HIGH FAT DIETS AND MOUSE COLON MUCOSAL MEMBRANES: A CENTRIFUGATION STUDY

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SUMMARY

Female Swiss mice were fed on a control diet (laboratory chow, 6% fat) or high fat diets (laboratory chow with added fat, principally corn oil, to produce a fat content of 16% or 23%). Homogenate of colon mucosa was fractionated by isopycnic banding on a linear sucrose gradient and the distribution of 5'-nucleotidase determined. Two main peaks were detected which we interpret as belonging to basolateral plasma membrane (BLPM) and brush borders. A high fat diet did not affect the relative sizes of the peaks or the median density of the BLPM peak but did significantly reduce the density of the brush border peak from 1.208 g/ml to 1.197 g/ml.

INTRODUCTION

Data from epidemiology points to a role for a high fat diet in colon cancer [1]. Evidence from animal experimentation has been generally consistent with this view [18] although a report published recently failed to observe any effect on colon carcinogenesis in rats induced by gavage with dimethylhydrazine [14].

If a high fat diet does indeed enhance colon cancer, a possible mechanism by which this could be mediated is via alterations in the BLPM and brush border of the target cells in the colon (i.e. the mucosa). Such alterations might raise membrane permeability so increasing the vulnerability of the cell to fecal carcinogens. Alternatively, there might be changes in receptors or the level of membrane based enzymes.

A previous report from this laboratory described the rise in the cholesterol and phospholipid content of mouse liver after feeding a high fat diet [10]. This probably reflects changes in membrane composition. Others have reported that in rats, manipulation of dietary fat induces changes in the lipid

composition of heart sarcolemma [2] and in the phospholipid fatty acids of intracellular membranes (liver microsomes and mitochondria and heart mitochondria) [21]. It seems quite likely that colon plasma membranes will respond in a similar fashion.

The lipid content of membranes exerts a strong influence on membrane function. This has been demonstrated with respect to both the permeability characteristics of liposomes [6,9,19] and to various physical properties of plasma membranes from rat small intestinal enterocytes [4,5].

In this investigation we have taken a novel approach to the question of how dietary fat alters membrane characteristics. Homogenate of mouse colon mucosa has been subjected to isopycnic banding by centrifugation on a sucrose density gradient and alterations in membrane equilibrium density determined.

MATERIALS AND METHODS

Diets

The following diets were used: (1) Control diet. This was rodent laboratory chow (Ralston Purina Co., St. Louis, MO) (6% fat). (2) Diet F16. This contained chow (89%) plus fat (11%) to give a 16% fat content on a dry weight basis. The fat consisted of 98.5% corn oil ('Mazola'. Best Foods, San Juan, Puerto Rico) and 1.5% oleic acid (Fisher Scientific Co., Fair Lawn, NJ). The diet was prepared approximately once per week by mixing the ingredients with water and was stored at 4°C. (3) Diet F23. This was the same as diet F16 except that the dry weight fat content was 23%. It contained 81% chow and 19% fat.

Mice

Female Swiss mice were used from a colony maintained in our animal house. Experimental details concerning the mice are indicated in Table 1.

Fractionation procedure

The colon and rectum of the mouse were removed and cut open longitudinally. The tissue was washed under a cold tap and rinsed in homogenization medium (20 mM Tris—HCl (pH 7.4) containing 10% w/v sucrose). It was placed on a glass plate on ice, lumen side up, and the mucosal layer scraped off with microscope slides. The mucosa was homogenized with 2.9 ml of the above medium in a glass-teflon Potter-Elvejhem homogenizer.

The homogenate, except a sample which was kept for later analysis, was fractionated by isopycnic banding. Immediately after preparation, 2 ml of homogenate was loaded on an 8.5 ml linear sucrose gradient (14—49% w/w) resting on a 0.6 ml cushion (52% w/w). Centrifugation was carried out for 80 min at 40,000 rev./min and at 4°C in a SW41 rotor (g_{av}- min = 15.7×10^6). The gradient was then unloaded using an Isco Density Gradient Fractionator by upward displacement with 54% w/w sucrose. About 12 fractions were collected.

TABLE 1

EFFECT OF DIET ON 5'-NUCLEOTIDASE DISTRIBUTION

Group	Diet period 1[a]	Diet period 2[b]	Size brush border peak[c,d,e]	Median density BLPM peak[d,e]	Median density brush border peak[d,f]	No. of mice
I	Control	Control	29.7 ± 12.1	1.114 ± 0.015	1.208 ± 0.006	6
II	F16	F16	23.2 ± 0.6	1.115 ± 0.012	1.192 ± 0.007	4
III	F16	F23	32.8 ± 9.3	1.110 ± 0.011	1.199 ± 0.010	6
II, III	F16	F16, F23	29.0 ± 8.5	1.112 ± 0.011	1.197 ± 0.009	10

[a]Diet consumed by mouse for 7.5 months after weaning. The same diet had been fed to the parents of the mice since weaning.
[b]Diet consumed for 6.5 months before killing commencing at end of diet period 1. Control, F16 and F23 diets contain 6%, 16% and 23% fat, respectively (see Materials and Methods).
[c]Area of brush border peak as a percentage of total area covered by BLPM and brush border peaks.
[d]Mean ± S.D.
[e]Comparison of group I vs. II and III shows no significant difference.
[f]Results significantly different ($P < 0.01$ for group I vs. II; $P < 0.02$ for group I vs. II, III pooled).

The refractive index of each fraction was measured with a refractometer (Bausch & Lomb). From this and standard tables [11] the density was estimated. The volume of each fraction was calculated from the weight. Fractions and a sample of homogenate were equilibrated to 20% w/w sucrose by addition of water or 60% w/w sucrose and were kept in an ice-bath. All samples were analyzed for 5'-nucleotidase. This is a well characterized marker for the plasma membrane [7]. Analyses were completed within about 12 h of homogenate preparation.

5'-Nucleotidase

The assay procedure was as described by Murer et al. [17]. The incubation volume of 0.5 ml contained 4 mM $MgCl_2$, 4 mM 5'- or 3'-AMP, 50 mM Tris—HCl (pH 7.4) and 0.25 ml of sample. After incubation at 37°C for 1 h the reaction was ended by addition of 0.5 ml of 20% w/v TCA. P_i was then determined [3]. 5'-Nucleotidase corresponds to 5'-AMP hydrolysis minus 3'-AMP hydrolysis.

Statistical analyses

Statistical significance of differences between diet groups were calculated using the 2-tailed Student's t-test; P values <0.05 were considered significant. The data from the 2 groups of mice fed a high fat diet were analyzed both with and without pooling them.

RESULTS

Data were obtained from 16 mice. Six were fed a control diet (group I). The other 10 received a high fat diet for the 14 months after weaning. (Their parents also received such a diet) (groups II and III; see Table 1). In each case the distribution of 5'-nucleotidase after isopycnic banding on a sucrose density gradient was determined.

The enzyme distribution was consistently trimodal. A typical result is shown in Fig. 1. At the top of the gradient (the high speed supernatant fraction) a peak of negative activity was detected. This indicates the presence of an enzyme activity which hydrolyzes 3'-AMP at a faster rate than 5'-AMP.

Peaks were also observed in the center and in the dense region of the gradient. We believe the former is localized on BLPM and the latter on brush borders (see Discussion). The peaks had median densities of 1.096—1.126 g/ml (BLPM peak) and 1.188—1.215 g/ml (brush border peak).

The results from the different diet groups were compared in several ways. A high fat diet did not significantly alter either the relative sizes of the BLPM and brush border peaks or their shapes or affect the median density of the BLPM peak. However, the median density of the brush border peak was reduced by a high fat diet (compare group I vs. II and III; Table 1).
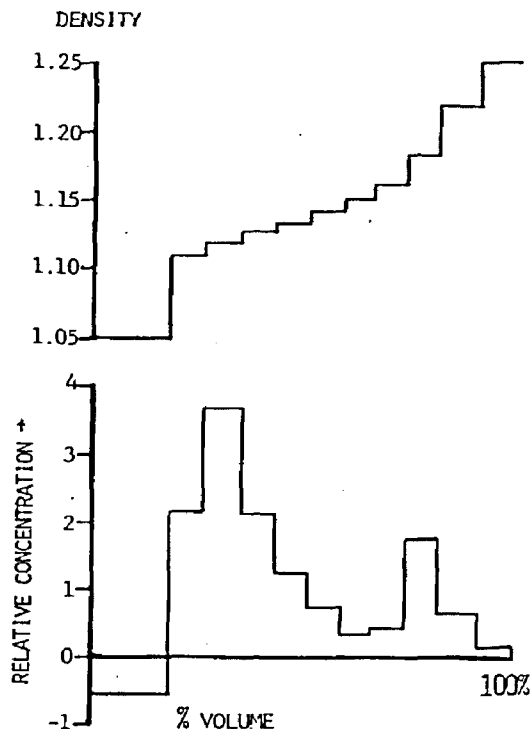

Fig. 1. Distribution of 5'-nucleotidase after isopycnic banding of mouse colon mucosal homogenate. The results are presented as described by De Duve [8].

The recovery of enzyme activity was between 210% and 674% for every gradient except one (i.e. several times more activity was detected in the fractions than was present in the original homogenate). Possibly an inhibitor of 5'-nucleotidase is present in homogenate but is separated from the enzyme by fractionation. In the single case where a low recovery was observed (76%) the mouse had a tumor in the reproductive tract and an unusually small amount of feces in its colon and rectum.

DISCUSSION

The experimental strategy with mice fed high fat diets (diets F16 and F23) resembled, in several respects, the situation in Western countries where colon cancer is common [1]. The diets were fed for 14 months after weaning. This corresponds to roughly 90% of the mean life expectancy of the mice used here [2]. The parents of the mice fed a high fat diet also received such a diet [3]. The high fat diets were prepared by simple addition of fat to chow rather than by isocaloric substitution. In Western society, also, the high fat content of the diet is generally at the expense of nutrients and fiber. For the same number of calories diets F16 and F23 contain approximately 23% and 36%, respectively, less vitamins, minerals, protein and fiber than does the control diet.

The quantity of feces in the colon and rectum of mice fed diets F16 and F23 is about half of that found in mice fed the control diet. Thus the deficit in feces is greater than the deficit in fiber. This might reflect a mild laxative action of polyunsaturated fat [20], the major lipid type in corn oil.

Isopycnic banding caused the particles carrying 5'-nucleotidase to be resolved into 2 peaks with median densities of 1.096—1.126 and 1.188—1.215 g/ml, respectively. Our identification of the former as BLPM and the latter as brush border is based largely on fractionation studies of 5'-nucleotidase, Na^+/K^+-ATPase and K^+-p-nitrophenyl phosphatase in the mucosa of the human rectum [7] and guinea-pig small intestine [13,15,16]. It should be noted, however, that the median densities reported in the present work are all appreciably lower than those reported in the literature cited above.

Our findings are consistent with those of Jackson et al. [12] on rat colon mucosa. They prepared a 'microsomal' sample (i.e. a sample rich in BLPM but containing few brush borders) by differential pelleting. It was then subfractionated by isopycnic banding. Consistent with our findings 5'-AMPase had a median density of about 1.12 g/ml. Unfortunately, no analyses were done for 3'-AMPase. Analyses were carried out for Na^+/K^+-ATPase and K^+-p-nitrophenyl phosphatase and these enzymes had a median density of roughly 1.15 g/ml (i.e. higher than that of 5'-AMPase but still lower than the values in other tissues). Detailed fractionation experiments with a range of marker enzymes are clearly needed to clarify our suppositions.

The results presented here indicate that the density of the colon mucosa brush border falls if the mice are fed a high fat diet. This conclusion is, of

course, tentative. Fractionation procedures can often generate misleading results. This is illustrated here both by the extremely high recoveries for 5'-nucleotidase (210—674%) and by the apparent negative activity in the fraction containing high speed supernatant. The latter observation demonstrates that the assay is insufficiently specific.

The most plausible factors by which our observations are explained are the low fecal bulk, the presence of dietary fat in the feces and secondary changes in fecal composition, notably bile acid levels [18]. Quite possibly there is an interaction so that, for instance, a raised fecal bile acid output and a low fecal bulk together cause a high concentration of bile acids which, in turn, then affects the mucosa.

We cannot discount the possibility that alterations in the brush border may be systemically mediated. In this respect it is relevant that diets F16 and F23 induce spontaneous obesity in the strain of mice used here (unpublished observations).

How relevant our observations are to colon carcinogenesis can, at present, only be speculated on. It is conceivable that the alteration in the brush borders makes the mucosal cell more susceptible to the effects of fecal initiators and promoters of carcinogenesis. This could come about by way of a raised lipid content in the membrane thereby facilitating the diffusion of non-polar chemicals into the mucosa. It is also possible that changes in membrane density may be unimportant in themselves but are associated with altered membrane function (e.g. cell surface enzymes).

In another study Swiss mice were fed the control diet or diets F16 or F23. A sample rich in BLPM and brush borders was prepared from the colon mucosa of each mouse and analyzed for 5'-nucleotidase. A raised level of the enzyme was induced by the high fat diets (Temple and El-Khatib, unpublished data). These various dietary effects on the colon mucosa membrane should be investigated further.

ACKNOWLEDGEMENTS

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