A high-protein formula increases colonic peptide transporter 1 activity during neonatal life in low-birth-weight piglets and disturbs barrier function later in life

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Abstract
Dietary peptides are absorbed along the intestine through peptide transporter 1 (PepT-1) which is highly responsive to dietary protein level. PepT-1 is also involved in gut homeostasis, both initiating and resolving inflammation. Low-birth-weight (LBW) neonates are routinely fed a high-protein (HP) formula to enhance growth. However, the influence of this nutritional practice on PepT-1 activity is unknown. Intestinal PepT-1 activity was compared in normal-birth-weight (NBW) and LBW piglets. The effect of HP v. normal-protein (NP) formula feeding on PepT-1 activity and gut homeostasis in LBW piglets was evaluated, during the neonatal period and in adulthood. Flux of cephalexin (CFX) across the tissue mounted in Ussing chambers was used as an indicator of PepT-1 activity. CFX flux was greater in the ileum, but not jejunum or colon, of LBW than NBW piglets during the neonatal period. When LBW piglets were formula-fed, the HP formula increased colonic CFX during the 1st week of life. Later in life, intestinal CFX fluxes and barrier function were similar whether LBW pigs had been fed NP or HP formula. However, colonic permeability of HP- but not NP-fed pigs increased when luminal pH was brought to 6.0. The formyl peptide N-formyl methionyl-leucyl-phenylalanine conferred colonic barrier protection in HP-fed piglets. Heat shock protein 27 levels in the colonic mucosa of HP-fed LBW pigs correlated with the magnitude of response to the acidic challenge. In conclusion, feeding a HP formula enhanced colonic PepT-1 activity in LBW pig neonates and increased sensitivity of the colon to luminal stress in adulthood.

Key words: Peptide absorption: Low-birth-weight neonates: N-Formyl methionyl-leucyl-phenylalanine: Formulas
Neutrophil chemotactic substances. PepT-1 was therefore believed to promote inflammation. As such, it has been reported that PepT-1 is highly expressed in a chronically inflamed colon in adults. Infusion of the model peptide N-formyl methionyl-leucyl-phenylalanine (f-MLP) elicits neutrophil infiltration and inflammation in the jejunum of adult rats within 4 h. Inhibition of PepT-1 reduced this effect. Infusion of f-MLP also increases ileal permeability in rats through a neutrophil-derived oxidant-dependent mechanism. However, participation of formyl peptide receptors 1 and 2 in gut homeostasis and repair mechanisms against oxidative and inflammatory damage by f-MLP through heat shock protein (Hsp) 27 induction has also been demonstrated. Cell protection mechanisms against oxidative and inflammatory damage by f-MLP seems to be involved in this protective effect.

Recent data indicated that changes in gut homeostasis during early life have a durable impact on gut function. This was first exemplified by early life stress that disturbs the cross-talk between gut microbiota, barrier function and neurons in rat neonates and is followed by greater response to gut inflammation or visceral hypersensitivity in adults. Our group recently demonstrated that HP formula feeding influences distal intestinal homeostasis in pigs both during the neonatal period and in adulthood. Based on these literature data and our published data on the developmental pattern of PepT-1 mRNA in LBW piglets, we hypothesised that LBW piglets have increased PepT-1 activity in the distal intestine that might be accentuated by HP formula feeding. Furthermore, we speculated that changes in PepT-1 activity would have long-term consequences on distal intestine barrier function and homeostasis. The objectives of the present study were therefore (1) to investigate PepT-1 activity in LBW and NBW piglets at different ages, (2) to examine the effect of HP formula feeding on PepT-1 activity in LBW piglets and (3) to evaluate whether distal intestine barrier function is disturbed in adult LBW pigs fed a HP formula during neonatal life.

Materials and methods

Animal procedures

The experimental protocol was designed in compliance with the recommendations of the French and European law (Décret: 2001-664 29/05/01, 86/609/CEE) for the care and use of laboratory animals under the certificate of authorisation to experiment on living animals no. 35-69. Cross-bred (Pétrain × Landrace) piglets from the experimental herd of INRA (St-Gilles, France) were used in two separate experiments.

Expt 1: comparison of peptide transporter 1 activity in low-birth-weight and normal-birth-weight suckling piglets

A total of fifteen LBW piglets (average birth weight 1·01 kg, range 0·74–1·21 kg) and fifteen NBW piglets (average birth weight 1·5 kg, range 1·32–1·64 kg) were selected at birth based on birth weight. They originated from eight different litters representative of our herd (number of piglets per litter 15±5 (SEM 0·6) and average birth weight 1·24 (SEM 0·05) kg). LBW piglets were chosen with a birth weight 30% lower than the average birth weight of the litter. Due to this constraint in birth weight, it was not possible to take sex into account. However, the number of same-sex and non-same-sex pairs of piglets was balanced among the three different age groups. Piglets suckled their mother until PND21 and had no access to creep feed. Of the piglets, one-third was killed at PND2, one-third at PND7 and one-third at PND21. Piglets were anaesthetised with isoflurane, delivered through a veterinary anaesthesia ventilator. They were then euthanised by intracardiac T-61 injection (Elvetis). After laparotomy, 15 cm segments of jejunum (20 cm distal from the Treitz ligament), ileum (20 cm proximal to the ileo-caecal junction) and colon (15 cm distal from the ileo-caecal junction) were collected, rinsed with cold 0·9% NaCl solution and immediately placed in Ringer’s bicarbonate (composition in mmol/l: Na+ 145, Cl− 128, PO4− 3 0·32, Ca2+ 2 1·25, Mg2+ 1·1, HCO3− 25, SO4− 2 1, K+ 6·3, pH 7·4) for the Ussing chamber experiment (cephalexin (CFX) flux experiment, without electrophysiological measurements).

Expt 2: effect of a high-protein formula on peptide transporter 1 activity and mRNA expression in low-birth-weight piglets

A total of forty-eight LBW piglets were fed from PND2 to PND28 a normal-protein (NP) formula (50 g protein/l) or a HP formula (77 g protein/l; Table 1), and then a pig-dedicated diet until PND160 as described already. Piglets were killed at PND7 (n 6 per group), PND28 (n 6 per group) and PND160 (n 12 per group). They were euthanised by electrocution followed by exsanguination. After laparotomy, the segments of the distal ileum and proximal colon were collected as described above. Pieces of tissue were collected for the Ussing chamber experiment (CFX flux experiment, without electrophysiological measurements at PND7, PND28 and PND160 and barrier function and electrophysiological measurements at PND160). Pieces of tissue (100 mg) were also collected and either placed in RNAlater (Applied Biosystems) for 24 h at 4°C and stored at −20°C until RNA extraction or snap-frozen in liquid N2 and stored at −80°C until Western blot analysis.

Ussing chamber experiment

Intestinal tissues were stripped of the external muscle layers (except for colonic tissues at PND7 and PND28 where the muscle layers were left intact to avoid deteriorating the mucosa when stripping) and opened along the antimesenteric border and then mounted in the Ussing chamber (World Precision Instrument). The chamber opening exposed 0·67 cm2 of tissue surface area to 8 ml of circulating oxygenated buffer (see below for composition) at 39°C.

Table 1. Composition of the neonatal formula

<table>
<thead>
<tr>
<th>Composition</th>
<th>NP</th>
<th>HP</th>
<th>Sow milk</th>
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</thead>
<tbody>
<tr>
<td>Energy (MJ/l)</td>
<td>4·75</td>
<td>5·03</td>
<td>4·70</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>51</td>
<td>77</td>
<td>50</td>
</tr>
<tr>
<td>Fat (g/l)</td>
<td>82</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>Carbohydrate (g/l)</td>
<td>49</td>
<td>46</td>
<td>51</td>
</tr>
</tbody>
</table>

NP, normal protein; HP, high protein.
Peptide transporter 1 activity in different segments of the intestine was evaluated by measuring mucosal-to-serosal fluxes of CFX (CFX, an aminocephalosporin transported through PepT-1(18) and not metabolised by the intestine(19)). One piece of proximal jejunal, distal ileal and proximal colonic tissue per piglet (PND2, PND7 and PND21 for Expt 1 and PND7, PND28 and PND160 for Expt 2) was bathed in 10 mM-HEPES Tris buffer (pH 7.4) on the serosal side. In preliminary experiments, we evaluated CFX passage across the intestinal tissues of NBW piglets in all chambers, and serosal samples were collected every 30 min for 120 min. Concentrations of horseradish peroxidase (5'-CATCCATGACACTACCTCGC-3'; 5'-GCATGGACTGTGGTCATGAGTC-3') were used as internal standards. CFX was added in the mucosal compartment of the Ussing chambers (World Precision Instrument) after a 20 min equilibration period. Serosal sample was collected after 60 min to measure CFX concentration by HPLC.

At PND160, we also evaluated ileal and colonic barrier function in standard or challenged conditions. Briefly, three adjacent pieces of ileum or colon per pig were mounted in Ussing chambers (World Precision Instrument). The transepithelial potential difference of the intestinal tissue was clamped at 0 mV by an external current (short-circuit current, Isc). Transepithelial resistance (R) was calculated from Ohm’s law from current deflections in response to 3 mV transepithelial voltage pulses of 300 ms every 10 s. We used a mild acidic stress (change from pH 7.4 to 6.0) associated or not with the inflammatory peptide f-MLP. Acidic conditions up to pH 6.0 (as opposed to pH 5.5) do not disturb intestinal epithelial cell barrier function (transepithelial resistance and flux of sulphonic acid) in normal conditions(20), but enhances epithelial barrier sensitivity to inflammatory stimuli(21). On both sides, one segment was mounted with Ringer buffer (pH 7.4), supplemented with 10 mM-mannitol and 10 mM-glucose on the luminal and serosal sides, respectively. Thereafter, two adjacent segments of tissues were mounted with 10 mM-HEPES Tris buffer (pH 6.0) on the luminal side and 10 mM-HEPES Tris buffer (pH 7.4) on the serosal side. After a 20 min equilibration period, 100 mM-f-MLP was added to the mucosal side of one of these later chambers. Since f-MLP absorption through PepT-1 requires acidic conditions on the mucosal side, we omitted the pH 7.4 + f-MLP condition. After 10 min, 10 µM-horseradish peroxidase and fluorescein isothiocyanate-dextran 4000 (FD-4) were added to the luminal side in all chambers, and serosal samples were collected every 30 min for 120 min. Concentrations of horseradish peroxidase and FD-4 were determined as described already(16).

Western blot analysis

Colonic mucosa protein was extracted in a TEX 1X(10 mM-Tris-HCl, 0.1 mM-EDTA, 0.01% Triton X-100), 60 mM Tris-base, pH 6.8, 10% glycerol, 3% SDS, 5% β-mercaptoethanol and a protease inhibitor cocktail (104 mM-4-(2-aminoethyl)-benzenesulfonyl fluoride, 80 µM-aprotinin, 4 mM-bestatin, 1.4 mM-E-64, 2 mM-leupeptin, 1.5 mM-pepstatin A, Sigma-Aldrich) buffer. Levels of Hsp27 and Hsp70 were then determined by Western blot as described previously(22). Densitometry of blots was measured and expressed relatively to β-actin density of the corresponding sample.

Statistical analysis

Data were analysed using the program GraphPad Prism (GraphPad Software, Inc.). In Expt 1, a two-way ANOVA was performed, testing piglet age, piglet birth weight status and the interaction between these two factors. Data from Expt 1 and Expt 2 (PND7 and PND28) were combined and analysed by one-way ANOVA. The t-test with Bonferroni correction was used as a subsequent multiple comparison test. Data from piglets at PND160 in Expt 2 were analysed using a t-test for basal parameters. Colonic barrier function responses to luminal stress were analysed using a two-way ANOVA, testing the neonatal diet, luminal treatment and the interaction between these two factor effects. The t-test with Bonferroni correction was used as a subsequent multiple comparison test. Data are presented as means with their standard errors. A P-value ≤ 0.05 was considered significant.

Results

Peptide transporter 1 activity in suckling low-birth-weight and normal-birth-weight piglets during the neonatal period

CFX fluxes across the proximal jejunal mucosa were similar in LBW and NBW piglets, irrespective of their postnatal age (Fig. 1(a)). In the ileum, no difference in CFX flux was observed between the NBW and LBW piglets at PND2. However, LBW piglets exhibited a 5-fold greater flux of CFX (P = 0.035; Fig. 1(b)) at PND7. Flux of CFX across the ileum
tended to stay greater in LBW than NBW piglets (P = 0.09; Fig. 1(b)) at PND21. In the proximal colon, although the CFX flux was increased 3-fold in LBW compared with NBW piglets at PND2, the difference did not reach significance (P = 0.17; Fig. 1(c)). No difference was observed thereafter.

Effect of a high protein formula on peptide transporter 1 activity and barrier function in low birth weight piglet ileum and colon

During the neonatal period. Formula feeding did not change CFX flux across the ileal tissue of LBW piglets at PND7, irrespective of the level of protein in the formula (Fig. 2(a)). At PND28, no difference in ileal CFX flux was observed between the NP- and HP-fed piglets (Fig. 2(a)). In the colon, NP formula feeding did not alter CFX flux across the mucosa compared with suckled LBW piglets. However, feeding a HP formula resulted in greater CFX flux across the colonic mucosa, especially at PND7 where CFX flux was enhanced 3.6-fold in HP- compared with NP-fed piglets (Fig. 2(b)). PepT-1 mRNA relative expression was unchanged by the type of formula in both segments and at both ages (Table 2).

Later in life. At PND160, neither ileal barrier function nor CFX flux across the ileum was different between the NP- and HP-fed pigs (Table 3). Stress conditions (pH 6.0 buffer or pH 6.0 buffer + f-MLP) did not modify ileal permeability (data not shown). Basal colonic barrier function parameters as well as CFX flux across the colonic mucosa of NP or HP formula-fed piglets were also similar between the NP- and HP-fed pigs (Table 3). When luminal pH was brought to 6.0, colonic flux of FD-4 dramatically increased compared with pH 7.4 in the colonic tissue of HP- but not NP-fed pigs (Fig. 3). The addition of f-MLP to the mucosal side of the colonic tissue restored FD-4 flux in HP-fed pigs (P < 0.001, Fig. 3). Horse-radish peroxidase flux across the colonic mucosa was not significantly altered by acidic pH or by the addition of f-MLP, irrespective of the pig neonatal diet (data not shown).

Fig. 1. Flux of cephalexin (CFX) across the different intestinal segments of normal-birth-weight (NBW) and low-birth-weight (LBW) suckling piglets at different postnatal ages. CFX fluxes across the (a) proximal jejunum, (b) ileum and (c) proximal colon were determined at postnatal day (PND) 2, PND7 and PND21 in NBW (A) and LBW (B) piglets. Values are means (n = 5–6 per group), with their standard errors represented by vertical bars. * Mean values with unlike letters were significantly different (P < 0.05).

Fig. 2. Flux of cephalexin (CFX) across the ileal and colonic mucosa of low-birth-weight (LBW) suckled piglets or piglets fed formulas differing in protein content. CFX flux across the (a) ileal and (b) proximal colon was determined at postnatal day (PND) 2, PND7, PND21 and PND28 in LBW suckled piglets (D) or in LBW piglets fed either a normal-protein (E) or a high-protein formula (F) from PND2 to PND28. Values are means (n = 5–6 per group), with their standard errors represented by vertical bars. * Mean values with unlike letters were significantly different (P < 0.05).
respectively). No such correlation was observed in NP-fed
decrease induced by the addition of f-MLP correlated
also correlated negatively with Hsp27 levels, whereas the
(Fig. 4(a)). The flux increase from neutral to acidic conditions
correlated negatively with FD-4 fluxes across the colonic
mucosa in luminal acidic conditions in HP-fed pigs
P
(mean values with their standard errors)

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<th>PND7</th>
<th>PND28</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Ileum NP</td>
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</tr>
<tr>
<td>Ileum HP</td>
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<tr>
<td>Colon NP</td>
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<td>Colon HP</td>
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<td>0·4</td>
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</table>

NP, normal protein; HP, high protein.
*Levels of PepT-1 mRNA (normalised to that of glyceraldehyde-3-phosphate
dehydrogenase (Gapdh)) were measured at PND7 and PND28 in the ileum and
colon of low-birth-weight piglets that were fed the NP or HP formula from PND2
to PND28.

To evaluate whether Hsp was involved in the protective
effect of f-MLP on barrier function, we measured the levels
of Hsp27 and Hsp70 in the colonic mucosa by Western blot
at PND160. No difference between the dietary groups was
observed (Hsp27: NP 0·42 (SEM 0·08) v. HP 0·41 (SEM 0·07),
P = 0·92 and Hsp70: NP 0·95 (SEM 0·09) v. HP 0·93 (SEM 0·10),
P = 0·88). Interestingly, Hsp27 levels in colonic mucosa
correlated negatively with FD-4 fluxes across the colonic
mucosa in luminal acidic conditions in HP-fed pigs
(Fig. 4(a)). The flux increase from neutral to acidic conditions
also correlated negatively with Hsp27 levels, whereas the
decrease induced by the addition of f-MLP correlated
positively with Hsp27 levels in HP-fed pigs (Fig. 4(b) and (c),
respectively). No such correlation was observed in NP-fed
animals or with Hsp70 levels (data not shown).

Discussion

PepT-1 is a singular intestinal transporter exhibiting multi-
faceted function, from peptide and amino-acid supply to
bacterial–host communication controlling gut homeostasis
and inflammation. It has also unique developmental features
with transient high expression in the colon of neonatal
animals during very early life and many regulatory pathways
in health and disease. The present study extends this pattern
of peculiar regulatory pathways to LBW neonates whose nutri-
ent requirements, intestinal development and gut homeostasis
characteristics are overlooked but yet of great importance for
neonatal care givers. We demonstrated that LBW neonatal
piglets exhibited greater PepT-1 activity in the ileum during
the neonatal period compared with NBW piglets. Further-
more, increasing dietary protein supply with HP formula
feeding increased PepT-1 activity in the colon. Later in life,
colonic barrier function of HP-fed pigs was more sensitive to
luminal stress (modelled here by a mild change in luminal
pH) than that of NP-fed pigs. Furthermore, f-MLP conferred
protection to HP colonocyte barrier function. This protective
effect of the bacterial peptide was unlikely accounted for a
higher entry into colonocytes since PepT-1 activity was similar
in the colon of NP- and HP-fed pigs at that age. Conversely,
a role for Hsp27 is conceivable as demonstrated by the negative
correlations between Hsp27 levels in the colon and the colonic
response to stress and the positive correlation between Hsp27
levels and barrier function restoration by f-MLP.

Peptide transporter 1 activity in low-birth-weight piglets

LBW piglets are characterised by delayed maturation of
intestinal functions during the 1st days of life compared with
NBW piglets(14,23) followed by catch-up for some but not all intestinal functions, resulting in no apparent gross
anatomical difference between LBW and NBW piglets but
showing transcriptomic, proteomic and functional differ-
ences at the end of the neonatal period or immediately
after weaning(24–26). Our data demonstrate increased
PepT-1 activity, illustrated with CFX flux, in the ileum of
LBW compared with NBW piglets with a significant differ-
ence at PND7. One could argue that CFX crosses intestinal
mucosa not only through PepT-1 but also through tight
junctions in the paracellular space. However, paracellular
transport of CFX is unlikely for two reasons. First, we
(G Boudry and I Le Huerou-Luron, unpublished results)
and others(18) demonstrated that CFX transport is a saturating
transport of CFX is unlikely for two reasons. First, we
(G Boudry and I Le Huerou-Luron, unpublished results)
and others(18) demonstrated that CFX transport is a saturating
process, whereas paracellular leak would be non-saturating.
Second, we previously observed lower paracellular

Table 2. Ileal and colonic peptide transporter 1 (PepT-1) mRNA levels at postnatal day (PND) 7 and PND28*
(Mean values with their standard errors)

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<th>PND7</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
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<tr>
<td>Ileum NP</td>
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<tr>
<td>Ileum HP</td>
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<td>Colon NP</td>
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<td>0·6</td>
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<tr>
<td>Colon HP</td>
<td>1·6</td>
<td>0·4</td>
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</table>

NP, normal protein; HP, high protein.
*Levels of PepT-1 mRNA (normalised to that of glyceraldehyde-3-phosphate
dehydrogenase (Gapdh)) were measured at PND7 and PND28 in the ileum and
colon of low-birth-weight piglets that were fed the NP or HP formula from PND2
to PND28.

Table 3. Ileal and colonic barrier function parameters and cephalaxin (CFX) fluxes at postnatal day (PND) 160*
(Mean values with their standard errors)

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<th></th>
<th>Ileum</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
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<tr>
<td>FD4 flux (ng/cm² per h)</td>
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<td>64</td>
</tr>
<tr>
<td>HRP flux (ng/cm² per h)</td>
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</tr>
<tr>
<td>CFX flux (μmol/cm² per h)</td>
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<td>26</td>
</tr>
<tr>
<td>Isc (mA/cm²)</td>
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<td>10</td>
</tr>
<tr>
<td>R (mS/cm²)</td>
<td>21</td>
<td>2</td>
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</table>

NP, normal protein; HP, high protein; Isc, short-circuit current; R, transepithelial resistance; FD4, fluorescein isothiocyanate-
dextran 4000; HRP, horseradish peroxidase.
*Intestinal barrier function parameters (Isc, R, flux of FD4 (4 kDa) and HRP (40 kDa)) as well as flux of CFX across the ileum and
colon mounted in Ussing chambers were measured in PND160 pigs that had been fed the NP or HP formula from PND2
to PND28.
permeability in the ileum of LBW compared with NBW piglets at PND28\(^{27}\), which would not fit with enhanced CFX flux if it was a paracellular passage.

Our previous data showed that the delay in PepT-1 mRNA maturation was transient in early life and that by PND5, PepT-1 mRNA levels were similar in NBW and LBW piglets in the ileum and colon\(^{14}\). Functionally, this did not translate into enhanced PepT-1 activity at PND2 but a few days later (PND7) in the ileum with no significant difference in PepT-1 activity in the colon. Discrepancy between mRNA levels, protein expression and activity of PepT-1, especially in the colon, has already been observed either in healthy or diseased states\(^{28}\). A very recent report has demonstrated increased PepT-1 mRNA but not protein expression in LBW rat pups born to dams fed a low-protein diet compared with NBW pups\(^{29}\), corroborating differences between LBW and NBW neonates at the gene expression level that do not translate into difference in function.

**Effect of high-protein formula during the neonatal period**

Several studies have demonstrated that HP or high peptide intake increases PepT-1 expression *in vitro* in intestinal epithelial cell lines\(^{30,31}\) or *in vivo* in the small intestine of adult rodents\(^{32,33}\). PepT-1 regulation by its own substrate during in vivo experiments has been demonstrated by the spectacular fall in mRNA level between 3-week-old unweaned and 6-week-old fully weaned Yucatan piglets\(^{35}\) and cannot be further enhanced by dietary protein. In the colon, PepT-1 regulation by protein might be more flexible and the 25% increase of protein concentration in the luminal content was greater in our HP-fed piglets at least during the 1st week of HP formula feeding. The reason why PepT-1 activity was not enhanced in the small intestine by the increased substrate availability is unclear. It can be speculated that PepT-1 expression and activity in the small intestine are maximal during the neonatal period as illustrated by the spectacular fall in PepT-1 mRNA level between 3-week-old unweaned and 6-week-old fully weaned Yucatan piglets\(^{35}\) and cannot be further enhanced by dietary protein. In the colon, PepT-1 regulation by protein...
flowing through the ileo-caecal junction might be sufficient to enhance PepT-1 activity. Another possible pathway for specific up-regulation of PepT-1 activity in the colon could be pro-inflammatory signals since PepT-1 expression and activity have been shown to be induced by TNF-α and interferon-γ. However, our previous data did not show any sign of inflammation in the colon of piglets fed a HP formula, weakening this hypothesis.

**Effect of the high-protein formula on colonic barrier function later in life**

The present study provides evidence that feeding a HP formula during the neonatal life has long-term consequences on colonic homeostasis. We show that a mild stress (slightly acidic pH) alters barrier function in the colonic mucosa of HP- but not NP-fed pigs. In Caco-2 cells, a decrease in pH from 7.4 down to 6.0 does not modify epithelial permeability, whereas a pH of 5.5 dramatically increases monolayer permeability, suggesting that intestinal epithelial cells are able to maintain epithelial barrier integrity up to a certain level of metabolic stress. Our data suggest that neonatal HP feeding alters the sensitivity of intestinal epithelial cells to metabolic stress. The effect of pH change was rapid since a change in permeability was observed within the 2 h of the Ussing chamber experiment. Colonic barrier function in basal conditions was not different between the NP- and HP-fed pigs, suggesting that expression of tight junction proteins was similar between the two groups. The change in colonic permeability with the acidic pH was therefore either linked to the change in tight junction localisation or phosphorylation and cytoskeleton reorganisation as already established with other metabolic stresses, or to altered metabolic rescue pathways required under metabolic stress. These data are in line with our previous data showing that female colonic mucosa of pigs fed a HP formula during early life was more sensitive to oxidative stress or inflammatory mediators than the colonic mucosa of NP-fed pigs.

Interestingly, addition of f-MLP, a pro-inflammatory peptide transported by PepT-1, reduced the colonic hyper-permeability observed with acidic conditions in HP-fed animals. Previous reports showed that pretreatment with f-MLP protected intestinal epithelial cells from oxidant- or inflammatory mediator-induced depolymerisation of actin and a decrease in transepithelial resistance. This effect was probably mediated by the induction of Hsp27 into epithelial cells since silencing Hsp27 expression inhibited the protective effect of f-MLP. A similar Hsp27-driven protective effect by f-MLP is plausible in the present study since Hsp27 levels correlated negatively with the magnitude of response to the acidic challenge and positively with the magnitude of response to f-MLP. However, we must stress out that Hsp27 measured here is constitutive Hsp27 and not Hsp27 induced by the acicid challenge or f-MLP incubation. Moreover, these correlations were observed only in HP- not NP-fed animals that displayed no reaction to the acidic challenge. Finally, the mean level of Hsp27 in colonic mucosa was not different between the NP- and HP-fed pigs. A complex relationship between priming of epithelial cells during the neonatal period induced by a greater invasion of bacterial peptides into colonocytes through PepT-1 and response to f-MLP and induction of Hsp27 later in life in HP-fed pigs can therefore be hypothesised.

Taken together, these experiments demonstrate that LBW neonates have unique intestinal peptide transport capacity that can be greatly influenced by the diet, especially in the colon. The nutritional management of LBW infants that are routinely fed a HP formula may result in enhanced activity of the transporter in the colon during early life. This practice seems also to have consequences on colonic homeostasis later in life. Considering the high level of potentially harmful bacterial peptides present in that part of the gastrointestinal tract at both periods of life, immunological consequences and mechanisms warrant further investigations.

**Acknowledgements**

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The authors’ contributions are as follows: G. B. and I. L. H.-L. formulated the research question; G. B., A. J., G. S. and I. L. H.-L. designed the study; G. B., V. R., C. P., A. J., G. S. and I. L. H.-L. carried out the study; G. B., V. R., C. P. and G. S. analysed the data; G. B. and I. L. H.-L. wrote the paper.

The authors declare that they have no conflict of interest.

**References**


