

# Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability

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## A B S T R A C T

Non-steroidal anti-inflammatory drugs (NSAIDs) are effective analgesics but cause gastrointestinal injury. Present prophylactic measures are suboptimal and novel therapies are required. Bovine colostrum is a cheap, readily available source of growth factors, which reduces gastrointestinal injury in rats and mice. We therefore examined whether spray-dried, defatted colostrum could reduce the rise in gut permeability (a non-invasive marker of intestinal injury) caused by NSAIDs in volunteers and patients taking NSAIDs for clinical reasons. Healthy male volunteers ( $n = 7$ ) participated in a randomized crossover trial comparing changes in gut permeability (lactulose/rhamnose ratios) before and after 5 days of 50 mg of indomethacin three times daily (tds) *per oral* with colostrum (125 ml, tds) or whey protein (control) co-administration. A second study examined the effect of colostrum and control solutions (125 ml, tds for 7 days) on gut permeability in patients ( $n = 15$ ) taking a substantial, regular dose of an NSAID for clinical reasons. For both studies, there was a 2 week washout period between treatment arms. In volunteers, indomethacin caused a 3-fold increase in gut permeability in the control arm (lactulose/rhamnose ratio  $0.36 \pm 0.07$  prior to indomethacin and  $1.17 \pm 0.25$  on day 5,  $P < 0.01$ ), whereas no significant increase in permeability was seen when colostrum was co-administered. In patients taking long-term NSAID treatment, initial permeability ratios were low ( $0.13 \pm 0.02$ ), despite continuing on the drug, and permeability was not influenced by co-administration of test solutions. These studies provide preliminary evidence that bovine colostrum, which is already currently available as an over-the-counter preparation, may provide a novel approach to the prevention of NSAID-induced gastrointestinal damage in humans.

## INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most commonly prescribed medicines used worldwide. Although of undoubted efficacy for the

treatment of musculoskeletal injury, chronic administration of NSAIDs results in both gastric and intestinal damage. This includes peptic ulceration and injury to the small and large intestine causing increased permeability with blood and protein loss and stricture formation [1–4].

**Key words:** gastrointestinal tract, intestinal injury, repair, nutrition.

**Abbreviations:** NSAID, non-steroidal anti-inflammatory drug; tds, three times daily.

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Current strategies to reduce gastrointestinal side effects of NSAIDs consist of co-administration of acid suppressants or prostaglandin analogues. Although they are beneficial in reducing peptic ulceration, they are less efficacious in limiting small intestinal damage [3]. In addition, diarrhoea can be a troublesome side effect from the use of prostaglandin analogues, and these drugs are also relatively contraindicated in young women because of their pro-abortive and teratogenic activity [5]. Novel therapeutic approaches are therefore required.

Colostrum, the milk produced for the first few days after birth, is a rich natural source of nutrients, antibodies and growth factors for the suckling neonate. Some studies suggest it may be of value in eliminating infection and stimulating growth of the neonatal gastrointestinal tract [6,7]. Its value in the prevention and treatment of adult gastrointestinal injury is, however, largely unexplored. We have shown recently [8], using a combination of *in vitro* and *in vivo* animal models, that a commercially available defatted bovine colostrum preparation can reduce NSAID-induced gut injury in rats and mice.

We have now further examined its potential clinical value for the prevention and treatment of NSAID-induced enteropathy by measuring changes in gut permeability in normal volunteers taking clinically relevant doses of the NSAID indomethacin and also in patients taking long-term NSAIDs for clinical reasons.

## METHODS

### Materials and ethics

Chemicals were obtained from Sigma unless otherwise stated. Local ethical approval and patient consent was obtained for all studies.

### Preparation of colostrum and whey protein solutions

The test solutions were identical to those used for the previously published *in vitro* and *in vivo* studies [8], and were prepared by Viable Bioproducts, Turku, Finland. The initial colostrum and milk whey protein solutions were treated in an identical fashion and were passed through a microfilter (0.2 mm pore). The final colostrum whey solution ('Bioenervi') is free of fat (including polar lipids) and lactose, and is reduced in most of the major milk proteins, including casein and lactalbumin, with the remaining protein being relatively rich in immunoglobulins and growth factors. The total protein content of the colostrum solution was 4.3 mg/ml. The concentrations of the various growth factors present in the colostrum preparation are incompletely defined, but include: insulin-like growth factor-I and -II, at approx. 2 mg/l each; transforming growth factor  $\beta$  at 25  $\mu$ g/l; and epidermal growth factor at 6  $\mu$ g/l (data supplied by

SHS International Ltd, personal communication). The milk whey (placebo) solution provided an iso-proteinaceous solution (4.3 mg/ml), which has a similar appearance to the colostrum preparation, but is free of growth factor constituents (data supplied by SHS International Ltd, personal communication).

### Assessment of permeability

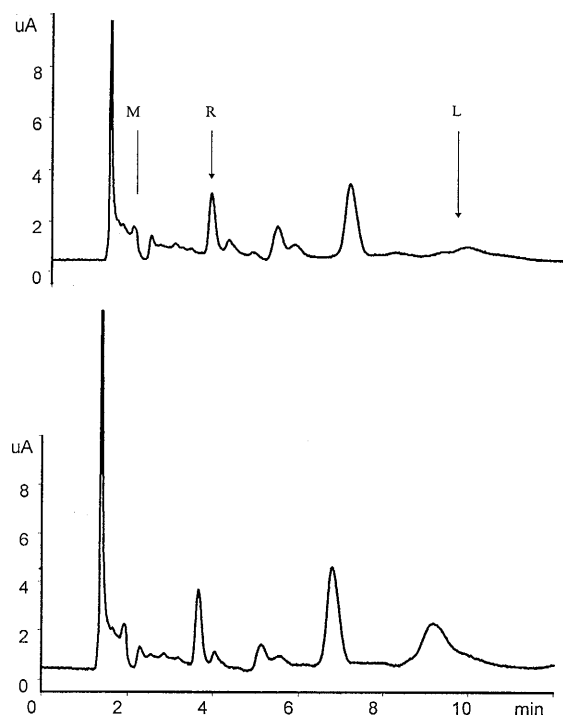
Assessment of intestinal permeability by quantifying unmediated absorption of at least two sugars of different sizes provides a sensitive index of intestinal damage [9]. We used lactulose as the disaccharide probe, and rhamnose and mannitol as two alternative monosaccharide probes in a hypo-osmolar formulation. Both rhamnose and mannitol have been widely used and provide similar information regarding changes in 'paracellular pathways'. However, as there are minor differences in their processing *in vivo* [9], both sugars were included in the test drink.

Following an overnight fast, subjects emptied their bladders and then drank a standardized sugar solution containing 5 g of lactulose, 2 g of mannitol and 1 g of rhamnose in a total of 450 ml of water (calculated osmolality 69 osmol/kg of water). Subjects were allowed unlimited intake of fluid after the first hour of the test to ensure adequate urine output. The urine was collected and pooled over the next 5 h and total volume recorded. Aliquots were centrifuged briefly to remove gross debris and the supernatant frozen at  $-25^{\circ}\text{C}$  until later analysis.

Analyses of sugar content within the urine were based on the method of Sørensen et al. [10], with minor modifications. The various sugars were separated using HPLC and quantified using a pulsed amperometric detector. Using this technique, sugars are oxidized on the gold electrode at the working potential ( $E'_0 = 0.05\text{ V}$ ), the current produced being a measure of the amount of sugar present in the sample [11].

The system comprised of a Hewlett Packard 1100 series HPLC system using a Dionex CarboPac PA10 anion-exchange analytical column ( $4.5 \times 250\text{ mm}$ ) with an equivalent guard column. NaOH (50 mM, low in carbonate; BDH-Merck Ltd) was used as the mobile phase (1 ml/min, isocratic conditions). Sugars were detected using a Hewlett Packard 1049A Electrochemical Detector with a gold working electrode and solid reference electrode. The potentials were set as follows:  $E'_0 = 0.05\text{ V}$ ,  $E'_1 = 0.6\text{ V}$  and  $E'_2 = -0.8\text{ V}$ ; and  $t_1 = 120\text{ ms}$ ,  $t_2 = 120\text{ ms}$ ,  $t_3 = 400\text{ ms}$ . Data analyses were performed using the Hewlett Packard Chemstation software.

Typically, the mannitol peak eluted from the column at approx. 2 min, rhamnose at 4 min and lactulose at 9 min. Analysis of single- and mixed-sugar standards in the concentration range 0.05 mg/ml to 50 mg/ml showed



**Figure 1** Elution profiles of urinary samples from a volunteer who has taken a hypo-osmolar drink containing test sugars

Samples were run on an isocratic HPLC column and sugars detected using pulsed amperometric detection. The peaks relating to lactulose (L) and rhamnose (R) are well resolved, whereas the mannitol (M) peak is lost in the non-specific early peak. Lower panel: the equivalent result from the same subject who had a repeat test after 5 days of indomethacin (50 mg, tds). In this second test, there is a marked increase in the lactulose peak.

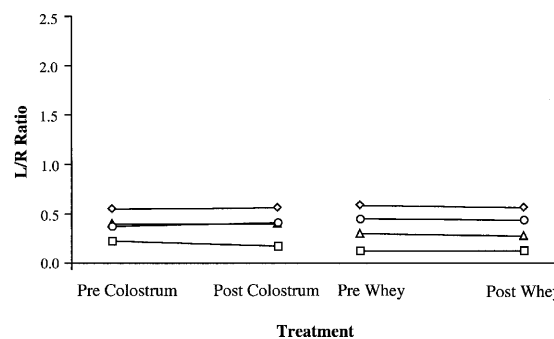
good chromatographic separation and proportional changes in the area under the curve (results not shown).

Initial baseline urine samples, analysed following the preliminary studies, showed no sugar peaks in these areas. However, when the same separation protocol was used for the subsequent full studies, the mannitol peak was sometimes obscured by an overlap from other urinary constituents (Figure 1). We therefore used the lactulose/rhamnose ratio as our index of intestinal injury, a combination that has been recommended for assessing enteropathy induced by NSAIDs [12].

## Study protocols

### Normal volunteers (Study 1)

To determine the reproducibility of results, a single individual performed permeability studies for 6 separate days (while not taking any test treatments or NSAIDs). These samples were assayed to determine intra-patient variation. In addition, a single sample was measured six times to determine intra-assay variation.



**Figure 2** Colostrum or control solution does not influence permeability when given alone to normal volunteers

Four volunteers ingested control and test solution for 5 days without taking NSAIDs. Permeability studies were performed at the beginning and end of the study periods. Each subject's individual results are shown by a different symbol.

To examine whether the colostrum or control preparation influenced permeability under basal conditions, four subjects underwent an initial permeability assessment and then ingested either the colostrum or whey protein preparation [125 ml, three times daily (tds)] for 5 days with a further assessment on the final day. No change in permeability was seen (Figure 2).

Seven male volunteers (26–38 years old) who were not intolerant of milk products, taking NSAIDs, or suffering from conditions likely to affect intestinal permeability (e.g. coeliac disease or previous intestinal surgery), were entered into the study.

Subjects abstained from alcohol consumption and ingestion of any NSAID, including aspirin, for 1 week prior to starting the study and throughout the remainder of the test period. Following an initial baseline permeability assessment, they received, in random order, the colostrum preparation (125 ml, tds) or control solution for 7 days. For the last 5 days of each study arm, they also took indomethacin 50 mg, tds. At the end of the test period intestinal permeability was reassessed. A 2 week 'washout' period was left between the two stages of the study. Throughout the study, volunteers and patients were asked about symptoms and compliance. Formal dyspepsia scoring was not undertaken, however, as the standard validated questionnaires are not appropriate for short-term studies.

### Patient group (Study 2)

A total of 15 patients (seven male, eight female; mean age 61 years, range 43–75) were recruited from the community (predominantly from Latham House Surgery, Melton Mowbray, Leicestershire, U.K.). All had been regularly taking a stable, substantive dose of a non-selective NSAID without additional prophylaxis (e.g. acid suppressant) for at least 1 year. This comprised of Voltarol (75 mg or higher) in two patients, Piroxicam

(20 mg or higher) in three patients, Naproxen (500 mg twice daily or higher) in five patients and Ibuprofen (1.2 g or higher) in five patients. The underlying conditions necessitating NSAID usage were: osteoarthritis, in 11 patients; rheumatoid arthritis, in three patients; and psoriatic arthropathy, in one patient. None of the patients were taking any other drugs likely to alter intestinal permeability or had previously suffered any clinical adverse events due to NSAIDs. Patients did not undergo endoscopy prior to starting the trial.

Patients were randomized to receive, in a double-blinded, randomized control fashion, colostrum or control solution (125 ml, tds for 7 days) with a 2 week washout period between the two study arms. For both arms of the study (colostrum or control), an initial baseline assessment of intestinal permeability was performed prior to starting the test solutions. Permeability was re-assessed after 7 days treatment. Patients were assessed by telephone interview on day four and at the end of treatment with a structured questionnaire to document consumption of their NSAID, and test treatment and identify side effects (if any).

### Statistics

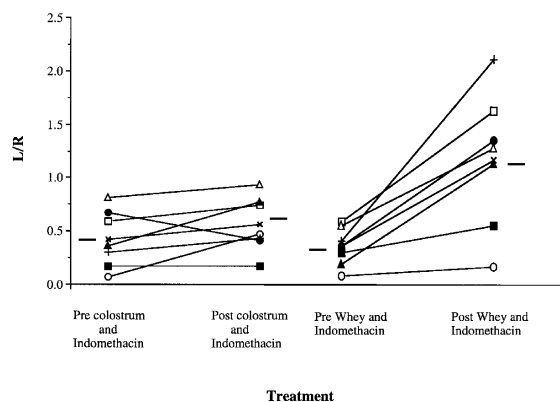
Data were analysed by one- or two-way ANOVA as appropriate, using presence of indomethacin and test solution as factors. When a significant effect was found ( $P < 0.05$ ), individual comparisons were performed using *t* tests based on the residual and degrees of freedom obtained from the ANOVA, a method equivalent to repeated-measures analyses, which takes account of the fact that the data are paired. Comparison of baseline values of Study 1 versus initial values of patients in Study 2 were performed using a two-tailed unpaired *t* test.

## RESULTS

### Normal volunteers (Study 1)

For multiple measurements of a single sample, the coefficient of variation was 5.9%. Measurement of the six serial samples from the same individual gave a coefficient of variation of 7.7%. Administration of colostrum or control solution to the individuals not taking NSAIDs had no effect on their permeability results (Figure 2). Therefore although there was a relatively broad normal range (0.17–0.81, see Figure 2 and baseline values of Figure 3), in keeping with other published works (for example [3]), intra-volunteer variation was small. It was for this reason that each subject acted as their own control.

All seven subjects completed the study without protocol violations. One developed mild nondescript upper-abdominal discomfort while taking the indomethacin



**Figure 3** Influence of colostrum on indomethacin-induced increases in permeability

Seven volunteers participated in this double-blinded crossover study. Each subject's individual results are shown by a different symbol. The group means from each stage are also marked. Baseline permeability values were similar prior to each arm of the study. In the control (whey) arm, 5 days of indomethacin caused a 3-fold rise in lactulose/rhamnose ratios ( $P < 0.01$ ). Co-administration of the colostrum preparation prevented this increase.

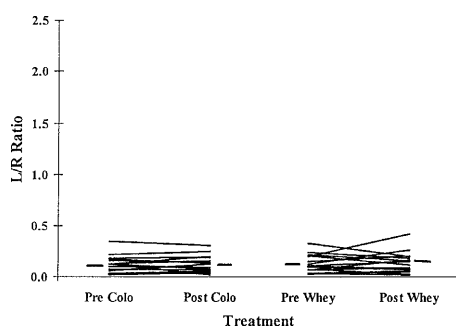
(control) arm but completed the course. No side effects due to ingestion of the colostrum preparation were reported.

Baseline permeability values were similar at the beginning of each study arm (lactulose/rhamnose ratio  $0.36 \pm 0.07$  versus  $0.42 \pm 0.10$ , means  $\pm$  S.E.M.,  $P > 0.05$ ; Figure 3). Permeability increased approx. 3-fold in response to indomethacin in the control arm ( $P < 0.01$  versus baseline value), but showed no significant rise when colostrum was co-administered (Figure 3). The order in which control and colostrum were administered did not appear to influence results (although numbers are too small to perform detailed statistical analysis).

### Patient group (Study 2)

One subject developed a non-specific viral-type illness during the washout period and was therefore withdrawn from the study. Of the remaining patients, one developed mild oesophageal reflux-type symptoms during their placebo-treatment week. A second patient reported improvement in long-standing vague lower-abdominal pain while taking colostrum.

Baseline permeability assessments (assessed while patients continued to take their regular NSAID therapy) gave results which were actually lower than those seen in the volunteers of Study 1 prior to them starting indomethacin (lactulose/rhamnose ratio  $0.13 \pm 0.02$ , means  $\pm$  S.E.M., in patients compared with  $0.36 \pm 0.07$  volunteers,  $P < 0.05$ ). There was however, some overlap between individuals in the two studies. Co-administration of the colostrum or control solution to patients



**Figure 4** Influence of colostrum on permeability in patients ( $n = 14$ ) taking NSAIDs long-term for clinical reasons

Individual results and group means are shown. Baseline permeability values were low, despite all of the patients continuing to take their NSAIDs. Values were actually somewhat lower than those seen in volunteers not given NSAID (see Figure 2). Co-administration of the colostrum or control solution had no effect on these values.

taking NSAIDs had no significant effect on their gut permeability (Figure 4).

## DISCUSSION

We have used changes in gut permeability, a well validated indirect method of investigating small intestinal injury, to examine the potential clinical value of a commercial defatted colostrum preparation in reducing NSAID-induced enteropathy. The colostrum preparation, but not a similarly prepared whey protein solution, significantly reduced the increase in permeability caused by short-term (5 day) exposure to indomethacin in normal subjects. Patients taking long-term NSAIDs for clinical reasons had initial permeability values that were low, being similar or lower than those seen in normal subjects not given NSAIDs, and were not influenced by co-administration of the colostrum preparation.

Several methods are available to determine the degree of small intestinal injury induced by NSAIDs, all of which have their drawbacks: enteroscopy is an invasive procedure;  $^{111}\text{In}$ -labelled white cells require radioactive exposure; and measurement of the neutrophil marker, calprotectin, in the stool is still at a relatively early stage of development [13]. Measurement of gut permeability is a safe and simple investigation to perform, but is an indirect method of assessing small intestinal injury. Assessment of excretion of two molecules of different sizes, such as a monosaccharide and a disaccharide, by HPLC with pulsed amperometric detection, provides high sensitivity and allows correction for potential confounding factors, such as changes in the rate of gastric emptying and small intestinal transit.

Measurement of intestinal permeability has been used previously to assess the degree of small intestinal damage in patients with coeliac disease [14] and Crohn's disease [15], as well as injury caused by NSAIDs. Several studies

have shown that short term (1–7 days) administration of clinically relevant doses of NSAIDs, such as indomethacin, naproxen and ibuprofen, increase gut permeability by approx. 3-fold (e.g. [16]). Our results from the control arm, using normal volunteers, are therefore in keeping with published works.

NSAIDs are one of the most widely prescribed group of drugs used worldwide. Point-prevalence studies, however, suggest that 10–30% of unselected patients taking NSAID therapy have peptic ulceration [17], which can often be asymptomatic [18]. In addition, up to 70% of patients taking NSAIDs have some degree of enteropathy associated with low-grade blood and protein loss [13,19–21], although it is only of clinical significance in a much smaller percentage of patients. Specific cyclooxygenase-2 inhibitors have reduced gastric toxicity but are expensive and cannot replace the use of aspirin as an anti-platelet agent, the use of which continues to be a major contributor to the development of significant gastrointestinal bleeding [22,23].

In the present study, the baseline permeability values of patients taking long-term NSAIDs were low, being similar or lower than those seen in control subjects not given an NSAID. This result is in keeping with the report of Struthers et al. [24], but is at variance with the report of Sigthorsson et al. [25] who found an approx. 2-fold increase in permeability in patients taking similar doses of NSAIDs to those in the present study. The reasons behind these different results are unclear, although variations exist in the details of the probes used and the osmolality of the test solutions. We specifically ensured that our test solution was hypo-osmolar, as this has been reported to maximize alterations in permeability changes induced by NSAIDs [25]. As all of the volunteers in the short-term study showed a marked rise in permeability, it is possible that the small intestine of our patients taking NSAIDs chronically underwent adaptation. Adaptation is a well recognized phenomenon, with regards to the disappearance of gastric erosions, in patients who continue to take NSAIDs [26]. All of our patients were selected on the basis of having taken NSAIDs long-term, without the requirement for additional prophylaxis. It is therefore possible that those patients who suffer serious side effects (such as chronic blood loss) fail to adapt appropriately. Alternatively, because of the selection criteria, our patients may represent a group who are not susceptible to the initial effects of the NSAID which were seen in the volunteers. This idea is less likely, however, as although the number of volunteers were small, all responded in a similar manner when acutely exposed to indomethacin. Additional differences existed between the patients and volunteers; the mean age of patients was greater than that of volunteers and consisted of both males and females. This last point was probably not of major relevance as sub-analyses of the males alone showed similar results. To address

this area further, additional larger studies should probably examine gut permeability in patients prior to starting NSAID therapy, with serial measurements of side effects (dyspepsia scores etc.) and permeability following randomization to control, or colostrum solution, at the same time as NSAID therapy is started.

Colostrum is the milk produced by the mother for the first few days after birth and is much richer in growth factors and antibodies than ordinary milk [6,7,27]. Bovine colostrum preparations are currently available in the U.S.A. and throughout Europe as 'over-the-counter' health food supplements. They do, however, contain large amounts of potent growth factors which are biologically active [8]. Products such as these are also termed 'functional foods' or 'nutraceuticals' based on the realization that the distinction between food and drugs is becoming blurred.

NSAIDs such as indomethacin cause damage to the gastrointestinal tract by several mechanisms, including reduction of mucosal prostaglandin levels, reduction of mucosal blood flow, stimulating neutrophil activation and, possibly, also stimulating apoptosis [28]. It is likely that many of these mechanisms will be influenced by the numerous growth factors present in the colostrum preparation. There is now increasing evidence that administration of multiple peptides can result in additive or synergistic activity [29]. Orally administered colostrum-derived preparations, therefore, appear to be an attractive therapeutic option as they contain multiple growth factors in a formulation that provides inherent protection against proteolytic digestion. Further, long-term clinical studies appear warranted to examine its value in the prevention/treatment of NSAID-induced gut injury, and also possibly for other ulcerative conditions of the bowel, such as necrotizing enterocolitis and inflammatory bowel disease, where therapies are sub-optimal and novel approaches are required.

## ACKNOWLEDGMENTS

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## Declaration of interest

The use of bovine colostrum for the prevention of NSAID-induced gut injury has been patented by SHS International Ltd (No. 9619634.0), who partially funded this work.

## REFERENCES

- 1 Allison, M. C., Howatson, A. G., Torrance, C. J. et al. (1992) Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. *N. Engl. J. Med.* **327**, 749–754

- 2 Bjarnason, I., Zanelli, G., Smith, T. et al. (1987) Non-steroidal anti-inflammatory drug induced intestinal inflammation in humans. *Gastroenterology* **93**, 480–489
- 3 Bjarnason, I. (1988) Non-steroidal anti-inflammatory drug induced small intestinal inflammation in man. In *Recent Advances in Gastroenterology* (Pounder, R., ed.), pp. 23–46. Churchill Livingstone Press, London
- 4 Morris, J., Madhok, R., Sturrock, R. D., Capell, H. A. and Mackenzie, J. F. (1991) Enteroscopic diagnosis of small bowel ulceration in patients receiving non steroidal anti-inflammatory drugs. *Lancet* **337**, 520
- 5 Pastuszak, A. L., Schuler, L., Speck-Martin, C. E. et al. (1998) Use of misoprostol during pregnancy and Mobius' syndrome in infants. *N. Engl. J. Med.* **338**, 1881–1885
- 6 Xu, R. J. (1996) Development of the newborn GI tract and its relation to colostrum/milk intake: a review. *Reprod. Fertil. Dev.* **8**, 35–48
- 7 Xanthou, M., Bines, J. and Walker, W. A. (1995) Human milk and intestinal host defence in newborns: an update. *Adv. Paediatr.* **42**, 171–208
- 8 Playford, R. J., Floyd, D. N., Macdonald, C. E., Calnan, D. P., Adenekan, R. O., Johnson, W., Goodlad, R. A. and Marchbank, T. (1999) Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. *Gut* **44**, 653–658
- 9 Bjarnason, I., Macpherson, A. and Hollander, D. (1995) Intestinal permeability: an overview. *Gastroenterology* **108**, 1566–1581
- 10 Sørensen, S., Proud, F. J., Adam, A., Rutgers, H. C. and Batt, R. M. (1993) A novel HPLC method for the simultaneous quantification of monosaccharides used in tests of intestinal function and permeability. *Clin. Chim. Acta* **221**, 115–125
- 11 Hardy, M. R., Townsend, R. R. and Lee, Y. C. (1998) Monosaccharide analysis of glycoconjugates by anion exchange chromatography with pulsed amperometric detection. *Anal. Biochem.* **170**, 54–62
- 12 Bjarnason, I. (1994) Intestinal permeability. *Gut Suppl.* **1**, S18–S22
- 13 Tibble, J. A., Sigthorsson, G., Foster, R. et al. (1999) High prevalence of NSAID enteropathy as shown by a simple faecal test. *Gut* **45**, 363–366
- 14 Greco, L., D'Adamao, G., Truscelli, A., Parrilli, G., Mayer, M. and Budillon, G. (1991) Intestinal permeability after single dose gluten challenge in coeliac disease. *Arch. Dis. Child.* **66**, 870–872
- 15 Koltun, W. A., Tilberg, A. F., Page, M. J. and Poritz, L. S. (1998) Bowel permeability is improved in Crohn's disease after ileocelectomy. *Dis. Colon Rectum* **41**, 687–690
- 16 Bjarnason, I., Williams, P., Smethurst, P., Peters, T. J. and Levi, A. J. (1986) The effect of NSAIDs and prostaglandins on the permeability of the human small intestine. *Gut* **27**, 1292–1297
- 17 McCarthy, D. (1989) Nonsteroidal antiinflammatory drug-induced ulcers: Management by traditional therapies. *Gastroenterology* **96**, 662–674
- 18 Larkai, E. N., Smith, J. L., Lidsky, M. D. and Graham, D. Y. (1987) Gastroduodenal mucosa and dyspeptic symptoms in arthritic patients during chronic nonsteroidal anti-inflammatory drug use. *Am. J. Gastroenterol.* **82**, 1153–1158
- 19 Morris, A. J., Wasson, L. A. and MacKenzie, J. F. (1992) Small bowel enteroscopy in undiagnosed gastrointestinal blood loss. *Gut* **33**, 887–889
- 20 Bjarnason, I., Hayllar, J., Macpherson, A. J. and Russell, A. S. (1993) Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* **104**, 1832–1847
- 21 Bjarnason, I., Zanelli, G., Prouse, P., Smethurst, P., Smith, T., Levi, S., Gumpel, M. J. and Levi, A. J. (1987) Blood and protein loss via small intestinal inflammation induced by nonsteroidal antiinflammatory drugs. *Lancet* **ii**, 711–714
- 22 Stack, W. A., Hawkey, G. M., Atherton, J. C., Logan, R. F. and Hawkey, C. J. (1999) Interaction of risk factors for peptic ulcer bleeding. *Gastroenterology* **116**, G0419
- 23 Stack, W. A., Hawkey, G. M., Logan, R. F. and Hawkey, C. J. (1999) Low dose aspirin use and past history as the main determinants of site-specific ulcer bleeding. *Gastroenterology* **116**, G1392

- 24 Struthers, G. R., Andrews, D. J., Gilson, R. J. C., Reynolds, G. A. and Low-Ber, T. (1985) Intestinal permeability. *Lancet* **I**, 587–588
- 25 Sigthorsson, G., Tibble, J., Hayllar, J. et al. (1998) Intestinal permeability and inflammation in patients on NSAIDs. *Gut* **43**, 506–511
- 26 Konturek, J. W., Dembinski, A., Stoll, R. et al. (1994) Mucosal adaptation to aspirin induced gastric damage in humans. Studies on blood flow, gastric mucosal growth and neutrophil activation. *Gut* **35**, 1197–1204
- 27 Steimer, K. S., Packard, R., Holden, D. et al. (1981) The serum free growth of cultured cells in bovine colostrum and in milk obtained later in the lactation period. *J. Cell. Physiol.* **109**, 223–234
- 28 Levi, S. and Shaw-Smith, C. (1994) Non-steroidal anti-inflammatory drugs; how do they damage the gut? *Br. J. Rheumatol.* **33**, 605–612
- 29 Chinery, R. and Playford, R. J. (1995) Combined intestinal trefoil factor and epidermal growth factor is prophylactic against indomethacin-induced gastric damage in the rat. *Clin. Sci.* **88**, 401–403

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