An association between maternal diet and colonic diverticulosis in an animal model

L Wess, M Eastwood, A Busuttil, C Edwards, A Miller

Abstract

Background/Aims—Maternal diet may have an effect on the health of the offspring in middle and later life. This study used the laboratory rat as an animal model to examine whether the fibre content of the maternal diet during pregnancy affected subsequent development of colonic diverticula in the offspring fed lifelong fibre deficient or higher fibre diets.

Methods—The parents of experimental animals were fed either a diet that was known to predispose to colonic diverticulosis or a control diet for one month prior to mating. The offspring were fed one of these diets for 18 months. The incidence of colonic diverticulosis, submucosal collagen content, collagen solubility in weak acid, and the composition of intestinal contents were then measured.

Results—Offspring of rats fed a higher fibre diet from higher fibre diet fed parents had 0% incidence of colonic diverticulosis. When offspring (regardless of parental diet) were fed a low fibre diet for life the acid solubility was lowered compared with rats fed lifelong higher fibre diet mean (SD) (0.044 (0.0007) v 0.073 (0.0015) sigmoid colon (ratio of soluble:insoluble collagen)); 21.1% had diverticulosis and there was reduced fibre fermentation. However when the diet of the parents of the fibre deficient diet fed rats was considered, the animals whose mothers had a fibre deficient diet had lower acid solubility (0.032 (0.0007)) and an increased incidence of colonic diverticulosis (42.1%) than the animals fed a fibre deficient diet from higher fibre diet fed parents ($p < 0.01$ in all instances).

Conclusion—Maternal diet and the subsequent nutrition of the progeny seem to be of importance in the development of colonic diverticulosis in the rat.

(Gut 1996; 39: 423-427)

Keywords: maternal diet, colonic diverticulosis, collagen, crosslinking, short chain fatty acid.

Several studies have now established that maternal diet plays a major part in the development of chronic disease in the adult offspring.¹⁻³ All studies in this area have concentrated on respiratory diseases and heart disease. The effect of maternal diet on the gastrointestinal tract of the offspring has not been considered before.

Diverticulosis is a very common disease in Western humans. Colonic mucosal herniations

occur through the muscular wall probably in response to a fibre deficient diet, which causes small inspissated stools that require high pressures to move and evacuate.⁴ Part of the pathogenesis is a progressive deterioration in the strength of the colonic wall in aged subjects.⁵ In a previous study we have measured an increase in colonic wall collagen crosslinkage in aged human subjects.⁶

A high fibre diet has been shown to decrease the incidence of diverticula in a rat model⁷ and we have recently shown that a similar diet increased acid solubility of colonic collagen in the rat particularly in the distal colon.⁸ We have previously shown that weaning rats onto particular dietary fibres affects the way the colonic flora ferments each fibre and hence their effect on stool output in adult life.⁹ This suggested that events in early life may have longterm effects on colonic function.

Collagen is known to have inter and intramolecular crosslinks that stabilise and give strength to the tissue in which it is located. Two pathways of crosslinking have been identified in collagen, one based on lysine aldehydes and the other on hydroxylysine aldehydes. Lysine and hydroxylysine are deaminated to produce allysine and hydroxyallysine respectively. The aldehyde is then free to react spontaneously in a variety of ways. The reaction of either type of aldehyde with the ϵ -amino group of lysine or hydroxylysine results in the production of reducible intermolecular crosslinks. Initially all possible reactions produce a Schiff base type crosslink also known as an aldimine type linkage. These intermediate forms are susceptible to cleavage by dilute acid. Hydroxyallysine derived intermediate crosslinks can also undergo a further spontaneous reaction to form a ketoimine type structure in vivo. This is known as an Amadori rearrangement. The ability for the intermediate crosslink to form the acid stable ketoimine, depends on whether it was derived from allysine and hydroxylysine, or hydroxyallysine and lysine. The first of these is unable to form the ketoimine, the second is able to do this. The ketoimine type crosslink is stable to weak acids. The amount of acid labile crosslinks decreases with the maturation of the tissue.¹⁰ The initial aldehyde derived crosslinks undergo maturation to produce more stable forms, which are stable in weak acids. The mature products of crosslink formation are a complex area of study. By stabilising the molecular arrangement within collagen fibrils, intermolecular crosslinks confer stability to the collagen containing tissue.¹⁰⁻¹¹

Collagen solubility in weak acids is indirectly related to the amount of acid labile crosslink

Department of
Biological and
Molecular Sciences,
University of Stirling,
Stirling
L Wess
A Miller

Gastrointestinal Unit,
Western General
Hospital, Edinburgh
M Eastwood

Forensic Medicine
Unit, University of
Edinburgh Medical
School, Edinburgh
A Busuttil

Department of Human
Nutrition, University
of Glasgow, Yorkhill
Hospitals, Glasgow
C Edwards

Correspondence to:
Dr L Wess,
Department of Biological
and Molecular Sciences,
University of Stirling,
Stirling, FK9 4LA.

Accepted for publication
27 February 1996

forms in the collagen of the tissue under study. In this study acid solubility is measured as a ratio of acid soluble collagen to acid insoluble collagen. A lower solubility index indicates a higher degree of acid stable crosslink forms. We have previously carried out a study on the solubility of colonic collagen from the human large bowel in relation to aging and colonic diverticulosis.⁶ The solubility of collagen in weak acids is known to be decreased with advancing age in certain tissues – that is, skin, vascular adventitia, and chordae tendinae of heart valves,¹¹ and this is one of the bases for this study. Acid solubility gives an estimate of the strength of the collagen and hence the tissue in which it is located.

We therefore decided to investigate the influence of maternal diet on the extent of collagen crosslinkage and incidence of diverticula in elderly rats alongside our study of the effects of lifelong higher fibre on rats.

Methods

Animals

Sixty male Wistar rats bred at the Western General Hospital Animal Unit were maintained in cages, on refined cat litter to avoid ingestion of the bedding. They were housed in groups of five and maintained on one of two diets for 18 months. There were 20 rats in each dietary group fed fibre deficient or higher fibre diets⁸ similar to those used by Fisher,⁷ (Special Diet Services, Lavender Mill, Poole, Dorset, prepared from one batch).

There were three groups comprising: (a) low fibre diet fed young, bred from low fibre diet fed parents (familial low fibre group (FLF)). (b) Low fibre diet fed young, bred from high fibre diet fed parents (weaned low fibre group (WLF)). (c) Control group, high fibre diet fed young, bred from high fibre diet fed parents (familial high fibre group (FHF)).

Non-starch polysaccharides contained in the diet were measured by the Englyst method.¹² The composition of the diets has already been outlined in our previous publication.⁸

The rats were bred specifically for this study and the parents of experimental animals were also fed the appropriate diet for a period of one month prior to mating. Each group of rats were weaned onto one of the two diets after 17 days suckling and remained on that diet thereafter.

All rats were weighed and monitored at weekly intervals and the terminal body weight was measured. The health of the animals at the end of the experiment was assessed at post-mortem examination and weekly examinations of their fur, teeth, gait, mobility, and general activity were carried out for the duration of the study.

After 18 months the rats were killed by overdose of ether. Immediately after death a postmortem examination was carried out by a consultant pathologists (AB). For details of tissue collection and histological assessment consult our previous publication.⁸ Colonic wall collagen content and the acid solubility index were measured as previously⁶⁻⁸ using the

Kivirikko method.¹³ Short chain fatty acids were analysed by gas liquid chromatography.¹⁴

Statistics

The effect of colonic diverticulosis, rat body weight, and the presence of tumours and any other abnormalities on the total collagen content and the acid solubility of collagen was determined by multiple regression analysis and analysis of covariance.

Results

All animals remained healthy throughout the experiment, with the exception that three of the FLF group rats and one of the WLF group rats had to be removed from the study because of middle ear disease. This is a common disease in rodents and is not known to be related to the diet fed. The animals from the two fibre deficient diet groups were significantly heavier than those in the higher fibre diet group at the end of 18 months mean (SD) (FLF 912 (56) g, WLF 740 (45) g, and FHF 590 (30) g ($p > 0.001$)).

Diverticula and other abnormalities

At death the percentage of animals with colonic diverticulosis in the FHF group was 0% (of 20 animals), in the WLF it was 21.1% ($p < 0.001$), and in the FLF it was 42.1% ($p < 0.001$). All of the diverticula were found in the mid and left side of the colon (as in humans).⁴ Histological examination showed no colonic muscle thickening or tinctorial changes in the collagen of the lamina propria or submucosa. No diffuse inflammatory changes of the acute type were seen in the bowel. Some of the diverticula showed chronic inflammatory cells at some part of their structure; inflammation was not a prominent feature.

The number of animals with tumours and abnormalities was different between the three groups. The FHF rats had a total of nine tissue abnormalities compared with 34 tissue abnormalities in the WLF group and 48 tissue abnormalities in the FLF group. In the FHF group there were eight testicular tumours (of interstitial cell origin) and one mesenteric fibrous nodule (a fibroma). In the WLF group all rats had a small caecum, two rats had nodular and reticulated lungs (due to histologically confirmed desquamative pneumonitis), five rats had a pale or nodular liver (caused by fatty infiltration and excessive portal fibrosis), three rats had a pale or nodular pancreas (because of interlobular fibrosis), and no rats had testicular tumours. In the FLF group all rats had a small caecum, six rats had nodular and reticulated lungs, 14 rats had a pale or nodular liver, eight rats had a pale or nodular pancreas, and 12 rats had testicular tumours of similar type. These results illustrate that tissue abnormalities are more prevalent in the fibre deficient diet groups than in the higher fibre group and that the FLF group rats had a higher number of abnormalities than the WLF group, which were fed identical diets.

TABLE I *Caecal and colonic tissue weights and wet and dry weight of contents. The caecum and colon were carefully dissected and divested of any fat and then rinsed in isotonic saline, blotted, and weighed. The caecal and colonic contents were collected, weighed wet, and then freeze dried and reweighed*

	FHF (n=20)	FLF (n=17)	WLF (n=19)	Pooled (SD)
Caecum				
Tissue weight (g)	1.45	1.17**	1.06***†	0.11
Contents:				
Wet weight (g)	5.93	3.49***	3.63***	1.03
Dry weight (g)	1.90	1.35**	1.97‡	0.54
% Dry	32.1	37.5*	54.5***†	6.3
Colon				
Tissue weight (g)	2.05	1.40***	1.31***	0.21
Colon length (cm)	21.4	18.6***	17.7***	1.9
Contents:				
Wet weight (g)	3.56	2.05***	1.43***	0.99
Dry weight (g)	1.56	1.15*	0.99**	0.55
% Dry	43.9	55.1***	69.9***†	8.1

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ FLF and WLF compared with FHF. † $p < 0.001$, ‡ $p < 0.01$, WLF compared with FLF. Results are compared by Student's *t* test after analysis of variance (using pooled SD).

FHF rats had heavier caecums, and larger and heavier colons, than the FLF and WLF rats. FHF rats had heavier wet and dry caecal contents and increased water content compared with FLF and WLF rats (Table I). WLF rats had lighter caecal and colonic tissue weights but greater dry weight of caecal contents than FLF rats.

Caecal and colonic short chain fatty acids

Ten animals from each group were used for analysis of caecal and colonic SCFA. They were selected as alternate animals in the numbered sequence for each group. Total SCFAs in both caecum and colon were higher in the FHF group compared with the two fibre deficient diet groups ($p < 0.001$, Table II). There were also the expected changes in the percentages of the individual SCFAs with higher butyrate and lower propionate than the fibre deficient fed rats. When the FLF and WLF rats were compared there were small but significant changes in caecal and colonic SCFAs (Table II). In the caecum the only significant difference was a lower proportion of acetic acid and a higher proportion of isovaleric acid in the WLF group. In the colon, however, there was a significantly higher concentration of SCFAs (per gram wet weight) and a higher percentage of propionic acid and lower percentage of butyric and isovaleric acid. This would seem to reflect more fermentation occurring in the colon of WLF rats.

Colonic collagen

Total collagen content (measured as mg/100 mg wet tissue weight) was similar in all sites and all feeding regimens, regardless of the presence or absence of colonic diverticulosis. The median value was 13.23 mg/100 mg (0.29) and the range was 12.95 mg/100 mg (0.30)–13.51 mg/100 mg (0.16).

Acid solubility of the FHF group was similar regardless of the site of the colon. Acid solubility (acid soluble:acid insoluble collagen) was lower in the sigmoid colon than the ascending

colon in the two low fibre groups (WLF and FLF) ($p < 0.001$ sigmoid colon compared with ascending colon) but the change was less in the WLF rats (Table III). The acid solubility was lower in those rats with colonic diverticulosis compared with their healthy counterparts ($p < 0.001$, for all four sites of the colon). The acid solubility was also lower in the FLF group compared with the WLF group in all of the sites examined and in the healthy and diverticulosis rats ($p < 0.001$) (Table III).

Statistical analysis

Multiple regression analysis showed that the total collagen content is not influenced by the presence of tumours or the weight of the animals in any of the three dietary groups. The same analysis shows that the acid solubility of collagen is not affected by the presence of tumours or the weight of the animal in any group and that the significant factor is the presence of colonic diverticulosis ($p < 0.001$) in all sections of the colon in the FLF and WLF groups).

Discussion

The role of maternal diet in the development of chronic disease in the child and later in the adult is becoming increasingly evident.¹ The mechanisms whereby diet during pregnancy can affect the health of the adult offspring are not clear. Although it is possible to monitor blood parameters, it is difficult to study the effects on tissue and organ structure and function in humans for ethical and practical reasons. One major problem being the lifelong development of many of the chronic diseases. In this study we therefore developed and used a rat model to study the effect of maternal diet on colonic wall structure, collagen solubility in weak acid, and bacterial fermentation of the adult offspring.

Colonic diverticulosis is a prevalent disease in humans and is believed to be linked to dietary fibre intake throughout life. The role of

TABLE II *Caecal and colonic short chain fatty acids (SCFAs). SCFAs were analysed by gas liquid chromatography¹⁴ and are shown as total SCFA dry weight and wet weight and are also shown individually as a percentage of the total*

	FHF (n=10)	FLF (n=10)	WLF (n=10)	Pooled (SD)
Caecal SCFAs				
μmol/g dry wt	296	60.2***	60.0***	42.2
μmol/g wet wt	96.4	22.7***	29.5***	15.3
μmol/caecum	597	88.4***	113.3***	101.7
%acetic	61.9	70.0**	57.6†	5.1
%propionic	13.0	16.1	18.0**	3.6
%butyric	20.3	5.9***	8.4***	3.0
%isovaleric	0.64	1.9***	3.3***†	0.8
Colonic SCFAs				
μmol/g dry wt	135.9	24.7***	32.3***	24.5
μmol/g wet wt	52.3	6.9***	22.1***‡	8.2
μmol/colon	168.0	19.9***	34.6***	39.4
%acetic	71.5	64.0*	76.1‡	7.8
%propionic	10.4	12.1*	15.5***‡	2.9
%butyric	14.6	6.3***	2.9***‡	3.1
%isovaleric	0.84	6.2***	1.7†	2.1

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ FLF and WLF compared with FHF. † $p < 0.001$, ‡ $p < 0.01$, WLF compared with FLF. Results are compared by Student's *t* test after analysis of variance (using pooled SD).

TABLE III Acid solubility of collagen. Acid solubility is an indirect measure of the degree of crosslinkage of collagen. It is calculated by measuring the collagen content of the phase that is soluble in weak acid and the phase that is insoluble and calculating these as a ratio to each other (soluble:insoluble). This ratio is shown for the ileum, caecum, and four resected sections of the colon for the healthy rats and the rats with colonic diverticulosis

	Ileum	Caecum	Ascending	Transverse	Descending	Sigmoid
Familial high fibre diet						
Healthy (n=20)	0.075 (0.0016)	0.075 (0.0013)	0.075 (0.0020)	0.073 (0.0015)	0.073 (0.0016)	0.073 (0.0015)
Familial low fibre diet						
Healthy (n=10)	0.065*** (0.0018)	0.058*¶ (0.0015)	0.047 (0.0007)	0.043* (0.001)	0.037*** (0.0006)	0.032*** (0.0007)
Diverticulosis (n=7)	0.054*¶ (0.0008)	0.051*± (0.0006)	0.039± (0.0012)	0.033*± (0.0003)	0.033*± (0.0003)	0.028***± (0.0002)
Weaned low fibre diet						
Healthy (n=15)	0.070* (0.002)	0.067* (0.002)	0.058 (0.001)	0.055 (0.0014)	0.052* (0.0008)	0.044*** (0.0016)
Diverticulosis (n=4)	0.056*± (0.0012)	0.051*± (0.0004)	0.048*± (0.0009)	0.043***± (0.001)	0.037***± (0.001)	0.033***± (0.0008)

***=p<0.001, **=p<0.01, *=p<0.05 compared with ascending region. †=p<0.001, ‡=p<0.01, diverticulosis compared with healthy colon. ||=p<0.001, ¶=p<0.01, ±=p<0.05 FLF and WLF compared with FHF group. The results were compared by Student's *t* test after analysis of variance.

maternal diet in this condition may not seem obvious but colonic development in utero and during the neonatal period, especially in relation to the establishment of a stable colonic bacterial microflora, may be critical.

Three groups of rats were set up to examine the effect of maternal diet on colonic diverticulosis, colonic collagen content, and solubility in weak acid, and on colonic and caecal contents. The animals were all of a similar birth weight, but the weight gain and final body weights were very different. The weight gain was in sequence FLF>WLF>FHF (p<0.001). This may have some bearing on the results as obesity may have a part to play in the development of colonic diverticulosis.¹⁵ However, there was no relation between body weight and the collagen acid solubility index.

The significant differences in the incidence of diverticula and the extent of collagen crosslinkage between the two low fibre diets was understandably less than the difference between either group and the rats fed a high fibre diet. There was however a difference between the two fibre deficient diet fed rats in terms of the collagen acid solubility and the incidence of colonic diverticulosis. The collagen acid solubility is lower in the FLF group compared with the WLF group (27% lower in the sigmoid colon of FLF than WLF) and the incidence of colonic diverticulosis is increased twofold in the FLF compared with WLF rats.

The acid solubility of the colonic wall collagen is lower in the proximal colon than the distal colon, which suggests a relation with either: (1) Fermentation of dietary fibre by the colonic microflora, which on a fibre deficient diet will occur mainly in the proximal colon whereas on a higher fibre diet fermentation may occur throughout the colon.¹⁶ (2) The mechanical work required to move small hard stools. The water in the colonic contents is absorbed mainly in the transverse colon so density of the stool is greatest in the distal colon where most work would be required for movement and evacuation.

The mechanism for protective action of maternal diet against the effects of a lifelong fibre deficient diet is unclear. However, the caecal and colonic SCFA were also affected by

maternal diet. Higher fibre maternal diet was associated with greater production of SCFAs, with a lower production of isovaleric acid in the colon of WLF indicating more fermentation at more distal sites than in the FLF rats. If bacterial fermentation is related to collagen structure this could explain some of the effect of maternal diet. We have previously shown that rats weaned on to a high fibre diet and then fed a low fibre diet as adults for four weeks had more SCFAs in the caecum than rats weaned on to the low fibre diet directly.⁹ This suggests that even on a low fibre diet there is potential for increasing SCFAs by manipulating the colonic microflora. If the physical and chemical environment of the colon promotes colonisation by a different population of bacteria during the neonatal and weaning period there may be differences in the fermentation capacity of the adult colonic microflora.

Maternal diet might affect the colonic environment by changing the physiology of the colon in terms of, for example, mucin production or buffering capacity either in utero or by differences in the composition of milk during suckling. There may be some substance or agent in the milk of higher fibre diet fed mothers, which affects the gut flora and collagen. It has been shown in studies of human babies that the constituents of formula milk can have substantial influences on the composition of faecal bacteria^{17, 18} and SCFAs.¹⁹

The effect of maternal diet on the physical properties of the colonic contents seems unlikely to be responsible for changes in collagen crosslinkage. Indeed in our study the WLF rats had a lower percentage water in their colonic contents, which would be predicted to increase the incidence of diverticula.

An alternative mechanism would be that antioxidants in the maternal diet protected the collagen in the fetus or neonate from free radical attack. However, free radical attack is a slow gradual assault on the collagen and it is difficult to envisage a longterm effect of antioxidants in the maternal diet.

In conclusion, we have shown a significant reduction in the incidence of diverticula in a rat model associated with a maternal diet that is high in dietary fibre. The mechanism of this action needs to be explored. These results suggest that maternal diet may have a significant role in the development of gastrointestinal disease.

- 1 Barker DJP, Osmond C. Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. *Lancet* 1986; i: 1077-81.
- 2 Barker DJP, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. *J Epidemiol Community Health* 1989; 43: 237-40.
- 3 Barker DJP, Martyn CN. The maternal and fetal origins of cardiovascular disease. *J Epidemiol Community Health* 1992; 45: 71-2.
- 4 Painter NS. *Diverticular disease of the colon*. London: William Heinemann Medical, 1975.
- 5 Watters DAK, Smith AN, Eastwood MA. Mechanical properties of the colon: comparison of the features of the African and European colon in vitro. *Gut* 1985; 26: 384-92.
- 6 Wess L, Eastwood MA, Wess TJ, Busuttill A, Miller A. Crosslinking of collagen is increased in colonic diverticulosis. *Gut* 1995; 37: 91-4.

- 7 Fisher N, Berry CS, Fern T, Gregory JH, Hardy J. Cereal dietary fibre consumption and diverticular disease: a lifespan study in rats. *Am J Clin Nutr* 1985; **42**: 788–804.
- 8 Wess L, Eastwood MA, Edwards C, Busuttill A, Miller A. Collagen alteration in an animal model of colonic diverticulosis. *Gut* 1996; **38**: 701–6.
- 9 Armstrong E, Eastwood MA, Edwards CA, Brydon WG. The effect of weaning diet on the subsequent colonic metabolism of dietary fibre in the adult rat. *Br J Nutr* 1992; **68**: 741–51.
- 10 Robins SP, Shimokomaki M, Bailey AJ. The chemistry of collagen crosslinks. *Biochem J* 1973; **131**: 771–80.
- 11 Monnier VM, Kohn RR, Cerami A. Accelerated age-related browning of human collagen in diabetes mellitus. *Proc Natl Acad Sci* 1984; **81**: 583–7.
- 12 Englyst H, Wiggins HS, Cummings JH. Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates. *Analyst* 1982; **107**: 307–18.
- 13 Kivirikko KI, Laitinen O, Prockop DJ. Modifications of a specific assay for hydroxyproline in urine. *Anal Biochem* 1967; **19**: 249–55.
- 14 Spiller GA, Chernoff MC, Hill RA, Gates JE, Nassar JJ, Shipley EA. Effect of purified cellulose, pectin and low residue diet on faecal volatile fatty acids, transit time and faecal weight in humans. *Am J Clin Nutr* 1980; **33**: 754–9.
- 15 Eastwood MA, Brydon WG, Baird JD, Elton RA, Helliwell S. Faecal weight and composition of serum lipids and diet amongst subjects aged 18–80 years not seeking health care. *Am J Clin Nutr* 1984; **46**: 628–34.
- 16 Edwards CA, Eastwood MA. Comparison of the effects of isphagula and wheat bran on rat caecal and colonic fermentation. *Gut* 1992; **33**: 1229–33.
- 17 Balmer SE, Scott PH, Wharton BA. Diet and faecal flora in the newborn: casein and whey proteins. *Arch Dis Child* 1989; **64**: 1678–84.
- 18 Balmer SE, Wharton BA. Diet and faecal flora in the newborn: iron. *Arch Dis Child* 1991; **66**: 1390–4.
- 19 Edwards CA, Parrett AM, Balmer SE, Wharton BA. Faecal short chain fatty acids in breast-fed and formula-fed babies. *Acta Paediatr* 1994; **83**: 459–62.