

## HIGH FAT AND NUTRIENT DEPLETED DIETS AND THE ENZYME PROFILE OF FECES AND COLON MUCOSA OF MICE

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*Swiss and C57BL/1 mice were fed one of the following diets: commercial laboratory chow (5.5 percent fat) or chow with added starch and/or corn oil so as to supply a fat level of 5.5 or 23 percent, while reducing the level of nutrients and dietary fiber per 100 calories to 61 percent of the level found in standard chow. Both nutrient depleted diets increased fecal pH whereas the level of dietary fat had no independent effect. In Swiss mice fed the above diet for 6.5 months, enzyme analyses revealed the following: (1) in colon mucosal samples there was no diet-induced change in the level of ouabain-insensitive ATPase,  $\beta$ -glucuronidase, phosphodiesterase I (PDI), or 5'-nucleotidase; (2) the non-specific esterase level in males was increased by a nutrient depleted diet; and (3) in females, a nutrient depleted diet reduced the fecal level of  $\beta$ -glucuronidase,  $\beta$ -glucosidase and non-specific reducing activity.*

### INTRODUCTION

An impressive body of epidemiological and experimental evidence associates a high fat diet with colon carcinogenesis (1,2). Among the mechanisms by which dietary fat might exert its effect is the alteration of the pH and chemical composition of the colon contents. Changes may also occur in the enzyme activity and membrane characteristics of the colon mucosa.

In a previous study, we reported that feeding a high fat diet to Swiss mice caused a rise in fecal non-specific reducing activity (3). This was determined using 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride hydrate (INT), a synthetic electron acceptor. Reducing activity is non-enzymic and is probably also non-bacterial. Its most likely origin is an intestinal wall secretion, mainly from the cecum (4). Since they might influence fecal metabolism, measurements were also made of the fecal level of  $\beta$ -glucuronidase and  $\beta$ -glucosidase, which have been reported to respond to dietary manipulation (2,5-7). It is possible that  $\beta$ -glucuronidase reactivates carcinogens which have been excreted by the liver as glucuronide conjugates.

In this study the effect of a high fat diet on fecal pH was tested in both Swiss and C57BL/1 mice. A lower pH may be protective against colon cancer by decreasing the activity of 7- $\alpha$ -dehydroxylase and thus the conversion of primary to secondary bile acids (8). It may also increase the absorption of bile acids to dietary fiber (9) and so reduce the exposure of the colon mucosa.

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A high fat diet induces an apparent decrease in the density of colon mucosal brush borders (10). This suggests an enhanced uptake of lipid into the membrane. We also observed that feeding this diet to Swiss mice caused an increase in the colon mucosal level of 5'-nucleotidase (3). The latter observation is being retested here. As an additional test for an altered function of the colon mucosa membrane we measured the level of PDI, an enzyme which, like 5'-nucleotidase, is localized on plasma membranes (11).

To help elucidate what other areas of colon biochemistry are altered by dietary fat, we also investigated whether fat induces an altered colon mucosal level of non-specific esterase, ouabain-insensitive ATPase and  $\beta$ -glucuronidase.

## MATERIALS AND METHODS

### *Mice, diets and environmental conditions*

These were the same as reported previously (3,4); male and female Swiss and C57BL/1 mice were used. The mice were fed on the diets indicated in Table 1.

Table 1. *Composition of diets.*

<i>Ingredients</i>	<i>Diet, % composition of weight</i>			
	<i>Control</i>	<i>F6</i>	<i>F23</i>	<i>F16</i>
Chow*	100	67	81	89
Corn oil		1.9	18.5	10.7
Oleic acid			0.28	0.16
Starch		31		
Fat content <sup>†</sup>	5.5	5.5	23	16
Nutrient/calories <sup>‡</sup>	100	61	61	75

\*Rodent Laboratory Chow (Ralston Purina Co., St. Louis, Mo). The diet is based mainly on vegetable foods and contains 23.4 percent protein, 16 percent neutral detergent fiber and an adequate concentration of all nutrients.

<sup>†</sup> Final content of fat calculated on a dry weight basis.

<sup>‡</sup> Relative content of vitamins, minerals, protein, and fiber per 100 calories.

### *Diet treatment and preparation of samples*

*Experiment I.* Starting at age 33 days, Swiss mice were fed the control diet or diets F6 or F23 for approximately 6.5 months. Organ and fecal samples were prepared essentially as described previously (3) except where indicated. The cecum was discarded and the contents of the final two-thirds of the remaining colon (ie by length from the ileo-cecal junction to the anus) were removed and mixed. Part was used for pH determination and the remainder to prepare a supernatant sample. The latter was prepared by extraction with 0.1% Triton X-100 except that the first centrifugation step was for 1 min.

Mucosal homogenate was prepared using the entire length of the colon (excluding the cecum). This initial homogenate was used for all mucosal assays except ATPase and 5'-nucleotidase which utilized the resuspended pellet after centrifugation (130 000  $\times$  g for 44 min).

*Experiment II.* Swiss mice, aged 18 days, were fed the control diet or diets F16 or F23 for 3, 6, 12, 14 or 21 months. With mice on diets F16 and F23, their parents also received diet F16 commencing 14 days before mating. Other mice received diet

F6 for 3 or 6 months. With the 3 month mice, the starch in diet F6 was replaced by a mixture of sucrose and glucose (1:1 by weight).

*Experiment III.* C57BL/1 mice received diets F6 or F23 either (a) for 4 or 23.5 months commencing at age 1.5 months or (b) for 4 months commencing at age 10 months.

In experiments II and III, after sacrifice of the mouse, the feces was taken from the entire length of the colon (excluding the cecum). After mixing, part was used for pH determination and the remainder for assay of bile acids and neutral sterols.

#### *Phosphodiesterase I (PDI)*

The PDI assay was based on that of Touster *et al.* (11). In a total volume of 0.375 ml containing 3 mM p-nitrophenyl thymidine 5'-phosphate (Sigma Chemical Co., St. Louis, Mo), 100 mM Tris-HCl (pH 9.0), 0.1% Triton X-100 and 0.15 ml of sample (initial homogenate or homogenization medium for controls). Incubation was for 11 min at 37°C and was terminated by adding 0.75 ml of 10% TCA. p-Nitrophenol was then determined (11).

#### *pH*

In experiment I, each sample was vigorously mixed with water (0.9 ml per 100 mg) and the pH measured with a glass calomel combination electrode (Sigma). In experiments II and III, the fecal dilution was greater (approximately 2-3 ml per 100 mg) and a glass-reference electrode (Beckman Instruments Inc., Palo Alto, Ca) was used.

#### *Bile acids and neutral sterols*

The method used was that of Cohen *et al.* (12-14). Samples in each diet-sex-age subgroup were pooled before analysis (due to the small sample size).

#### *Other assays*

Assays for ATPase (ouabain-insensitive), non-specific esterase (1-naphthyl acetate hydrolase),  $\beta$ -glucuronidase (in mucosa), 5'-nucleotidase (5'-AMP hydrolysis minus 3'-AMP hydrolysis) and protein were done as before (3) as were non-specific reducing activity ('INT reductase'),  $\beta$ -glucosidase and  $\beta$ -glucuronidase (in feces) (4).

#### *Statistical analysis*

Statistical significance of differences between diet groups was calculated with the use of the two-tailed Student's *t*-test; P-values less than 0.05 were considered significant.

## RESULTS

#### *Diet and the level of colon enzymes and reducing activity*

In experiment I, Swiss mice were fed the control diet or diets F6 or F23 for approximately 6.5 months. Samples of colon mucosa and of feces were analyzed for various activities (Table 2).

No clear diet-related effects were seen for ouabain-insensitive ATPase or 5'-nucleotidase in samples of resuspended pellets prepared from colon mucosal homogenate. The following observations were made using colon mucosal homogenate. Esterase in female mice was unaffected by diet but in males, diets F6 and F23 resulted in an activity significantly higher than in male mice fed the control diet. No significant effects due to diet were seen for  $\beta$ -glucuronidase or PDI.

Table 2. Effect of diet on enzyme levels and reducing activity in colon mucosa and feces.

Diet	Sex	No. of mice <sup>a</sup>	Colon Mucosal <sup>bc</sup>					Fecal <sup>b</sup>		
			ATPase	5'-Nucleotidase	Esterase	$\beta$ -Glucuronidase	PDI	Reducing activity <sup>d,e</sup>	$\beta$ -Glucuronidase <sup>e,f</sup>	$\beta$ -Glucosidase <sup>e,f</sup>
Control	F	15	10.2 ± 2.3	0.465 ± 0.169	36.4 ± 7.4	1.078 ± 0.482	1.17 ± 0.21	12.02 ± 2.56 <sup>g</sup>	5.35 ± 4.59 <sup>hi</sup>	18.48 ± 13.96 <sup>gh</sup>
F6	F	16	10.3 ± 1.6	0.405 ± 0.094 <sup>i</sup>	40.7 ± 10.3	1.132 ± 0.378	1.16 ± 0.17	10.02 ± 1.77 <sup>e</sup>	1.68 ± 1.33 <sup>h</sup>	7.11 ± 3.62 <sup>h</sup>
F23	F	17	10.8 ± 1.7	0.501 ± 0.161 <sup>j</sup>	35.6 ± 8.5	0.985 ± 0.316	1.14 ± 0.17	10.80 ± 3.30	2.44 ± 2.45 <sup>i</sup>	9.43 ± 4.89 <sup>e</sup>
Control	M	11	13.5 ± 2.9	0.498 ± 0.174	32.1 ± 7.4 <sup>gk</sup>	0.643 ± 0.087	1.02 ± 0.25	8.83 ± 4.45	2.96 ± 1.51	11.81 ± 6.53
F6	M	15	13.1 ± 1.7	0.480 ± 0.192	39.3 ± 6.9 <sup>gi</sup>	0.671 ± 0.156	1.12 ± 0.11	7.69 ± 1.85	2.77 ± 1.80	16.47 ± 17.73
F23	M	17	12.3 ± 1.3	0.420 ± 0.091	44.5 ± 6.6 <sup>ik</sup>	0.732 ± 0.151	1.15 ± 0.12	9.51 ± 3.34	3.23 ± 1.67	15.08 ± 11.98

<sup>a</sup>Assays of mucosal  $\beta$ -glucuronidase on female mice used 10(control diet), 12(diet F6) or 14(diet F23) individuals respectively; <sup>b</sup>Values are mean ± s.d. Values with the same suffix are significantly different. Only groups of the same sex were compared; <sup>c</sup>Units are  $\mu$ mol/h/mg protein; <sup>d</sup>Units are nmol/13 min/mg feces; <sup>e</sup>Quantity of feces refers to original feces from which the sample was extracted; <sup>f</sup>Units are nmol/h/mg feces; <sup>g</sup> $P < 0.02$ ; <sup>h</sup> $P < 0.01$ ; <sup>i</sup> $P < 0.05$ ; <sup>j</sup> $P = 0.05$ ; <sup>k</sup> $P < 0.001$ .

Fecal supernatant samples were analyzed for reducing activity,  $\beta$ -glucuronidase and  $\beta$ -glucosidase. With all three, no significant differences were seen in male mice but in females fed the control diet there was a significantly higher activity than with diet F6. In addition, with  $\beta$ -glucuronidase and  $\beta$ -glucosidase, a significantly higher activity was present in the control diet samples than in those from mice fed diet F23.

#### *Diet and fecal pH*

*Experiment I.* This study utilized approximately 15 female and six male Swiss mice in each diet group. Results in each sex were similar and the data were therefore pooled. Mice fed the control diet had a significantly lower fecal pH ( $7.22 \pm 0.42$ , mean  $\pm$  s.d.) than those fed diet F6 ( $7.64 \pm 0.37$ ;  $P < 0.01$ ) or diet F23 ( $7.54 \pm 0.50$ ;  $P < 0.05$ ).

*Experiment II.* A total of 99 male and 78 female Swiss mice were studied. There was no apparent difference in fecal pH between samples from mice fed diets F6, F16 and F23 but with mice fed the control diet, the pH was lower by an average of 0.23 pH units (data not shown).

*Experiment III.* In this study, 43 male and 40 female C57BL/1 mice were fed the specified diets for 4 or 12.5 months. No difference in fecal pH was seen between mice fed diets F6 and F23 (data not shown). For comparison, 25 adult C57BL/1 mice were studied which had been fed only the control diet. Their fecal pH was lower than those fed diets F6 and F23 by approximately 0.34 pH units.

#### *Diet and fecal sterols*

The fecal samples prepared in experiments II and III were analyzed for bile acids and neutral sterols. Despite the fact that samples in each diet-sex-age subgroup were pooled before analysis, the results show a high degree of random error between groups and this makes valid conclusions of diet effects impossible.

#### *Body weight*

In experiment I, male mice fed the control diet weighed about 12 percent more than the others (46.2 vs 41.4 g;  $P < 0.01$  for diet F6 and  $P < 0.001$  for diet F23). In females, those fed diet F23 weighed about 18% more than the others (51.2 vs 43.4 g;  $P < 0.01$ ). In experiment II, the high fat diets induced obesity in comparison with mice fed the control diet. Those fed diet F6 were of intermediate weight. In experiment III, diet F23 caused a slight excess weight gain after 4 months and a clear excess (about 23 percent) after 12.5 months in comparison with those fed diet F6 or the control diet.

## DISCUSSION

With both Swiss and C57BL/1 mice, the fecal pH after feeding diet F6 was the same as that after feeding diet F23. Since the two diets differ only in their level of fat and carbohydrate (diets F6 and F23 contain 5.5 and 23 percent fat respectively), we conclude that dietary fat has a negligible effect on fecal pH.

The data also show that in Swiss mice the control diet causes a lower fecal pH by about 0.2–0.4 pH units in comparison with mice fed diets F6, F16 and F23. This also appears to be true for diets F6 and F23 in C57BL/1 mice. Diets F6, F16 and F23 were prepared by simple addition of fat and starch to the control diet.

This causes a reduction in the level of vitamins, minerals, protein and dietary fiber per 100 calories to 75 percent (diet F16) or to 61 percent (diets F6 and F23) of that in the control diet. Supplementation of the diet with fiber has been shown to reduce fecal pH (15–19) and our results are also probably a reflection of this. The observation is potentially important since a lower pH may enhance colon carcinogenesis.

In our previous study the effect of a high fat diet was studied by comparing mice fed diets F16 and F23 with those fed the control diet (3). The results in this experiment permit differentiation between the effects of dietary fat per se (diet F23 vs diet F6) and that of nutrient and fiber depletion (diet F6 vs the control diet). It should be noted that in the studies of the levels of enzyme activities and of reducing activity, 48 group comparisons were made and a few apparently significant differences are therefore likely to arise by chance.

Previously, the level of mucosal ouabain-insensitive ATPase and non-specific esterase showed possible increases after feeding a high fat diet. On this occasion, however, ATPase showed no response to diet but the level of esterase (1-naphthyl acetate hydrolase) rose significantly with diets F6 and F23 as compared to the control diet (in males only). This increase in esterase with nutrient depleted diets warrants further investigation.

Combining the generally negative results on 5'-nucleotidase with the unresponsiveness of PDI to diet provides no evidence for an altered function of the colon mucosal plasma membrane, the site at which both enzymes are localized (10,11). It is possible, though, that other indices of membrane function, such as permeability and lipid composition, might be altered.

Mucosal  $\beta$ -glucuronidase, as in the previous work, was apparently unaltered by diet. The level of fecal enzyme activity, however, was changed by diet and parallels fecal  $\beta$ -glucosidase. In female mice, diet F23 caused a significant lowering of both enzyme activities compared to that with the control diet while diet F6 caused an even more significant reduction. No particular pattern was observed with male mice. The level of  $\beta$ -glucosidase may reflect changes in the fecal concentrations of chemicals with  $\beta$ -glucosidic bonds. In rats a diet rich in lean beef depresses fecal activity by at least 80 percent, compared with a grain-based diet (7), while cecal activity is halved by a fat-rich diet (20). On the other hand,  $\beta$ -glucuronidase is increased in feces and cecal contents by dietary fat (7,20). Lean beef (7) and a mixed Western diet (8) also induce the fecal activity in rats.

Non-specific reducing activity was little affected by diet apart from a significant but small depression in female mice fed diet F6 as compared with the control diet.

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