



## Sucrose and IQ induced mutations in rat colon by independent mechanism

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### Abstract

Sucrose-rich diets have repeatedly been observed to have co-carcinogenic actions in colon and liver of rats and to increase the number of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induced aberrant crypt foci in rat colon. To investigate a possible interaction between sucrose and IQ on the genotoxicity in rat liver and colon, we gave Big Blue rats<sup>TM</sup> a diet containing sucrose (0%, 3.45% or 13.4% w/w) and/or IQ (70 ppm) for a period of 3 weeks. Sucrose and IQ increased the mutation frequency in the colon. The effect of combined treatments with IQ and sucrose on the mutation frequencies was additive indicating that sucrose and IQ act independently. This was supported by the mutation spectra where sucrose expands the background mutations in the colon, whereas IQ, in other studies, more specifically has induced G:C → T:A transversions. In the liver IQ increased the mutation frequency, whereas addition of sucrose reduced the effect of IQ in a dose-dependent manner. The level of bulky DNA adducts in liver and colon was increased in animals exposed to either sucrose or IQ. In animals exposed to IQ, addition of sucrose had marginal effects on the level of bulky DNA adducts. Markers of oxidative damage and DNA repair were generally unaffected by the treatments. In conclusion, sucrose and IQ in the diet induced mutations in the colon by independent mechanisms, whereas an interaction was observed in liver leading to a decrease in mutations by the combined treatment.

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### 1. Introduction

Colon cancer is associated with diet and other lifestyle factors typical for the western countries,

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such as low intake of vegetables and fruit, a sedentary lifestyle, obesity, and probably increased intake of dietary energy, cooked meat, and sugar [1,2]. Epidemiological studies show a weak association between colon cancer and high intake of sucrose. In a recent review 16 of 18 relevant studies indicate that high intake of sucrose is associated with increased risk of colon cancer [3]. A high intake of sucrose has also been shown to increase the proliferation rate in the colorectal epithelium, and to expand the proliferative zone from less than 60% of the colonic crypt to the entire crypt in humans [4]. In the rat colon a sucrose-rich diet has increased the mutation rate, increased the number of IQ-induced aberrant crypt foci (ACF), a putative pre-neoplastic lesion in the colonic mucosa and increased the proliferation rate especially when it is given in bolus [5–8].

The heterocyclic aromatic amine (HAA) 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is formed in a condensation reaction between creatine and amino acids during cooking of meat and fish. IQ is a potent genotoxic rodent carcinogen, which induces tumours at several sites including colon [9]. In non-human primates IQ is a strong hepatic carcinogen. The mechanism includes metabolic activation followed by adduct formation with DNA [10]. In rats IQ is mutagenic in liver, colon and other organs [10,11]. Big Blue rats have been used to determine mutational events in several organs. After oral administration of IQ a trend towards increased G:C → T:A transversions and decreased G:C → A:T transitions in the colon has been observed [10,11].

In a recent study of possible interactions between IQ and sucrose, we found that increasing the concentration of sucrose from 3.4 to 6.4% in the diet enhanced the level of IQ specific adducts in the liver [12]. In the colon we only found that IQ increased DNA strand breaks but no other markers for genotoxicity, DNA repair or oxidative damage were affected by sucrose, IQ or the two substances given simultaneously. The doses in this experiment were very low and it cannot be excluded that there would be effects of sucrose and/or IQ if they were given at higher concentrations. The aims of the present study are therefore to evaluate the interaction between the genotoxic and oxidative effects of IQ and sucrose at a higher dose level and to include markers to investigate the nature of sucrose mutagenesis. We report here an experi-

ment with a wider range of sucrose concentrations (0, 3.4 and 13.45%) a higher IQ dose (70 ppm) and an enhanced design. In previous studies on sucrose mutagenesis we replaced the total diet with sucrose [5,12]. In the present study the design is improved by replacing added sucrose with cornstarch only, thereby keeping the concentration of carbohydrates and other groups of nutrients constant in all dosed groups. Furthermore, we have included markers for proliferation of the colon epithelium and determined the spectrum of sucrose-induced mutations in the colon. For this later analysis we have added material from a previous experiment [5] to determine the mutation spectrum in rats given very high doses of sucrose (34.45%).

## 2. Materials and methods

Chemicals were used as supplied, without further purification. Fluoresceinamine (isomer II), sodium cyanoborohydride, 4-morpholinoethane sulfonic acid, and SDS were from Aldrich Chemical Co. (Steinheim, Germany). If not otherwise stated, all other chemicals were from Merck (Darmstadt, Germany).

### 2.1. Animals and exposure

Forty-nine male Big Blue (Fischer) rats (9–12 weeks of age; 180–270 g) from AH Diagnostics (Aarhus, Denmark) were acclimatized for 6 days while being maintained on a purified diet without sucrose. The diet was modified from the standard diet formulated at the Danish Veterinary and Food Administration according to Meyer et al., by replacing sucrose with cornstarch [13]. Animals were stratified by weight and randomly assigned into six groups: control (0% w/w sucrose,  $n = 9$ ), 3.4% sucrose (w/w,  $n = 8$ ), 13.4% sucrose (w/w,  $n = 8$ ), IQ (70 ppm IQ,  $n = 8$ ), IQ/3.4% sucrose (70 ppm IQ and 3.45% w/w sucrose,  $n = 8$ ), and IQ/13.4% sucrose (70 ppm IQ and 13.4% w/w sucrose,  $n = 8$ ). Added sucrose replaced cornstarch. Animals were kept two in each cage as described previously [5]. The diet was administered for a total of 21 days and feed consumption was monitored. A 24 h urine sample was collected terminating on day 21 and stored at  $-80^{\circ}\text{C}$ . At day 21 each individual animal was weighed, anaesthetized

in a mixture of 40% carbon dioxide and 60% oxygen, and decapitated. Blood was collected through a polyethylene funnel into EDTA-coated tubes and separated into plasma and erythrocytes by centrifugation ( $3000 \times g$ , 10 min and  $4^\circ\text{C}$ ). Erythrocytes were mixed 1:1 with water and plasma and erythrocytes were stored at  $-80^\circ\text{C}$ . Liver and colon were handled as previously described, except that liver tissue was sampled from the left and right lateral lobe 1 cm from the ventral edge. Liver and colon tissue were fixed in 4% buffered formaldehyde for 48 h [5].

## 2.2. Mutation analysis

The mutation frequencies of the *cII* gene in liver and colon were determined as described previously [5]. The identified mutants in the colon were sequenced on an ABI 310 Genetic Analyser using the sequencing kit, BigDye™ Terminator Cycle Sequencing Ready Reaction from Applied Biosystems. All templates were sequenced with the primer 5'-cctctgccgaagttgagat-3'. Some of the templates were also sequenced with the primer 5'-ctcctgttgatagatccag-3' for resolving ambiguities. All available phages were sequenced from the animals in the control and 13.4% sucrose groups. Mutations detected in a previous study where groups of six animals were given 3.4% or 34.45% sucrose were included to allow study of a wider dose–response relationship [5].

## 2.3. Oxidative parameters and DNA damage

Biomarkers for oxidative stress in plasma included levels of ascorbic acid, lipid oxidation (MDA) and protein oxidation (2-amino adipic semialdehyde), all determined by HPLC as described previously [14–16]. Total protein was determined on a Cobas Mira analyser using a commercial kit (cat. # 0736783; Roche, Basel, Switzerland). Liver cytosol was prepared as described elsewhere [17] and the products of protein oxidation, 2-amino adipic semialdehyde and  $\gamma$ -glutamyl semialdehyde, were determined by HPLC [16]. The level of oxidative DNA damage in terms of 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) was determined by HPLC-EC in liver and colon mucosa cells [18] and by HPLC MS/MS in urine [19]. In liver and colon mucosa cells DNA strand breaks were determined by the comet assay [11].

## 2.4. DNA adducts, proliferation and apoptosis

The level of DNA adducts in colon and liver were determined by  $^{32}\text{P}$ -postlabelling with a standard method usually used to determine PAH adducts [20]. In liver and colon cell proliferation was assayed by immunohistochemistry of PCNA staining and apoptosis was detected by the TUNEL assay [5]. Data on cell proliferation in liver is given as the mean of the labelling index of the left and right lateral lobe. The colon samples were taken from the mid part of the colon and were treated like the liver samples with a few exceptions: the monoclonal mouse anti-PCNA (DAKO, M0879, clone PC10, Denmark) was diluted 1:1600 and EnVision+ (DAKO, K4000, Denmark) was used as secondary antibody. The labelling index was evaluated as previously described [21].

## 2.5. DNA repair

RNA purification, cDNA synthesis, ERCC1 and OGG1 analysis was performed as earlier described [11]. Quantification of MPG mRNA was performed under the same conditions and standards as for ERCC1 and OGG1 except for the modifications mentioned below.

For MPG the following oligonucleotides were used: Taqman probe: 5'-FAM-ACC TGT CCC AGA AAT GCA CGG GCT-Tamra-3', forward primer: 5'-AGT TGT GTT CCA TCA GCA AGT CG-3', reverse primer: 5'-GGC TAG GAC CGG AGT ATT TTG A-3'. The PCR reaction was run using 300 nM primers, 150 nM Taqman probe, 0.7  $\mu\text{l}$  cDNA from liver and 1–10  $\mu\text{l}$  cDNA from colon.

## 2.6. Statistics

Data were tested for homogeneity of variance using Levene's test and for normality using the Kolmogorov–Smirnov test. Log-transformations were performed for data, which did not meet these criteria. Effects of the single compounds and their eventual interaction were tested using two-way ANOVA ( $P < 0.05$ ). Dunnett's test was used to compare dosed groups with control where ANOVA indicate a significant single factor effect. The non-parametric Kruskal–Wallis test was used for datasets, which were not normally distributed or did not have homogeneity of variance

even after log-transformation. These statistical analyses were performed using the SAS Statistical Package, ver. 8.1; SAS Institute Inc., Cary, NC). The frequency of the different types of mutations were compared using Monte Carlo simulation models [22].

### 3. Results

There were no significant differences in weight gain between the different groups and all rats were healthy during the experiment (Table 1). There was a trend towards increased energy intake in the sucrose-dosed animals, which was significant in the 3.4% sucrose group.

In the liver IQ significantly increased the mutation frequency whereas sucrose had no significant effect of its own but reduced the effect of IQ in a dose-dependent manner (Fig. 1). In the colon mucosa the mutation frequency was significantly increased by sucrose, independent of IQ, whereas the effect of IQ was significant only by two-way ANOVA (Fig. 1). The combined treatment was additive in both colon and liver.

There was no significant change in the mutation spectrum in colon mucosa as a result of sucrose treatment (Table 2). In liver and colon the level of bulky DNA adducts increased dose-dependently in animals given either sucrose or IQ (Fig. 2). In animals exposed to IQ, addition of sucrose did not change the level of bulky DNA adducts in the colon. A small increase was observed in the liver but although the increase was statistically significant it was regarded as marginal, because the change was considered to be too small to be revealed reliably by  $^{32}\text{P}$ -postlabelling (Fig. 2).

The markers for protein oxidation in plasma and liver and lipid oxidation in plasma were not affected

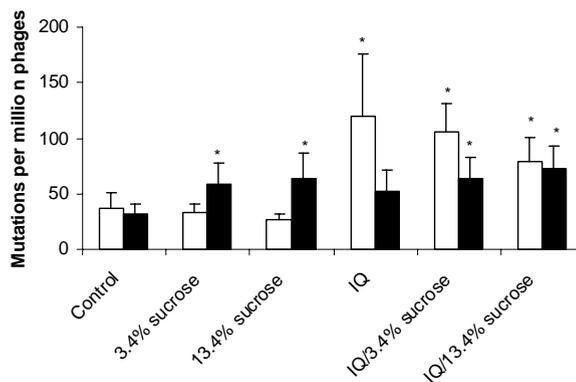


Fig. 1. Mutation frequency of *cII* in liver (□) and colon (■). Bars represent means, and lines denote 1 standard deviation. \* $P < 0.05$  from the control group.

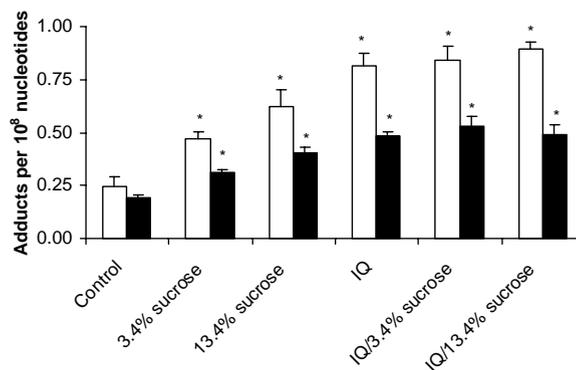


Fig. 2. Bulky DNA adduct level in liver (□) and colon (■). Bars represent means, and lines denote 1 standard deviation. \* $P < 0.05$  from the control group.

by the treatments (Table 3). There was a significant increase in total ascorbate in plasma in the IQ/3.4% sucrose group but because no trend was seen in the other groups, we do not consider this a treatment related

Table 1  
Energy intakes, body weight gain, and final body weights

Group	Control	3.4% sucrose	13.4% sucrose	IQ	IQ/3.4% sucrose	IQ/13.4% sucrose
<i>N</i>	9	8	8	8	8	8
Energy intake <sup>a</sup> (kJ/day)	261 ± 22	297 ± 8*	286 ± 22	272 ± 14	270 ± 5	284 ± 26
weight gain (g)	42.4 ± 9.6	37.0 ± 10.9	39.6 ± 9.6	34.4 ± 7.7	39.8 ± 12.1	41.0 ± 7.8
Final weights (g)	258 ± 23	255 ± 22	257 ± 31	253 ± 33	259 ± 38	258 ± 31

<sup>a</sup> The statistical calculations were based on the energy intake per cage.

\*  $P < 0.05$  compared to control.

Table 2

Number and type of mutations in phages isolated from colons of rats given feed containing different doses of sucrose

Mutation type		0% sucrose, <i>n</i> = 9	3.40% <sup>a</sup> sucrose, <i>n</i> = 6	13.40% sucrose, <i>n</i> = 8	34.45% <sup>a</sup> sucrose, <i>n</i> = 6
Transition	G:C → A:T	15 (33%)	21 (43%)	14 (28%)	23 (48%)
	A:T → G:C	2 (4%)	1 (2%)	1 (2%)	2 (2%)
Transversion	G:C → T:A	8 (17%)	9 (18%)	14 (28%)	10 (21%)
	G:C → C:G	3 (6%)	6 (12%)	9 (18%)	3 (6%)
	A:T → T:A	4 (9%)	0	1 (2%)	0
	A:T → C:G	0	3 (6%)	1 (2%)	2 (4%)
Insertion		11 (24%)	5 (10%)	8 (16%)	4 (9%)
Deletion		1 (2%)	2 (4%)	1 (2%)	4 (9%)
Complicated		2 (4%)	2 (4%)	1 (2%)	0
Total		46	49	50	48

<sup>a</sup> Materials from a previous experiment were used to determine number and types of mutation [5].

Table 3

Oxidative damage, DNA repair, apoptosis and proliferation

	Control	3.4% sucrose	13.4% sucrose	IQ	IQ/3.4% sucrose	IQ/13.4% sucrose
Vitamin C (plasma) (μM)	44.9 ± 5.4	50.6 ± 10.9	43.7 ± 5.0	47.6 ± 5.7	67.1 ± 20.3*	45.9 ± 5.8
AAS (plasma) (pmol/mg protein)	144 ± 14	152 ± 40	143 ± 17	148 ± 12	143 ± 9	144 ± 12
MDA (plasma) (pmol/mg protein)	87 ± 16	77 ± 20	90 ± 18	73 ± 5	78 ± 11	72 ± 11
8-oxodG (colon) (per 10 <sup>5</sup> dG)	1.2 ± 1.2	0.9 ± 0.4	1.4 ± 0.7	0.8 ± 0.4	0.8 ± 0.4	1.4 ± 0.9
Strand breaks (colon) (%DNA in tail)	15.4 ± 6.0	15.1 ± 8.2	14.9 ± 5.8	26.6 ± 8.8*	23.0 ± 4.6*	21.9 ± 9.1*
rERCC1 (colon) (per 10 <sup>5</sup> ) <sup>a</sup>	95 ± 85	65 ± 35	63 ± 28	77 ± 44	80 ± 72	67 ± 58
rOGG1 (colon) (per 10 <sup>6</sup> ) <sup>a</sup>	26 ± 24	23 ± 20	23 ± 25	24 ± 13	15 ± 13	14 ± 9
rMPG (colon) (per 10 <sup>6</sup> ) <sup>a</sup>	0.14 ± 0.08	0.16 ± 0.06	0.09 ± 0.05*	0.20 ± 0.14	0.22 ± 0.18	0.10 ± 0.05
PCNA (colon) <sup>b</sup>	12 ± 8	18 ± 13	17 ± 8	13 ± 9	18 ± 8	17 ± 13
PCNA (colon) <sup>c</sup>	4.5 ± 3.2	11.0 ± 9.5	7.3 ± 4.7	4.6 ± 6.5	5.2 ± 3.9	7.1 ± 5.3
AAS (liver) (pmol/mg protein)	82 ± 30	89 ± 28	80 ± 51	109 ± 25	59 ± 16	101 ± 38
8-oxodG (liver) (per 10 <sup>6</sup> dG)	0.26 ± 0.07	0.30 ± 0.06	0.34 ± 0.05*	0.31 ± 0.07	0.35 ± 0.06*	0.30 ± 0.04
Strand breaks (liver) (%DNA in tail)	6.9 ± 3.7	6.2 ± 2.2	5.1 ± 2.6	11.7 ± 3.3*	11.4 ± 4.5	13.4 ± 4.7*
GGs (liver) (pmol/mg protein)	144 ± 14	152 ± 40	143 ± 17	148 ± 12	143 ± 9	144 ± 12
rERCC1 (liver) (per 10 <sup>6</sup> ) <sup>a</sup>	39 ± 18	32 ± 11	26 ± 5	29 ± 8	26 ± 7	24 ± 5*
rOGG1 (liver) (per 10 <sup>6</sup> ) <sup>a</sup>	5.6 ± 2.0	3.8 ± 0.8*	3.3 ± 1.2*	2.8 ± 1.7*	2.4 ± 0.9*	3.1 ± 1.0*
rMPG (liver) (per 10 <sup>6</sup> ) <sup>a</sup>	0.58 ± 0.23	0.48 ± 0.20	0.34 ± 0.08	0.53 ± 0.22	0.60 ± 0.37	0.37 ± 0.14
Apoptosis (liver) <sup>d</sup>	1.50 ± 0.22	1.76 ± 0.46	1.76 ± 0.24	1.71 ± 0.31	1.76 ± 0.33	1.41 ± 0.43
PCNA (liver) <sup>d</sup>	0.99 ± 0.75	0.65 ± 0.53	0.98 ± 0.49	0.62 ± 0.36	1.18 ± 0.86	1.47 ± 0.91

<sup>a</sup> mRNA relative to *I8S*.<sup>b</sup> Percentage of cells staining positive in the lower third of the coloncrypt.<sup>c</sup> Percentage of cells staining positive in the mid third of the coloncrypt.<sup>d</sup> Percentage of cells staining positive.\* *P* < 0.05 compared to control.

effect. No effects were seen on DNA oxidation determined as 8-oxodG in colon mucosa cells (Table 3) or as 8-oxoguanine or 8-oxoguanosine in urine (data not shown). By two-way ANOVA, sucrose significantly increased liver 8-oxodG, independent of IQ (Table 3). The level of DNA strand breaks determined by comet assay was increased in colon and liver in the IQ treated animals, but no effect was seen in animals dosed only with sucrose (Table 3). Expression of the DNA repair gene OGG1 in the liver was down regulated in all treatment groups compared with the control group, whereas no consistent significant effects were seen in the expression of two other DNA repair genes, ERCC1 and MPG (Table 3). In the colon mucosa the expression of the DNA repair gene MPG was down regulated by 13.4% sucrose, whereas no significant changes were observed in the expression of ERCC1 and OGG1 (Table 3). There were statistically significant correlations between the expression of ERCC1 and the other two genes in both liver and colon ( $P < 0.0001$ , Pearson), but no correlation between MPG and OGG1. Cell proliferation, determined as the PCNA labelling index, was unchanged in the liver. In the colon mucosa there was no proliferative effect of IQ, whereas there was a trend towards increased cell proliferation in sucrose-treated animals in the basal third of the crypts (Table 3). When all sucrose-dosed animals were tested against the others, the average PCNA labelling index in the mid third of the colon crypts was significantly increased by sucrose.

#### 4. Discussion

It has previously been observed that sucrose increases the number rather than the complexity of ACF in colon induced by IQ [6,7], indicating a genotoxic effect of sucrose. Subsequently we have reported a mutagenic effect of sucrose in the rat colon, which was confirmed in the present study. We have analysed for interactions between IQ and sucrose on the mutation frequency data in the colon and found that the effects are best explained by the sum of the effects of IQ and sucrose. We therefore suggest that the mechanisms behind the mutagenic effects of sucrose and IQ are different and independent and that their interaction in carcinogenesis is only partly explained by their additive effects on mutagenesis.

In the liver, we found a decrease in the mutation frequencies with increased levels of sucrose relative to cornstarch when sucrose and IQ were co-administered. The mechanism could involve a sucrose-induced increase in the detoxification of IQ in the liver. This is supported by observations that a high sucrose diet can down regulate P450 1A1 in rat liver and decrease the *ex vivo* liver microsomal dependent mutagenicity of IQ [23]. However, this is not supported by the measurements for bulky DNA adduct, which show no decrease. An enhancing effect of cornstarch on liver mutagenesis seems less likely, although this would explain the lack of a similar effect in previous studies where sucrose was substituted for the total feed [5,12], and the reported trend towards an increased number of tumours in the liver of rats on a cornstarch based diet compared with rats on a sucrose based diet in a long-term study [24].

The level of bulky DNA adducts was increased by sucrose in the colon and the liver. The increase in the level of DNA adducts was highest in liver, in which sucrose decreased the mutation frequency, indicating that other factors influence the mutagenic potential of these adducts. An increased level of sucrose-induced bulky adducts in rat colon and liver is in keeping with our recent study with 3.4% sucrose [12], although we did not see such effects in our initial study with higher doses [5]. We ascribe this difference to design limitations in the initial study where large changes in all nutrients in the feed may have distorted the results. This is supported by the observation that a similar substitution of all feed components with lard gave rise to an even larger decrease in adduct levels [25]. We have previously found that 6.4% sucrose increased the level of bulky DNA adducts in the liver of rats given 20 ppm IQ [12]. In the present study sucrose, when co-administrated with IQ marginally increased the level of bulky adducts in the liver, whereas no effect was observed in the colon. The lack of complete concordance between data for levels of bulky DNA adducts, DNA strand breaks and mutation frequencies indicate that bulky DNA adducts are not solely responsible for the increased mutagenicity of sucrose in the colon. This is in agreement with other studies concluding that there are not necessarily strict correlation between total bulky DNA adduct level and other effects including late effects such as tumourigenicity [26].

To increase the information gained from the mutation spectrum analysis we included mutations from colons of rats fed diets with 3.4 and 34.45% sucrose from an earlier experiment [5]. The mutation spectrum (Table 2) across the groups was similar to the 3896 mutations registered in several organs in untreated mice and rats in a database at the Big Blue website (<http://eden.ceh.uvic.ca/bigblue.htm>, surveyed on 13 January 2003). E.g. 46% of the mutations in the database from untreated rats and mice were G:C → A:T mutations, 22% were G:C → TA and 9% were deletion mutations. This comparison may not be fully relevant because the mutation spectrum may vary between different organs and species. There are, however, very few studies published in the literature on mutations in the colon of Big Blue rats. We could not detect any difference in the mutation spectrum across the exposure groups. The mutation spectrum in the colon mucosa seen in the present sucrose-dosed animals was very broad and can be explained by an increase in the background mutations. This indicates that sucrose expands the background mutations and may therefore be an indirect mutagen. It has been suggested that sucrose induces insulin resistance resulting in increased secretion of insulin and insulin like growth factors [2]. In mice it has been observed that sucrose not only increased the number of ACF but also increased the proliferation of the colon epithelium [27]. In line with this we observed an apparent increase in proliferation rate in the colon epithelium in the lower third and mid third of the crypts but this was not statistically significant. Accordingly, no firm conclusions can be drawn concerning the mechanism behind sucrose mutagenesis.

We have previously reported that IQ tends to change the mutation spectrum with more G:C → T:A and less G:C → A:T mutations compared to control rats [11]. These changes were not statistically significant but consistent with the results of Bol et al. [10]. It would be interesting to study potential interactions between sugar and IQ with respect to the mutation spectrum. However, we estimated that we would not have power to resolve such an interaction statistically.

The lack of effect on oxidative damage parameters and DNA repair enzyme expression confirms previous findings that the mutagenic mechanism of sucrose in the colon is unlikely to be mediated by an oxidative pathway [5]. Moreover, despite enhanced DNA dam-

age in terms of adducts and strand breaks as well as mutations induced by IQ, DNA repair was not up-regulated. Nevertheless, correlation between the mRNA levels of the DNA repair enzymes suggests that these defences against different kinds of damage are partly co-regulated.

In conclusion, the present study with a more stringent design has confirmed a mutagenic effect of sucrose in the colon and not in the liver. The study indicates that sucrose and IQ in the diet induce mutations in the colon by independent mechanisms, whereas sucrose appears to decrease the mutagenic effect of IQ in the liver. The effects seem unrelated to markers of oxidative stress and DNA damage and to DNA repair enzyme expression. We were unable to resolve whether the mutagenic effect of sucrose might be influenced by enhanced cell proliferation in the colon, an explanation supported by the lack of changes in the mutation spectrum in sucrose-dosed animals.

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## References

- [1] R.M. Merrill, L.G. Kessler, J.M. Udler, G.C. Rasband, E.J. Feuer, Comparison of risk estimates for selected diseases and causes of death, *Prev. Med.* 28 (1999) 179–193.
- [2] W.R. Bruce, A. Giacca, A. Medline, Possible mechanisms relating diet and risk of colon cancer, *Cancer Epidemiol. Biomarkers Prev.* 9 (2000) 1271–1279.
- [3] E. Giovannucci, Insulin, insulin-like growth factors and colon cancer: a review of the evidence, *J. Nutr.* 131 (2001) 3109S–3120S.
- [4] R.M. Bostick, L. Fosdick, G.A. Grandits, T.J. Lillemoe, J.R. Wood, P. Grambsch, T.A. Louis, J.D. Potter, Colorectal epithelial cell proliferative kinetics and risk factors for colon cancer in sporadic adenoma patients, *Cancer Epidemiol. Biomarkers Prev.* 6 (1997) 1011–1019.

- [5] L.O. Dragsted, B. Daneshvar, U. Vogel, H.N. Autrup, H. Wallin, L. Risom, P. Moller, A.M. Molck, M. Hansen, H.E. Poulsen, S. Loft, A sucrose-rich diet induces mutations in the rat colon, *Cancer Res.* 62 (2002) 4339–4345.
- [6] E. Kristiansen, O. Meyer, I. Thorup, Refined carbohydrate enhancement of aberrant crypt foci (ACF) in rat colon induced by the food-borne carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), *Cancer Lett.* 105 (1996) 147–151.
- [7] A.M. Molck, O. Meyer, E. Kristiansen, I. Thorup, IQ (2-amino-3-methylimidazo[4,5-f]quinoline)-induced aberrant crypt foci and colorectal tumour development in rats fed two different carbohydrate diets, *Eur. J. Cancer Prev.* 10 (2001) 501–506.
- [8] C. Luceri, G. Caderni, L. Lancioni, S. Aiolfi, P. Dolara, V. Mastrandrea, F. Scardazza, G. Morozzi, Effects of repeated boluses of sucrose on proliferation and on AOM-induced aberrant crypt foci in rat colon, *Nutr. Cancer* 25 (1996) 187–196.
- [9] IARC. IQ (2-amino-3-methylimidazo[4,5-f]quinoline), in: IARC (Ed.), *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, Lyon, 1993, pp. 165–195.
- [10] S.A. Bol, J. Horlbeck, J. Markovic, J.G. de Boer, R.J. Turesky, A. Constable, Mutational analysis of the liver, colon and kidney of Big Blue rats treated with 2-amino-3-methylimidazo[4,5-f]quinoline, *Carcinogenesis* 21 (2000) 1–6.
- [11] P. Moller, H. Wallin, U. Vogel, H. Autrup, L. Risom, M.T. Hald, B. Daneshvar, L.O. Dragsted, H.E. Poulsen, S. Loft, Mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline in colon and liver of Big Blue rats: role of DNA adducts strand breaks DNA repair and oxidative stress, *Carcinogenesis* 23 (2002) 1379–1385.
- [12] P. Moller, M. Hansen, H. Autrup, J. Bornholt, U. Vogel, A.M. Molck, H. Wallin, L.O. Dragsted, L. Risom, H.E. Poulsen, S. Loft, Dietary low-dose sucrose modulation of IQ-induced genotoxicity in the colon and liver of Big Blue rats, *Mut. Res.* 527 (2003) 91–97.
- [13] O. Meyer, L. Blom, D. Sondergaard, The influence of minerals and protein on the nephrocalcinosis potential for rats of semisynthetic diets, *Lab. Anim.* 16 (1982) 271–273.
- [14] J. Lykkesfeldt, S. Loft, J.B. Nielsen, H.E. Poulsen, Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking, *Am. J. Clin. Nutr.* 65 (1997) 959–963.
- [15] S.T. Lauridsen, A. Mortensen, Probuco selectively increases oxidation of atherogenic lipoproteins in cholesterol-fed mice and in Watanabe heritable hyperlipidemic rabbits, *Atherosclerosis* 142 (1999) 169–178.
- [16] B. Daneshvar, H. Frandsen, H. Autrup, L.O. Dragsted, Gamma-glutamyl semialdehyde and 2-amino-adipic semialdehyde: biomarkers of oxidative damage to protein, *Biomarkers* 2 (1997) 117–123.
- [17] K.B. Kim, B.M. Lee, Oxidative stress to DNA protein and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene, *Cancer Lett.* 113 (1997) 205–212.
- [18] A. Wellejus, S. Loft, Receptor-mediated 17-ethynylestradiol-induced oxidative DNA damage in rat testicular cells, *FASEB J.* 16 (2002) 195–201.
- [19] A. Weimann, D. Belling, H.E. Poulsen, Measurement of 8-oxo-2'-deoxyguanosine and 8-oxo-2'-deoxyadenosine in DNA and human urine by high performance liquid chromatography-electrospray tandem mass spectrometry, *Free Rad. Biol. Med.* 30 (2001) 757–764.
- [20] H. Autrup, B. Daneshvar, L.O. Dragsted, M. Gamborg, M. Hansen, S. Loft, H. Okkels, F. Nielsen, P.S. Nielsen, E. Raffin, H. Wallin, L.E. Knudsen, Biomarkers for exposure to ambient air pollution—comparison of carcinogen-DNA adduct levels with other exposure markers and markers for oxidative stress, *Environ. Health Perspect.* 107 (1999) 233–238.
- [21] M. Poulsen, A.M. Molck, I. Thorup, V. Breinholt, O. Meyer, The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon, *Cancer Lett.* 167 (2001) 135–143.
- [22] D.B. Dunson, K.R. Tindall, Bayesian analysis of mutational spectra, *Genetics* 156 (2000) 1411–1418.
- [23] L.P. Peters, R.W. Teel, Effect of high sucrose diet on cytochrome P450 1A and heterocyclic amine mutagenesis, *Anticancer Res.* 23 (2003) 399–403.
- [24] R.H. Lindecrona, L.O. Dragsted, M. Poulsen, U. Vogel, H. Autrup, A.M. Molck, Effects of sucrose and cornstarch on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colon and liver carcinogenesis in F344 rats, *Cancer Lett.* 209 (2004) 17–24.
- [25] U. Vogel, B. Daneshvar, H. Autrup, L. Risom, A. Weimann, H.E. Poulsen, P. Moller, S. Loft, H. Wallin, L.O. Dragsted, Effect of increased intake of dietary animal fat and fat energy on oxidative damage, mutation frequency, *Free Rad. Res.* 37 (2003) 947–956.
- [26] M. Ochiai, K. Ishida, T. Ushijima, T. Suzuki, T. Sofuni, T. Sugimura, M. Nagao, DNA adduct level induced by 2-amino-3,4-dimethylimidazo[4,5-f]-quinoline in Big Blue mice does not correlate with mutagenicity, *Mutagenesis* 13 (1998) 381–384.
- [27] D. Stamp, X.M. Zhang, A. Medline, W.R. Bruce, M.C. Archer, Sucrose enhancement of the early steps of colon carcinogenesis in mice, *Carcinogenesis* 14 (1993) 777–779.