1997.

44. Zhao, LP, Kushi, LH, Klein, RD, and Prentice, RL: "Quantitative review of studies of dietary fat and rat colon carcinoma" *Nutr Cancer* **15**, 169-177, 1991.

45. Koohestani, N, Chia, MC, Pham, N-A, Tran, TT, Minkin, S, et al.: "Aberrant crypt focus promotion and glucose intolerance: correlation in the rat across diets differing in fat, n-3 fatty acids and energy. *Carcinogenesis*, in press 1998.

46. Higgins, JA, Miller, JC B., and Denyer, GS:

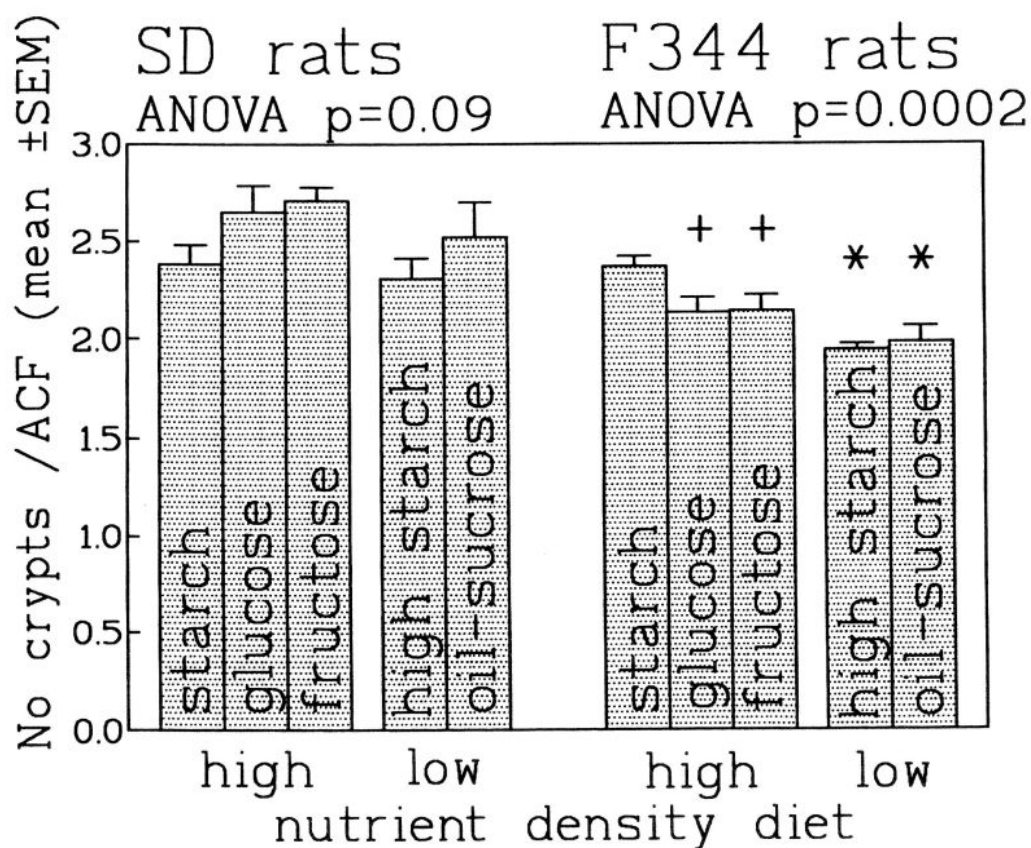


"Development of insulin resistance in the rat is dependent on the rate of glucose absorption from the diet" *J Nutr* **126**, 596-602, 1996.

47. Frayn, KN, and Klingman, SM: "Dietary sugars and lipid metabolism in humans" *Am J Clin Nutr* **62**, S250-S263, 1995.

48. Salmeron, J, Ascherio, A, Rimm, EB, Colditz, GA, Spiegelman, D, et al.: "Dietary fiber, glycemic load, and risk of NIDDM in men" *Diabetes Care* **20**, 545-550, 1997.

49. Hardman, WE, and Cameron, IL: "Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents." *Carcinogenesis* **17**, 1539, 1996



**Figure 2.** Multiplicity of aberrant crypt foci (No of crypts/ACF) in the colon of SD and F344 rats given diets with contrasting glycemic index or nutrient density, for 100 d, after a single dose of azoxymethane (20 mg/kg). Rats were given AIN76 diets of high nutrient density, based on starch (65%), glucose (65%), or fructose (65%), or diets of low nutrient density based on starch (82%) or oil and sucrose (39% of each). Means 9 or 12 rats (starch-fed controls), error bars are SEM. Groups that differ from the starch-fed control (first bar) are labelled with a + ( $p < 0.05$ ) or a \* ( $p < 0.01$ ).