

ORIGINAL ARTICLE

High faecal calprotectin concentrations in newborn infants

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Background: Calprotectin, a major component of soluble cytosolic proteins in human neutrophil granulocytes, is excreted in excess in stools during inflammatory bowel disease in adults and children. Faecal calprotectin concentrations are also higher during the first year of life than in adults.

Objectives: To measure faecal calprotectin concentrations in the neonatal period and define reference values according to the mode of feeding: standard infant formula, prebiotic infant formula (Calisma, Blédina SA, France), or breast feeding.

Patients and methods: A prospective study was carried out over three months in 69 full term, healthy newborns with a median gestational age of 39.8 weeks (range 37–41.5) and a birth weight of 3300 g (range 2600–4460). Three groups were formed depending on the mode of feeding: group 1 (n = 18) received a standard infant formula, group 2 (n = 19) the prebiotic infant formula, and group 3 (n = 32) was breast fed. One stool sample was taken from each newborn on day 4 (3–7), and faecal calprotectin analysed using a commercial enzyme linked immunoassay (Calprest, Eurospital, Italy).

Results: Faecal calprotectin concentrations (median 167 µg/g) were higher than reference values in healthy adults. The concentration was below the upper reference limit for adults (50 µg/g) for three infants only, one fed the standard formula and two fed the prebiotic formula. Concentrations did not differ significantly according to method of feeding.

Conclusions: Compared with healthy adults, newborns have high calprotectin concentrations in the first days of life. There is no obvious influence of the mode of feeding.

Calprotectin is a 36.5 kDa calcium and zinc binding protein which constitutes about 60% of soluble cytosol proteins in human neutrophil granulocytes.¹ When bound to calcium, it is remarkably resistant to proteolysis and is stable in the faeces for one week at room temperature.² Although its exact biological function is not known, it has been shown to have bactericidal and fungicidal properties,³ and various data suggest that it may also be involved in the regulation of the inflammatory process. Its faecal concentration has been associated with the degree of disease activity in inflammatory bowel disease in adults^{4–5} and children.^{6–7} It therefore has potential as a non-invasive diagnostic screening test in children, providing information on intestinal inflammation.⁸ Surprisingly, two recent studies^{9–10} have shown high concentrations of faecal calprotectin in healthy young infants during the first few weeks of life, which then decrease. No data are currently available during the first few days of life.

The aim of this study was to test whether high faecal calprotectin concentrations occur as early as the neonatal period. The mode of feeding is likely to interfere with gut behaviour during the first few days of life, especially in terms of microflora, now recognised to have a major effect on the composition and functional differentiation of the immune cells.¹¹ Thus, to define reference values, calprotectin was measured in breast fed and formula fed infants. Formula fed infants were given either a standard or a prebiotic formula (Calisma, Blédina SA, France).

PATIENTS AND METHODS

This prospective study was carried out over three months in healthy term infants (33 boys/36 girls) born in the maternity unit of Saint Vincent de Paul Hospital (Paris, France). All were born at term with a median gestational age of 39.8 weeks (range 37–41.5) and birth weight of 3300 g (range 2600–4460) (table 1). No neonates or mothers received antibiotics before or during the delivery.

Thirty two newborns were breast fed. When the mother declined to breast feed, parents were given the choice of a standard formula (n = 18) or a prebiotic formula (n = 19). Prebiotic formula (Calisma, Blédina SA, France) is a standard formula that has included in its manufacturing process fermentation by two probiotic strains—*Bifidobacterium breve* C50 and *Streptococcus thermophilus*. This process confers prebiotic activity through the active bacterial metabolites produced, such as non-digestible oligosaccharides and glycoproteins.¹² At the end of the process, the bacteria are inactivated by heat treatment to prevent any adverse effect of live bacteria on the neonates' immature intestinal system. In contrast with probiotics, which are health promoting viable micro-organisms, prebiotics are non-digestible ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon, especially bifidobacteria.¹³

Informed consent was obtained from each mother to collect a spot sample of faeces from their infant. Only stool samples were collected (meconium was discarded) at a median age of 4 days (range 3–7). About 1 g faeces was collected from the nappy and stored immediately at –20°C. Before analysis, frozen stool samples were thawed at room temperature, and the calprotectin concentration determined using a commercial enzyme linked immunoassay (Calprest, Eurospital, Italy). Results were expressed as µg/g stool. The upper reference limit in healthy adults (n = 125) supplied by the manufacturer is 50 µg/g stool. No significant difference between spot measurements were found when five samples from each of 47 stools were measured.¹⁴

Results are given as median and range. Data were analysed using the Statview (Abacus Concepts, Berkeley, California, USA) statistical software package. The results for the different groups were compared using analysis of variance and Fisher's exact test. Correlations were sought using Spearman's correlation test.

Table 1 Characteristics of the newborns

Characteristic	Breast fed (n = 32)	Formula fed	
		Standard (n = 18)	Calisma (n = 19)
Gestational age (weeks)	39.5 (37–41.5)	40 (37–41)	40 (38–41)
Birth weight (g)	3230 (2610–4180)	3515 (2660–4460)	3410 (2600–4290)
Boys/girls	18/14	9/9	6/13

Values are median (range). Calisma is a prebiotic infant formula.

RESULTS

The faecal calprotectin concentrations (median 167 µg/g) were mainly higher than the reference value for healthy adults. The concentration was below the upper reference limit for adults (50 µg/g) for three infants only, one fed the standard formula and two fed the prebiotic formula (fig 1). High interindividual variations were observed, ranging from 22 to 860 µg/g. No significant differences were found between boys and girls ($p = 0.85$), and no correlation was found between calprotectin concentration and day of sampling ($r = -0.037$).

No significant differences in faecal calprotectin concentration were found between the three different feeding groups: standard formula, 148 µg/g (range 45–480); prebiotic formula, 144 µg/g (22–514); breast milk, 204 µg/g (62–860).

DISCUSSION

This study found high faecal calprotectin concentrations in the neonatal period compared with concentrations in adults^{6,7} and healthy children.^{6,7}

Samples exhibited high interindividual variation, which probably reflects varying individual faecal excretion. However, medians and ranges were similar in the three groups, showing that faecal calprotectin concentration was not influenced by feeding. These interindividual variations may be attributed to the method of collection, in which the sample stayed in the baby's nappy. However, Olafsdottir *et al*⁹ showed that this sampling method, in which some water is absorbed by the nappy, increases the calprotectin concentration by no more than 30%, a variation far lower than that measured in this study.

These values found during the first week of life are similar to those observed by Olafsdottir *et al*⁹ (278 (105) µg/g) and

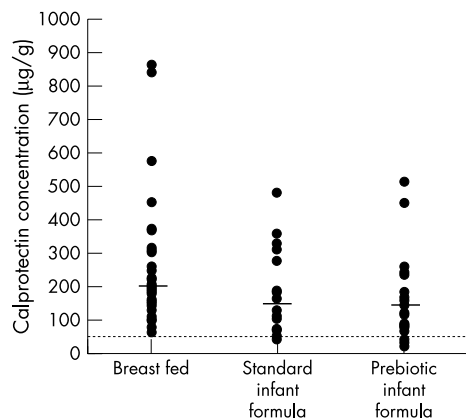


Figure 1 Faecal calprotectin concentrations in newborns fed by three different methods. Solid lines indicate median concentrations, and the dotted line indicates the upper reference limit of calprotectin concentration in adults (50 µg/g).

Rugtveit *et al*¹⁰ (269 µg/g (279–847)) in infants aged 2–10 weeks, and thus confirm that the calprotectin faecal pattern specific to early infancy dates back to the first days of life and is not influenced by formula feeding at that time. However, faecal calprotectin concentrations have been reported to be lower in breast fed than formula fed infants aged 1 week to 1 month.¹⁵

The high calprotectin concentrations may be related to the unusual physiology of neonatal gut. A specific pattern of functioning in the first weeks of life is characterised by, among other things, increased transmucosal leakage, as previously shown by Walker,¹⁶ a phenomenon that ends by the third trimester of life, in a process named “closure”. This leakage can be assessed by intestinal permeability studies.¹⁷ Indeed, the data of Olafsdottir *et al*⁹ and Rugtveit *et al*¹⁰ indicate that faecal calprotectin concentrations decrease to adult values with a time scale similar to that of the “closure” process. Increased permeability of the mucosal barriers may be associated with increased migration of granulocytes into the gut lumen, a phenomenon reflected by high faecal concentrations of calprotectin. As there is no accumulation of calprotectin-rich leucocytes in healthy mucosa in the first few months of life, the high calprotectin concentrations observed may reflect increased intestinal permeability related to transepithelial migration of neutrophils as observed in adults with inflammatory bowel disease.¹⁸ Furthermore, it has been suggested that increased intestinal permeability and intestinal inflammation are closely interrelated, especially in children.¹⁹

Interestingly, a preliminary study has been performed with the same methodology in preterm infants, which are known to have a more permeable intestine than term infants. In infants without any gastrointestinal symptoms, the concentration of calprotectin was very high (896 (572) µg/l). Furthermore, concentrations were even higher in infants suffering from gastrointestinal bleeding, an early stage of necrotising enterocolitis (1724 (1355) µg/g).²⁰ Similar results using a different assay were obtained by Carroll *et al*,²¹ with a significant difference between infants with necrotising enterocolitis and age matched controls.

Our data confirm that newborns in the first few days of life have high calprotectin concentrations compared with healthy adults. During the first weeks of life, the composition of the intestinal microflora, which is involved in mucosal immunity maturation, depends on various factors, mainly the type of feeding.¹¹ Although the number of infants in this study was small, differences in feeding mode did not appear to affect calprotectin concentrations in the first week of life.

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