European Journal of Clinical Nutrition (2008) 62, 978–984
 0954-3007/08 $30.00
www.nature.com/ejcn

ORIGINAL ARTICLE

Dietary supplementation with β-glucan enriched oat bran increases faecal concentration of carboxylic acids in healthy subjects

U Nilsson, M Johansson[1], Å Nilsson[2], I Björck and M Nyman

Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden

Background/Objective: Carboxylic acids (CAs), especially butyric acid, have been suggested to counteract colonic diseases, such as ulcerative colitis and colon cancer. Colonic formation of CAs can be influenced by the diet, but the concentrations and pattern formed need to be evaluated for different food products in humans. To elucidate how the colonic concentration of CAs in healthy subjects is influenced by dietary supplementation with oat bran, and whether the concentration varies over time and during consecutive days.
Subjects/Methods: Twenty-five healthy subjects (age 24 ± 1.3) were recruited to the study. The subjects were given 40 g β-glucan enriched oat bran per day, corresponding to 20 g dietary fibre, in 4 slices of bread. CAs were analysed in faeces during three consecutive days after 0, 4, 8 and 12 weeks on this diet.
Results: The concentration of acetic, propionic, butyric, isobutyric and isovaleric acid was higher ($P<0.05$–0.001) after 8 weeks on the oat bran diet as compared with values at entry, whereas that of lactic acid was lower ($P<0.05$). After 12 weeks, the concentrations of acetic, propionic and isobutyric acid were still higher and that of lactic acid lower. The variation between individuals was considerable, whereas in the same individuals there was little variation.
Conclusions: Oat bran increased the faecal concentration of CAs after 8 weeks, indicating an increased concentration also in the distal colon. The concentration of all main acids increased, except for lactic acid, which decreased. Oat bran may therefore have a preventive potential adjunct to colonic diseases.
European Journal of Clinical Nutrition (2008) **62,** 978–984; doi:10.1038/sj.ejcn.1602816; published online 23 May 2007

Keywords: carboxylic acids; humans; β-glucans; oat bran; dietary fibre

Introduction

There is mounting evidence that carboxylic acids (CAs) formed by colonic fermentation of indigestible carbohydrates have positive health effects (Wong *et al.*, 2006). In this context, butyric acid and, to some extent, propionic acid have mainly been emphasized. These acids are important energy sources for the colonocytes and may thus improve the condition of the colonic mucosa and, in consequence, decrease the risk of mucosal lesions. Especially butyric acid has been suggested to play a role in the prevention and treatment of colonic diseases, such as ulcerative colitis (Cummings, 1997) and colon cancer (Scheppach *et al.*, 1995), and to some extent Crohn's disease (Di Sabatino *et al.*, 2005). Current knowledge is summarized in recent reviews (Galvez *et al.*, 2005; Wong *et al.*, 2006).

Correspondence: Professor M Nyman, Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Center for Chemistry and Chemical Engineering, Lund University, SE-22100 Lund, Sweden.
E-mail: Margareta.Nyman@appliednutrition.lth.se
[1]Current address: AarhusKarlshamn Sweden AB, SE-374 82 Karlshamn, Sweden.
[2]Current address: Department of Clinical Sciences, Lund University, SE-221 85 Lund, Sweden.
Contributors: ÅN, IB and MN contributed to the conception and the design of the study and critically revising the paper. MJ contributed to the design of the study, the analyses and critically revising the paper. UN supervised all the analyses, did the interpretation of the data and was responsible for writing the paper.
Received 10 January 2007; revised 17 April 2007; accepted 18 April 2007; published online 23 May 2007

Different types of indigestible carbohydrates give rise to different amounts and patterns of CAs during colonic fermentation, and it may therefore be possible to regulate the CA formation by diet. The CAs formed may depend on for example the monomeric composition of the carbohydrates, the type of linkages between the carbohydrate monomers, their solubility and their molecular weight (Berggren *et al*., 1993; Casterline *et al*., 1997; Bird *et al*., 2000; Karppinen *et al*., 2000; Henningsson *et al*., 2002, 2003; Nilsson and Nyman, 2005). Studies examining CA formation have mainly been conducted *in vitro* by using human faecal inocula, or *in vivo* using animal models. The rat is the most common model when studying the CA formation *in vivo*, although there are also some studies available in pigs (Berggren *et al*., 1993; Roland *et al*., 1995; Brown *et al*., 1997; Djouzi and Andrieux, 1997; Bird *et al*., 2000; Henningsson *et al*., 2002, 2003; Nilsson and Nyman, 2005). Studies in man have mainly been performed by measurements of CAs in faeces and have been questioned on the premises that such measurements do not give information regarding the formation of CAs in proximal colon where most of the fermentation actually takes place. However, ulcerative colitis always affects the rectum with a variable extension in proximal direction, and colon cancer is most common in the distal colon. Faecal measurements of CAs can thus still be justified and can be expected to predict CA concentration at what appears to be a crucial colonic site. Interestingly, the concentrations of acetic, propionic and butyric acids in the distal part of colon of rats correlate with that in faeces, although the faecal concentrations were somewhat higher (Nilsson *et al*., 2006). Lactic acid, on the other hand, did not follow such a correlation. The use of a rat model is well motivated for mechanistic work and testing different experimental parameters that may influence CA formation, such as effects of molecular weight and processing conditions of β-glucans. However, quantitative faecal data in humans are fundamental, to select relevant food products intended for use in therapeutic or prophylactic trials and interventions in target groups.

The genesis of ulcerative colitis is unknown. Current hypotheses emphasize a gene environmental interaction causing a defect regulation of the normal inflammatory response to the normal colonic bacterial flora (Hanauer, 2004). Impaired oxidation of butyrate by the colonocytes has been suggested to be a contributing factor (Chapman *et al*., 1994). Although a decreased butyrate oxidation was observed in active ulcerative colitis (Den Hond *et al*., 1998), there was, however, no difference between controls and patients with quiescent colitis, speaking against a primary defect in colonic butyrate oxidation (Simpson *et al*., 2000). Some studies demonstrate a therapeutic effect of rectal butyrate and mixed CAs in distal colitis (Scheppach *et al*., 1992; Vernia *et al*., 2003). Although well-controlled clinical trials have not fully verified this finding (Scheppach, 1996; Steinhart *et al*., 1996), they suggest that butyrate may be effective in a subset of patients. Possibly, provision of exogenous butyrate may overcome the partial failure of butyrate oxidation in the diseased mucosa by mass action (Soergel *et al*., 1989). Furthermore, it has not been conclusively tested whether dietary changes that cause a defined increase in colonic CA formation decreases the risk of relapse in quiescent colitis.

Experimental studies *in vivo*, and in colonocytes, colon carcinoma cell lines and inflammatory cells indicate that butyrate may exert protective anti-inflammatory and anti-carcinogenic effects by several mechanisms (Smith *et al*., 1998; Luhrs *et al*., 2002; Menzel *et al*., 2004). Our own studies have shown that it was possible to increase the faecal butyrate level by giving patients with ulcerative colitis a diet supplemented with β-glucan enriched oat bran (Hallert *et al*., 2003). Unlike controls, subjects with symptoms showed no increase in gastrointestinal complaints during the trial. Subjects with ulcerative colitis have also an increased risk to develop colon cancer. However, studies in rats have shown that oat bran was less protective than wheat bran in experimental colon cancer models (McIntyre *et al*., 1993; Zoran *et al*., 1997; Reddy *et al*., 2000). An explanation to this could be that wheat bran is more slowly fermented than oat bran thus providing higher amounts of butyric acid in the distal colon. Another explanation could be that wheat bran is more resistant against fermentation, thus, binding the substance (dimethylhydrazine) that induces cancer (McIntyre *et al*., 1993).

The aim of the present study was to elucidate how the faecal concentrations of CAs vary in healthy individuals following dietary supplementation with β-glucans, and whether the concentration differs over time and during consecutive days. For this purpose 25 healthy subjects were recruited to the study. The subjects were given 40 g β-glucan-enriched oat bran as four slices of bread per day. CAs were analysed in faeces during 3 days at entry and after 4, 8 and 12 weeks on this diet.

Materials and methods

Experimental design

Twenty-five (10 men, 15 women) young healthy volunteers (aged 20–47 years, mean age 24.0 ± 1.3 years) participated in the study. The subjects were recruited by announcing in a local paper. All subjects fulfilled the inclusion criteria, thus, they were over 20 years old, had no known gastrointestinal or metabolic diseases, had not used antibiotics for the last 6 months and did not have any episodes of severe diarrhoea less than 6 months before the study. During the trial 40 g of a β-glucan-enriched oat bran, corresponding to 20 g fibre (10 g β-glucans) was added to the daily diet in the form of bread (four slices) without changing the normal diet to any greater extent. The subjects had regular contact with a dietician and every forth week they came to the department to receive a new package of deep-frozen bread and for reporting compliance. All subjects carried out two dietary registrations

Table 1 Dietary registrations in humans fed β-glucan-enriched oat bran bread

	At entry	*Week 8*	*Recommended*
Energy (kJ)	8800	8900	9200
Energy (kcal)	2100	2100	2200
Protein (g)	76	84	81
Fat (g)	73	73	74
Carbohydrates (g)	265	255	298
Dietary fibre (g)	23	39***	26
Vitamin C (mg)	143	106	60
Iron (mg)	14	18*	15
Calcium (mg)	1060	1060	800
Retinol eq (mg)	0.9	1.2*	0.80
Vitamin D (μg)	5	5	5
Vitamin E (mg)	9	8	8
Vitamin B_1 (mg)	1.5	1.5	1.1
Vitamin B_2 (mg)	2	2	1.3
Niacin eq (mg)	30	27	15
Vitamin B_6 (mg)	2	2	1.2
Vitamin B_{12} (μg)	5	5	2
Phosphorus (mg)	1400	1300	600
Magnesium (mg)	350	420*	280
Zink (mg)	11	13*	7
Folate (μg)	260	260	300
Selenium (μg)	34	38	40

Asterisks indicate significant difference from week 0: *$P<0.05$, ***$P<0.001$.

during 4 days, one before the study and one after 8 weeks. The intake of nutrients and energy is shown in Table 1. The dietary intake of most nutrients was similar at entry and after 8 weeks. As expected, the dietary fibre intake increased (from 23 to 39 g/day, $P<0.001$). Other differences include iron (14–18 mg/day) and retinol (0.9–1.2 mg).

Faecal samples ($\sim 2 \times 1$ g) from 3 consecutive days were collected at entry (time point 0), and then after 4, 8 and 12 weeks during the intervention period with oat bran. The faecal samples were frozen immediately, delivered to the Laboratory of Bacteriology at Lund University Hospital and stored at -20°C until analysed for the content of CAs.

Twenty subjects fulfilled the study. One subject dropped out because of gastrointestinal symptoms (diarrhoea), and three for personal reasons (lack of time). Owing to sampling error, the entry sample was lost from one subject. All subjects gave their informed consent and were aware that they could withdraw from the study at any time they desired. They were also informed that the high amount of dietary fibre could cause gastrointestinal problems, such as gases. The study was approved by the Ethics Committee for human studies at Lund University.

Oat bread

The oat bread was baked from 23.7 kg water, 10.8 kg oat bran (Swedish Oat Fiber, Väröbacka, Sweden), 5.6 kg white wheat flour (Nord Mills, Malmö, Sweden), 1.1 kg yeast, 1.8 kg gluten, 1.4 kg sugar, 0.3 kg E 472 and 0.3 kg salt. The dough was proofed in room temperature for 60 min and then divided into pieces and baked at 240°C for 40 min at a pilot bakery (Nordmills, Malmö, Sweden). After baking, the loaves were sliced (Skogaholm, Lund, Sweden), frozen and stored until use. Each slice of bread (~ 37 g bread) contained 5 g of oat fibre.

Analysis

β-Glucans. The content of β-glucans was quantified using an enzyme kit (Megazyme International Co., Wicklow, Ireland), based on the procedure developed by McCleary and Codd (1991) for mixed-linkage β-glucans, which has been approved by the AACC (method 32–23) and the AOAC (method 995.16).

Carboxylic acids. A gas-liquid chromatographic method was used to analyse the amount of short-chain fatty acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic and heptanoic acid). Other CAs quantified with this method were lactic acid and succinic acid (Richardson *et al*., 1989). The faecal samples collected were homogenized (using a Polytron, Kinematica, Switzerland) together with an internal standard (2-ethylbutyric acid, Sigma Chemical Company, St Louis, MO, USA). Hydrochloric acid was added to protonise the CAs, so that they could be extracted in diethylether. After being silylated with *N*-(tert-butyldimethylsilyl)-*N*-methyl trifluoroacetamide (MTBSTFA, Sigma Chemical Company), the samples were allowed to stand for 48 h to complete derivatization. Samples were then injected onto an HP-5 column (Hewlett Packard, GLC, HP 6890, Wilmington, DE, USA). Chem Station software (Hewlett Packard) was used for the analysis.

Statistical evaluation

Minitab statistical software (Release 13.32) was used for statistical evaluation of the results, and general linear model (analysis of variance (ANOVA)) followed by Dunnett's procedure, where the mean value of the 3 consecutive days from week 0 was compared with the corresponding values from the other weeks ($P<0.05$). All analyses were performed at least in duplicate and the maximum error of the analysis was $<5\%$. The coefficient of variation, that is the standard deviation divided with the mean value (%), was used to give a measure of the variation in faecal concentrations during the 3 consecutive days. Significant differences between these values were evaluated, using one-way ANOVA followed by Tukey's procedure.

Results

The concentration of CAs in faecal samples from the 20 out of 25 healthy volunteers that completed the study is shown in Table 2. No effects on CA concentration were seen after 4 weeks, except for a decreased formation of formic acid

Table 2 Concentrations (μmol/g wet content) of CAs in faeces of humans fed β-glucan-enriched oat bran bread[a,b]

Week	*0*			*4*			*8*			*12*		
	Mean±s.e.m.	*CV*	*Range*	*Mean±s.e.m.*	*CV*	*Range*	*Mean±s.e.m.*	*CV*	*Range*	*Mean±s.e.m.*	*CV*	*Range*
Formic	3.7±0.4	51	0.0–20.4	2.0±0.1***	33	0.2–5.3	3.0±0.2	29	1.2–6.2	3.4±0.2	35	0.0–9.9
Acetic	54.2±2.5	22	19.5–126.2	52.8±2.1	23	20.2–88.9	77.2±3.4***	19	22.9–125.6	67.0±3.3***	28	15.3–126.5
Propionic	11.6±0.5	23	4.5–22.5	12.5±0.7	24	4.4–28.5	15.0±0.9***	19	5.3–48.7	14.4±0.8**	24	5.8–30.8
Butyric	13.9±0.8	27	0.9–4.8	14.7±1.0	26	0.5–6.1	19.0±1.1***	27	0.2–7.1	16.0±1.0	34	0.3–7.0
Isobutyric	2.1±0.1	27	4.1–38	2.2±0.1	29	4.9–48.6	2.7±0.2***	28	5.4–37.1	2.5±0.2*	29	6–37.8
Isovaleric	1.5±0.1	35	0.2–4.1	1.5±0.1	28	0.3–4.7	1.9±0.2*	33	0.0–5.3	1.7±0.1	41	0.1–6
Valeric	2.2±0.2	33	0.3–14	1.8±0.1	27	0.1–3.7	2.2±0.2	24	0.1–5.5	2.2±0.1	31	0.2–5.6
Caproic	1.1±0.1	37	0.1–6.3	0.9±0.1	40	0.0–4.2	1.3±0.2	48	0.0–5.3	1.1±0.1	44	0.0–3.7
Heptanoic	0.2±0.0	58	0.0–0.7	0.2±0.0	65	0.0–0.7	0.2±0.0	41	0.0–1.0	0.4±0.1	73	0.0–5.7
Lactic	5.4±0.4	36	0.7–12.1	4.6±0.3	35	0.6–12.5	4.3±0.3*	28	0.3–11.4	3.5±0.2***	31	0.4–8.4
Succinic	1.9±0.2	46	0.1–6.8	2.0±0.6	47	0.2–33.3	1.8±0.2	39	0.1–7.2	1.8±0.4	43	0.1–21.9
Total CA	90.5±3.5	20	42.7–177.9	88.8±3.5	21	39.9–160.2	122.5±4.9***	18	40.4–198.4	108.7±5.1**	23	35.7–192.3

Abbreviations: CAs, carboxylic acids; CV, coefficient of variation.
Asterisks indicate significant difference from week 0: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
[a]Values are means±s.e.m., $n=20$.
[b]CV, that is standard deviation divided with the mean value during 3 consecutive days.

Table 3 Faecal proportion of CAs (%) in humans during dietary supplementation with β-glucan-enriched oat bran bread[a]

Week	*0*	*4*	*8*	*12*
	Mean±s.e.m.	*Mean±s.e.m.*	*Mean±s.e.m.*	*Mean±s.e.m.*
Formic	4±0	2±0***	2±0***	3±0*
Acetic	55±1	56±1	60±1***	58±1***
Propionic	12±0	13±0	12±0	12±0
Isobutyric	2±0	2±0	2±0	2±0
Butyric	14±1	15±1	15±1	14±0
Isovaleric	2±0	2±0	1±0	2±0
Valeric	2±0	2±0	2±0	2±0
Caproic	1±0	1±0	1±0	1±0
Heptanoic	0±0	0±0	0±0	0±0
Lactic	6±0	5±0	3±0***	4±0***
Succinic	2±0	2±1	2±0	2±0

Abbreviation: CAs, carboxylic acids.
Asterisks indicate significant difference from week 0: *$P<0.05$, ***$P<0.001$.
[a]Values are means±s.e.m., $n=20$.

(Table 2). However, after 8 weeks the mean concentration of acetic, propionic and butyric acid had increased ($P<0.001$). Thus, the mean concentration of acetic acid increased from 54.2 to 77.2 μmol/g, that of propionic acid from 11.6 to 15.0 μmol/g and that of butyric acid from 13.9 to 19.0 μmol/g, respectively. Further, in 19 of the 20 subjects that completed the study, the total faecal concentration of CAs was higher ($n=17$) or similar ($n=2$) compared with values at entry. Similar profiles were seen with acetic, propionic and butyric acid (Figure 1). The concentration of isobutyric ($P<0.001$) and isovaleric acid ($P<0.05$) was also higher after 8 weeks on the oat bran diet, whereas that of lactic acid was lower ($P<0.05$). After 12 weeks, the faecal concentrations were still higher and that of lactic acid lower, exceptions being butyric and isovaleric acid.

The interindividual range of faecal concentrations of CAs was considerable (Figure 1). However, at entry data regarding the concentrations of acetic, propionic and butyric acid were similar and there were only a few deviating values (two to three out of 20 subjects), whereas the individual variation was higher with lactic acid. After 8 weeks on the oat bran-enriched diet, the interindividual variation was considerably higher, as judged from the higher s.e.m. values (Table 2).

The faecal concentration of CAs was also determined during 3 consecutive days during the intervention. No significant differences in the concentration of CAs could be seen in any of the subjects during these days (Table 2).

The faecal proportion of acetic acid was higher after 8 and 12 weeks on the oat bran diet ($P<0.001$) compared with at entry of the study, whereas that of lactic acid was lower ($P<0.001$) (Table 3).

Discussion

The present study shows that it is possible to increase colonic CA formation in healthy subjects by adding β-glucans to the diet. Most of the CAs formed in the human colon are absorbed and it may be argued that faecal concentrations is a complex function of rate of formation, absorption and utilization. However, as most colonic diseases occur in the distal colon and faecal concentrations of CAs are likely to reflect the concentrations of CAs, to which this part of the colon is exposed, we consider faecal measurements relevant. Studies in rats have also shown that there is a good correlation between the distal and faecal concentrations of acetic, propionic and butyric acid. However, the faecal concentrations generally were somewhat higher than distal concentrations (Nilsson *et al*., 2006). Thus, the absorption of water was relatively faster than that of CA in distal colon.

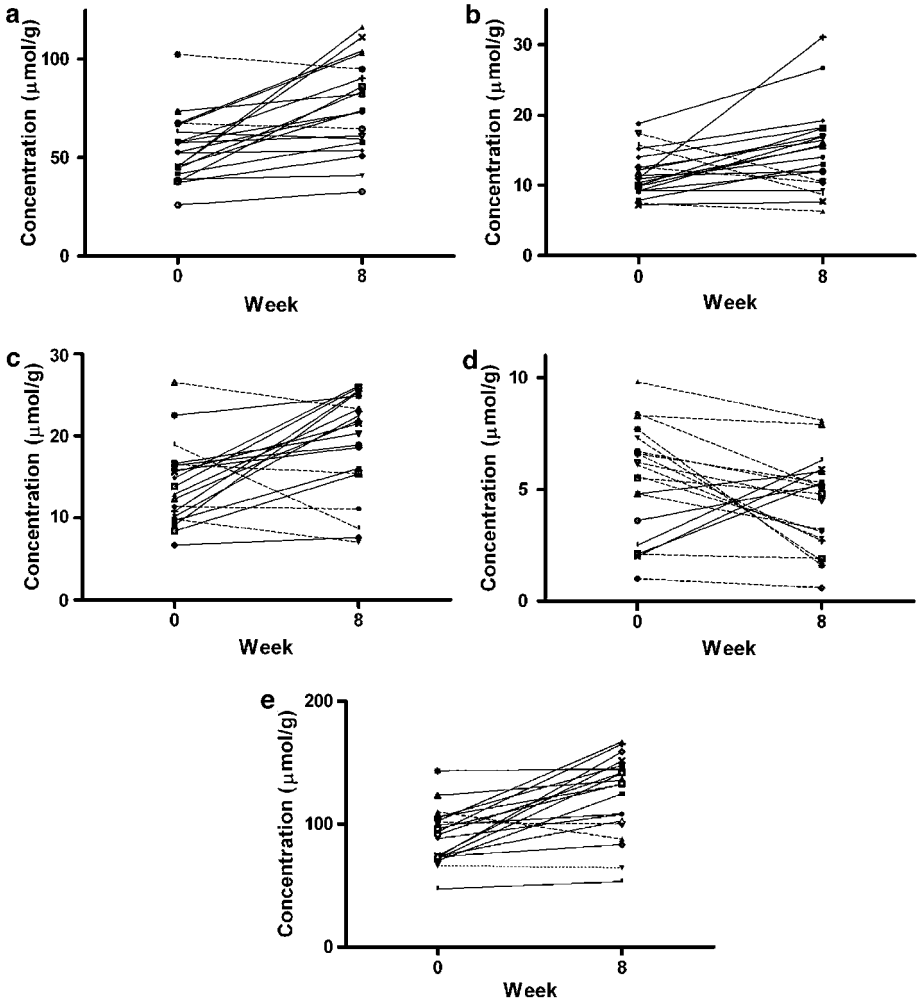

Figure 1 Individual concentrations in faeces of (**a**) acetic acid, (**b**) propionic acid, (**c**) butyric acid, (**d**) lactic acid and (**e**) total concentrations of carboxylic acids (μmol/g) at weeks 0 and 8.

Considering that addition of β-glucans to the diet tend to increase faecal weight, unchanged or moderately increased CA concentrations in faeces is likely to be associated with a larger increase in the mass of CAs that reaches the distal colon. If so, the faecal concentrations seen in our study may also reflect a considerable increase in CA utilization in distal colon, although this was not quantified in the present work.

The total faecal concentrations of CAs at entry of the study were similar to that measured in previous studies on subjects with ulcerative colitis (Hallert *et al.*, 2003) and irritable bowel syndrome (Molin G, Noeback S, Johansson M-J, Berggren A, Nyman M, Björck I and Jeppsson B, unpublished results). Interestingly, the concentration of butyric acid was higher in the healthy subjects in the present study than in patients with ulcerative colitis (13.9 versus 11.1 μmol/g), whereas the concentration of lactic acid was lower (5.4 versus 15.9 μmol/g) (Hallert *et al.*, 2003). Studies by others (Vernia *et al.*, 1988) showed that faecal concentrations of butyric acid decreased, and concentrations of lactic acid increased with severity of ulcerative colitis, and that high colonic concentrations of lactic acid were associated with increased risk for diarrhoea and mucosal inflammation (Cummings, 1995). In the present study, the concentrations of lactic acid decreased during the intervention period with oat bran, whereas in our previous study in patients with ulcerative colitis, the faecal level of lactic acid was similar at entry of the study and after 4, 8 and 12 weeks of dietary supplementation with β-glucan-enriched oat bran (Hallert *et al.*, 2003). However, in that study, there was a large variation between different subjects, and of the individual subjects 65–70% had lower lactic acid concentration during the intervention with oat bran than at entry (unpublished results).

Generally, the faecal butyric acid concentrations are in a range known to have a variety of biological effects in cell cultures and other experimental model systems. The importance of a lowered faecal concentration of lactic acid,

as observed in the present work, is not known. Lactic acid can be further metabolized for example by propionibacteria to propionic acid and acetic acid (Macfarlane and Cummings, 1991). Furthermore, butyric acid can be produced from lactic acid through the acetyl-CoA pathway (Bourriaud *et al*., 2005). A decreased lactate level may thus reflect decreased formation as well as increased metabolism. Overall, it is thus not farfetched to postulate that the changed exposure of CAs observed in the present study may be biologically important in disease prevention. Another product believed to be formed in the initial fermentation by many micro-organisms in colon is formic acid (Pryde *et al*., 2002).

The concentration of CAs did not increase until after 8 weeks. The reason for this is not known, but may be due to a quite stable and balanced composition of the colonic microbiota in healthy subjects, thus, requiring a comparatively longer time to increase the number of bacteria that can ferment the increased amount of available substrate. This may also explain the smaller range in the concentration of the different CAs compared with a previous study in patients with ulcerative colitis consuming a similar type of oat bran (Hallert *et al*., 2003). An increase in the faecal excretion of CAs has also been seen in other studies in humans, for example, when dietary fibre from fruits and vegetables were added to the diet during a period of 2 weeks (Jenkins *et al*., 2001). In the present work with healthy subjects, there was a decrease in the concentration of total CAs after 12 weeks compared with 8 weeks ($P<0.05$). This was not observed in our previous intervention with oat bran in ulcerative colitis patients. Whether this reflects differences in metabolic adaptation of the colonic microbiota between the different target groups, or a higher compliance in the ulcerative colitis patients who may be more motivated, is not known.

The increase in the concentration of CAs seen after 8 weeks resulted from an increase of all the main acids that is acetic, propionic and butyric acids. Similar results, with an increase of acetic, propionic and butyric acid, have been obtained in rats with β-glucans from barley (Lambo-Fodje *et al*., 2006). However, in the study in humans with ulcerative colitis in remission with the same type of β-glucan-enriched oat bran as in the present study, there was a specific increase of butyric acid already after 4 weeks (Hallert *et al*., 2003). The differences could be due to patients with ulcerative colitis having diminished capacity to utilize butyric acid and therefore the excretion of this specific acid increases, or that the transit time in colon is faster in colitis patients. Another difference could be that patients with ulcerative colitis have another composition of the microbiota than healthy people, leading to the use of different metabolic pathways by the microbiota and different CAs. Thus, subjects with ulcerative colitis have been shown to have higher number of sulphate-reducing bacteria (Cummings, 1995) and in another study, it was shown that in the active phase of the disease, the number of lactobacilli was significant lower than in remission (Bullock *et al*., 2004). The differences may also be due to the oat bran as such, since the bread was much denser in the present study, indicating a different structure of the oat β-glucans and differences in baking properties, which might have affected the CA profile. In rats, fructo-oligosaccharides of different molecular weights were shown to give different short-chain fatty-acid patterns during fermentation (Nilsson and Nyman, 2005). Thus, fructo-oligosaccharides with a low molecular weight gave high proportions of butyric acid and those with high molecular weight were especially prone to yield propionic acid. Further, Wood (2004) has found that β-glucans with different physico-chemical properties behave differently when frozen, which may be important to keep in mind when comparing these results with others, since the bread in this study was frozen.

The average faecal proportion of butyric acid at entry was high (14%) compared with levels in patients with ulcerative colitis (11%) given the same type of oat bran in a similar dose. By adding oat bran to the diet, it was possible to increase the concentration further from 13.9 to 19.0 μmol/g. This must be considered as advantageous as butyric acid has been suggested to prevent against colonic diseases. No significant differences in CA concentrations could be seen between consecutive days, justifying the validity of the faecal levels of CAs analysed. An experimental design, with 20 subjects and a dietary supplementation with the test food product for 8 weeks, thus appears suitable for screening of potential differences in faecal CA patterns achievable by dietary means with other fibre sources giving high amounts of butyric acid for example wheat bran and different types of resistant starches. Our main conclusion is that β-glucan-enriched oat bran increases the long-term exposure of the distal colon to CAs. It thus supports other motifs to conduct a conclusive clinical trial regarding the effect of β-glucans as additional relapse preventive therapy in ulcerative colitis. One may speculate whether a combination with wheat bran will increase the distal and/or faecal concentrations of CAs. Studies in rats have shown that by combining a highly fermentable type of resistant starch with the more resistant wheat bran shifted the fermentation and release of CAs to the distal part of colon (Henningsson *et al*., 2002).

Acknowledgements

We thank Kjell Damstedt, Cerealia RD for baking the bread and dietician Ulrika Koppers-Watting for help with the diet registration. This study was financially supported by the VL- and SL-foundation.

References

Berggren AM, Björck IME, Nyman EMGL, Eggum BO (1993). Short-chain fatty-acid content and pH in cecum of rats given various sources of carbohydrates. *J Sci Food Agric* **63**, 397–406.
Bird AR, Hayakawa T, Marsono Y, Gooden JM, Record IR, Correll RL *et al*. (2000). Coarse brown rice increases fecal and large bowel

short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *J Nutr* **130**, 1780–1787.
Bourriaud C, Robins RJ, Martin L, Kozlowski F, Tenailleau E, Cherbut C *et al*. (2005). Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J Appl Microbiol* **99**, 201–212.
Brown I, Warhurst M, Arcot J, Playne M, Illman RJ, Topping DL (1997). Fecal numbers of bifidobacteria are higher in pigs fed Bifidobacterium longum with a high amylose cornstarch than with a low amylose cornstarch. *J Nutr* **127**, 1822–1827.
Bullock NR, Booth JC, Gibson GR (2004). Comparative composition of bacteria in the human intestinal microflora during remission and active ulcerative colitis. *Curr Issues Intest Microbiol* **5**, 59–64.
Casterline JL, Oles CJ, Ku Y (1997). *In vitro* fermentation of various food fiber fractions. *J Agric Food Chem* **45**, 2463–2467.
Chapman MA, Grahn MF, Boyle MA, Hutton M, Rogers J, Williams NS (1994). Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. *Gut* **35**, 73–76.
Cummings J (1995). *The Large Intestine in Nutrition and Disease*. Institut Danone: Bruxelles.
Cummings JH (1997). Short-chain fatty acid enemas in the treatment of distal ulcerative colitis. *Eur J Gastroenterol Hepatol* **9**, 149–153.
Den Hond E, Hiele M, Evenepoel P, Peeters M, Ghoos Y, Rutgeerts P (1998). *In vivo* butyrate metabolism and colonic permeability in extensive ulcerative colitis. *Gastroenterology* **115**, 584–590.
Di Sabatino A, Morera R, Ciccocioppo R, Cazzola P, Gotti S, Tinozzi FP *et al*. (2005). Oral butyrate for mildly to moderately active Crohn's disease. *Aliment Pharmacol Ther* **22**, 789–794.
Djouzi Z, Andrieux C (1997). Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human faecal flora. *Br J Nutr* **78**, 313–324.
Galvez J, Rodriguez-Cabezas ME, Zarzuelo A (2005). Effects of dietary fiber on inflammatory bowel disease. *Mol Nutr Food Res* **49**, 601–608.
Hallert C, Björck I, Nyman M, Pousette A, Granno C, Svensson H (2003). Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. *Inflamm Bowel Dis* **9**, 116–121.
Hanauer SB (2004). Update on the etiology, pathogenesis and diagnosis of ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* **1**, 26–31.
Henningsson AM, Björck IM, Nyman EM (2002). Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats. *J Nutr* **132**, 3098–3104.
Henningsson AM, Nyman MEGL, Björck IM (2003). Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats. *Br J Nutr* **89**, 319–328.
Jenkins DJ, Kendall CW, Popovich DG, Vidgen E, Mehling CC, Vuksan V *et al*. (2001). Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism* **50**, 494–503.
Karppinen S, Liukkonen K, Aura AM, Forssell P, Poutanen K (2000). *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *J Sci Food Agric* **80**, 1469–1476.
Lambo-Fodje AM, Öste R, Nyman ME (2006). Short-chain fatty acid formation in the hindgut of rats fed native and fermented oat fibre concentrates. *Br J Nutr* **96**, 47–55.
Luhrs H, Gerke T, Muller JG, Melcher R, Schauber J, Boxberge F *et al*. (2002). Butyrate inhibits NF-kappaB activation in lamina propria macrophages of patients with ulcerative colitis. *Scand J Gastroenterol* **37**, 458–466.
Macfarlane GT, Cummings JH (1991). The colonic flora, fermentation, and large bowel digestive function. In: Phillips SF (ed). *The Large Intestine: Physiology, Pathophysiology, and Disease*. Raven Press, Ltd.: pp 51–92.
McCleary BV, Codd R (1991). Measurement of (1-3),(1-4)-beta-D-glucan in barley and oats – a streamlined enzymatic procedure. *J Sci Food Agric* **55**, 303–312.
McIntyre A, Gibson PR, Young GP (1993). Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* **34**, 386–391.
Menzel T, Luhrs H, Zirlik S, Schauber J, Kudlich T, Gerke T *et al*. (2004). Butyrate inhibits leukocyte adhesion to endothelial cells via modulation of VCAM-1. *Inflamm Bowel Dis* **10**, 122–128.
Nilsson U, Nyman M (2005). Short-chain fatty acid formation in the hindgut of rats fed oligosaccharides varying in monomeric composition, degree of polymerisation and solubility. *Br J Nutr* **94**, 705–713.
Nilsson U, Nyman M, Ahrne S, Sullivan EO, Fitzgerald G (2006). Bifidobacterium lactis Bb-12 and Lactobacillus salivarius UCC500 modify carboxylic acid formation in the hindgut of rats given pectin, inulin, and lactitol. *J Nutr* **136**, 2175–2180.
Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ (2002). The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett* **217**, 133–139.
Reddy BS, Hirose Y, Cohen LA, Simi B, Cooma I, Rao CV (2000). Preventive potential of wheat bran fractions against experimental colon carcinogenesis: implications for human colon cancer prevention. *Cancer Res* **60**, 4792–4797.
Richardson AJ, Calder AG, Stewart CS, Smith A (1989). Simultaneous determination of volatile and non-volatile acidic fermentation products of anaerobes by capillary gas-chromatography. *Lett Appl Microbiol* **9**, 5–8.
Roland N, Nugon-Baudon L, Andrieux C, Szylit O (1995). Comparative study of the fermentative characteristics of inulin and different types of fibre in rats inoculated with a human whole faecal flora. *Br J Nutr* **74**, 239–249.
Scheppach W (1996). Treatment of distal ulcerative colitis with short-chain fatty acid enemas. A placebo-controlled trial. German-Austrian SCFA Study Group. *Dig Dis Sci* **41**, 2254–2259.
Scheppach W, Bartram HP, Richter F (1995). Role of short-chain fatty acids in the prevention of colorectal cancer. *Eur J Cancer* **31A**, 1077–1080.
Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S *et al*. (1992). Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* **103**, 51–56.
Simpson EJ, Chapman MA, Dawson J, Berry D, Macdonald IA, Cole A (2000). *In vivo* measurement of colonic butyrate metabolism in patients with quiescent ulcerative colitis. *Gut* **46**, 73–77.
Smith JG, Yokoyama WH, German JB (1998). Butyric acid from the diet: actions at the level of gene expression. *Crit Rev Food Sci Nutr* **38**, 259–297.
Soergel KH, Harig JM, Loo FD, Ramaswamy K, Wood CM (1989). Colonic fermentation and absorption of SCFA in man. *Acta Vet Scand Suppl* **86**, 107–115.
Steinhart AH, Hiruki T, Brzezinski A, Baker JP (1996). Treatment of left-sided ulcerative colitis with butyrate enemas: a controlled trial. *Aliment Pharmacol Ther* **10**, 729–736.
Vernia P, Annese V, Bresci G, d'Albasio G, D'Inca R, Giaccari S *et al*. (2003). Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: results of a multicentre trial. *Eur J Clin Invest* **33**, 244–248.
Vernia P, Caprilli R, Latella G, Barbetti F, Magliocca FM, Cittadini M (1988). Fecal lactate and ulcerative colitis. *Gastroenterology* **95**, 1564–1568.
Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ (2006). Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* **40**, 235–243.
Wood PJ (2004). Relationships between solution properties of cereal beta-glucans and physiological effects – a review. *Trends Food Sci Technol* **15**, 313–320.
Zoran DL, Turner ND, Taddeo SS, Chapkin RS, Lupton JR (1997). Wheat bran diet reduces tumor incidence in a rat model of colon cancer independent of effects on distal luminal butyrate concentrations. *J Nutr* **127**, 2217–2225.