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Effect of osmolality on net fluid absorption in non-infected and ETEC-infected piglet small intestinal segments

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Abstract

In the small intestinal segment perfusion model the effect of osmolality on net fluid absorption in enterotoxigenic *Escherichia coli* (ETEC)-infected and non-infected small intestinal segments of piglets was investigated. In ETEC-infected segments net fluid absorption was reduced. Lowering the osmolality from 375 to 150 mOsmol/l by reducing sodium chloride concentrations increased net fluid absorption. There was a linear relationship between osmolality and net fluid absorption for both non-infected and ETEC-infected segments. Below 150 mOsmol/l the inverse relationship between osmolality and net fluid absorption disappeared. Substitution of sodium chloride with mannitol decreased net fluid absorption since mannitol cannot be actively absorbed. In ETEC-infected segments perfused with oral rehydration solution net fluid absorption significantly increased compared to a sodium chloride solution of similar osmolality, probably because of the high glucose concentration. Supplying hypotonic sodium chloride fluids to piglets can be beneficial in replenishing water and electrolyte losses in case of infectious diarrhoea.

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1. Introduction

Weaning is a critical phase in pig production. At a young age piglets are confronted with separation of the sow and a change in diet. At the same time moving to another accommodation with another climate and mixing of different littermates frequently occurs. As a consequence, feed intake is reduced and does not meet energy requirement during the first days post weaning. The integrity of the gastrointestinal tract is impaired, resulting in post-weaning problems such as growth depression and occurrence of diarrhoea.

Diarrhoea is often associated with different *Escherichia coli* bacteria, and colibacillosis is the most common cause of post-weaning mortality, killing 1.5–2.0% of the weaned piglets (Hampson, 1994) and causing major economic losses (van Beers-Schreurs and Bruininx, 2002). Enteric diseases caused by strains of enterotoxigenic *E. coli* (ETEC) are not only the most common type of colibacillosis in pigs, but also occur widely in humans (Nagy and Fekete, 1999) and are the predominant causative micro-organism of severe diarrhoea in children in developing countries (Bhan, 2000).

Various strategies for controlling diarrhoea in young piglets, such as use of vaccination strategies, in-feed antibiotics, spray-dried plasma proteins, pro- and prebiotics, organic acids, and zinc and copper salts, have been tried with mixed results (Fairbrother et al., 2005; Owusu-Asiedu et al., 2003), and diarrhoea still occurs. Infants and children with dehydration due to acute diarrhoea are treated

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effectively with oral rehydration solution (ORS), and clinical studies show that reducing the osmolality of ORS reveals beneficial effects on the clinical course of acute diarrhoea in children (Thillainayagam et al., 1998).

In this study, we have investigated the effect of osmolality on net fluid absorption in piglets, using the small intestine segment perfusion (SISP) test (Nabuurs et al., 1993) with ETEC-infected and non-infected segments.

2. Materials and methods

All procedures involving animal handling and testing were reviewed and approved by the Animal Care and Ethics Committee of the Animal Sciences Group Lelystad, The Netherlands.

2.1. Animals

Piglets (crossbred Yorkshire × (Large White × Landrace)) were weaned at three weeks of age. They were transported to the institute and fed a standard piglet feed. Water containing 60 mg/l colistinsulphate (Colistine 1200WO, Eurovet, Bladel, The Netherlands) was applied ad libitum. Two weeks after weaning a biopsy from the duodenal mucosa was taken using a fiberscope (Olympus GIF XP10, Olympus, Hamburg, Germany) and F4 receptor status was determined (Sellwood et al., 1975). Piglets that expressed F4 receptors involved in binding of the ETEC strain were used in the experiments three weeks after weaning.

2.2. Bacterial strain

Enterotoxigenic *E. coli* (ETEC) (O149:K91:K88^{ac}), expressing the F4 fimbriae and producing heat-labile toxin (LT) and heat-stable toxin b (STb), was grown for 16–18 h at 37 °C in brain-heart infusion. The culture was centrifuged and the pellet was suspended in phosphate-buffered saline (PBS) to an absorption value of 1.0 measured at 600 nm spectrophotometrically, corresponding to 1×10^9 colony forming units/ml.

2.3. Perfusion fluids

In the first experiment with eight piglets sodium chloride based solutions in the range of 150–375 mOsmol/l (C150, C225, C300 and C375; Table 1) were used in two repetitive Latin Square designs. The range was enlarged to 0–600 mOsmol/l (C0, C75, C150, C300 and C600; Table 1) in the second experiment with five piglets in a Latin Square design. All perfusion solutions contained 1 g/l glucose and 1 g/l amino acids to assure bacterial growth (Nabuurs et al., 1993). In the third experiment with four piglets, sodium chloride perfusion fluids (C150 and C300; Table 1) were compared with solutions in which half of the sodium chloride was replaced by mannitol (C150M and C300M; Table 1) in a Latin Square design, while in

Table 1
Composition of the perfusion fluids

Code	NaCl (g/l)	Glucose (g/l)	Casamino acids (g/l)	D-Mannitol (g/l)	Osmolality ^a (mOsmol/l)
C0	0	1.0	1.0	0	16 ± 3
C75	2.18	1.0	1.0	0	88 ± 1
C150	4.35	1.0	1.0	0	157 ± 1
C225	6.53	1.0	1.0	0	227 ± 3
C300	8.70	1.0	1.0	0	288 ± 1
C375	10.88	1.0	1.0	0	360 ± 1
C600	17.40	1.0	1.0	0	566 ± 4
C150M	2.18	1.0	1.0	13.65	167 ± 1
C300M	4.35	1.0	1.0	27.30	312 ± 1

^a Values are means ± standard deviation ($n = 4$).

the same experiment the sodium chloride C300 was compared to the standard ORS (3.5 g/l sodium chloride, 1.5 g/l potassium chloride, 2.9 g/l sodium citrate dihydrate and 20 g/l glucose; 311 mOsmol/l) of the World Health Organization (Nalin et al., 2004).

2.4. Surgical procedure

The anaesthetic and surgical procedures used were essentially as described before (Nabuurs et al., 1993). The animal was tranquillized with 2 mg/kg bodyweight azaperon (Stresnil®, Janssen-Cilag BV, Tilburg, The Netherlands). Anaesthesia was induced and maintained throughout the whole experiment with sevoflurane (Sevoflurane®, Abbott BV, Amstelveen, The Netherlands) and nitrous oxide. The piglets were placed in dorsal recumbency on a heated surface, to control body temperature. The abdominal cavity was opened and the first intestinal segment was prepared approximately 300 cm caudal to the stomach. A small cranial tube (inflow; inner diameter 3 mm, outer diameter 5 mm) was placed and a wide tube (outflow; inner diameter 5 mm, outer diameter 9 mm) was placed about 20 cm distal from the first. Caudal from and adjacent to this first segment nine other segments were prepared in the same way. In this way the ten segments were situated on average at 50% of the total length of the small intestine. Between odd and even segments 2 cm pieces of the intestine were removed for measurement of the circumference.

2.5. Perfusion procedure

Fifteen minutes before perfusion started segments 2, 4, 6, 8 and 10 were injected with 5 ml of PBS and the other segments were injected with 5 ml of the ETEC suspension in PBS. Simultaneously, the 10 segments were perfused with the different fluids all perfusing a non-infected as well as the adjacent ETEC-infected segment. Each segment was perfused with 64 ml of perfusion fluid over 8 h, by injecting 2 ml of fluid every 15 min. The non-absorbed fluid passed through the outflow tubes into corresponding drainage bottles placed at the same level as the piglet's abdomen.

At the end of the experiment the fluid remaining in the segments was blown out into the drainage bottles (outflow). The piglets were killed by injection of 200 mg/kg body-weight sodiumpentobarbital (Dolethal®, Vétquinol B.V., 's-Hertogenbosch, The Netherlands). The segments were cut from the mesenterium and the length was measured.

Sodium and chloride concentrations of the perfusion fluids and the outflow were determined using an Electrolyte 4⁺ analyzer (Noval Biomedical, Waltham, MA, USA). Osmolality was determined using a cryoscopic osmometer (Osmomat, Gonotec, Berlin, Germany).

Net fluid absorption was calculated as the difference between the volume of inflow and the volume of outflow, divided by the surface area (length times circumference) of the segment. The net absorption of sodium and chloride was calculated from the volume and concentration in the perfusion fluid and the volume and concentration in the outflow, divided by the surface area of the segment.

2.6. Statistics

Results of the first experiment were analyzed using linear regression (GraphPad, 2004). Results from non-infected and ETEC-infected segments perfused with the same perfusion fluid were compared using the Student's paired *t* test, whereas comparisons between different perfusion fluids tested in the Latin Square design were done with one way analysis of variance (ANOVA). Net absorption of fluid, sodium and chloride are presented as the mean $\pm$ standard error of the mean.

3. Results

Lowering the osmolality from 375 to 150 mOsmol/l by reducing sodium chloride concentrations resulted in an increased net fluid absorption, both in non-infected and ETEC-infected segments (Fig. 1). There was a linear relationship between the osmolality of the perfusion fluids and the average net fluid absorption for both non-infected and ETEC-infected segments. The slopes of the two lines were not different, but there was a significant difference in the elevations ($P < 0.0001$). For a perfusion fluid of 300 mOsmol/l this generated a net fluid absorption of 587 and 203 $\mu\text{l}/\text{cm}^2$ for non-infected and ETEC-infected segments, respectively, corresponding with a reduction in net fluid absorption of 65% upon ETEC infection.

Fig. 1. The relationship (with 95% confidence intervals) between osmolality (osm) and net fluid absorption (fl) for non-infected (fl = $1162 - 1.91 \times \text{osm}$; $r^2 = 0.96$) and ETEC-infected (fl = $947 - 2.48 \times \text{osm}$; $r^2 = 0.98$) segments.

Decreased sodium and chloride concentration and hence decreased osmolality of the perfusion fluid resulted in significant decreased sodium and chloride concentrations and osmolality of the outflow of both non-infected and ETEC-infected segments (Table 2). There were no significant differences between sodium and chloride concentration and osmolality in the outflow of non-infected and ETEC-infected segments. Net sodium and chloride absorption was significantly higher for non-infected segments compared to ETEC-infected segments (Table 3).

Lowering the sodium and chloride concentration of the perfusion fluid below 150 mOsmol/l, did not result in a further increase of net fluid absorption (Fig. 2A) while absorption of both sodium and chloride decreased (Fig. 2B). At osmolalities higher than 150 mOsmol/l only a small increase in sodium absorption was seen. Osmolality of the outflow increased during perfusion with fluids of increasing osmolality (Fig. 2C). In the range of 0–150 mOsmol/l, osmolality in the outflow of non-infected segments was lower compared to ETEC-infected segments, whereas for high osmolality perfusion fluids, the osmolalities of the outflow were identical for non-infected and ETEC-infected segments.

Substitution of half of the sodium chloride for mannitol in perfusion fluids with total osmolalities of 150 and 300 mOsmol/l (fluids C150 versus C150M and C300 versus C300M) resulted in major reductions in average net fluid absorption both in non-infected and ETEC-infected seg-

Table 2

Osmolality and sodium and chloride concentration in the outflow after perfusion of non-infected and ETEC-infected segments with solutions with osmolalities of 150–375 mOsmol/l

	Na (mM)		Cl (mM)		Osmolality (mOsmol/l)	
	Non-infected	ETEC-infected	Non-infected	ETEC-infected	Non-infected	ETEC-infected
C150	116 $\pm$ 3	123 $\pm$ 3	106 $\pm$ 6	102 $\pm$ 1	244 $\pm$ 10	258 $\pm$ 8
C225	132 $\pm$ 1	132 $\pm$ 1	117 $\pm$ 3	113 $\pm$ 2	267 $\pm$ 2	274 $\pm$ 4
C300	143 $\pm$ 1	141 $\pm$ 1	127 $\pm$ 3	124 $\pm$ 2	286 $\pm$ 3	288 $\pm$ 2
C375	156 $\pm$ 2	151 $\pm$ 2	141 $\pm$ 5	132 $\pm$ 4	309 $\pm$ 3	302 $\pm$ 2

Table 3

Net sodium and chloride absorption after perfusion of non-infected and ETEC-infected segments with solutions with osmolalities of 150–375 mOsmol/l

	Na absorption ($\mu\text{mol}/\text{cm}^2$)		Cl absorption ($\mu\text{mol}/\text{cm}^2$)	
	Non-infected	ETEC-infected	Non-infected	ETEC-infected
C150	68 $\pm$ 4	27 $\pm$ 11**	70 $\pm$ 4	35 $\pm$ 9**
C225	76 $\pm$ 8	40 $\pm$ 12*	76 $\pm$ 8	48 $\pm$ 9*
C300	100 $\pm$ 15	46 $\pm$ 8**	99 $\pm$ 14	53 $\pm$ 8*
C375	101 $\pm$ 10	39 $\pm$ 9***	96 $\pm$ 11	47 $\pm$ 7**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for ETEC-infected versus non-infected.

Fig. 2. Net fluid absorption (A), net sodium absorption (B) and outflow osmolality (C) after perfusion of non-infected and ETEC-infected segments with fluids differing in sodium chloride concentrations resulting in osmolalities of 0–600 mOsmol/l.

ments (Table 4). Doubling the osmolality using sodium chloride (C150 versus C300) resulted in a decrease of net fluid absorption conform the data shown in Figs. 1 and

Table 4

Net fluid absorption in non-infected and ETEC-infected segments perfused with five perfusion fluids differing in composition and/or osmolality

Osmolality (mOsmol/l)	Perfusion fluid	Non-infected	ETEC-infected
± 150	C150	901 $\pm$ 124	568 $\pm$ 96
	C150M	628 $\pm$ 134	348 $\pm$ 92
± 300	C300	536 $\pm$ 231	44 $\pm$ 33
	C300M	99 $\pm$ 88	-193 $\pm$ 62*
	ORS	490 $\pm$ 201	240 $\pm$ 67***

* $P < 0.05$, versus C300.** $P < 0.01$, versus C300M.

2A, while doubling osmolality using mannitol (C150 versus C300M) resulted in a significant larger decrease in net fluid absorption, respectively 901 $\pm$ 124 versus 99 $\pm$ 88 $\mu\text{l}/\text{cm}^2$ for non-infected and 568 $\pm$ 96 versus -193 $\pm$ 62 $\mu\text{l}/\text{cm}^2$ for ETEC-infected segments. Average net fluid absorption was not statistically different ($P = 0.89$) for ORS perfusion fluid and sodium chloride based perfusion fluid C300 in non-infected segments (Table 4). ETEC-infected segments perfused with the ORS perfusion fluid showed significant ($P = 0.04$) higher net fluid absorption compared to segments perfused with sodium chloride based perfusion fluid C300, respectively 240 $\pm$ 67 versus 44 $\pm$ 33 $\mu\text{l}/\text{cm}^2$.

4. Discussion

The SISF model enables us to study the effect of different perfusion solutions on net fluid absorption in non-infected and ETEC-infected small intestinal segments within one piglet. Studying the effect of osmolality on net fluid absorption, we observed a linear increase in net fluid absorption when fluids of decreasing osmolality (sodium chloride concentrations) were used. This corresponds well to data obtained for the rat small intestine (Thillainayagam et al., 1993; Wall et al., 1997). A similar relationship was shown in ETEC-infected segments, corresponding with the correlation between osmolality and net fluid absorption for cholera toxin-induced secreting rat intestine (Cunha Ferreira et al., 1992; Pillai et al., 1994). Below 150 mOsmol/l the inverse relationship between fluid absorption and osmolality of the perfusion solution disappeared, as was shown before in human jejunum (Soergel et al., 1968). In our study we observed a high net fluid absorption at very low osmolality despite low net sodium absorption or even a net sodium secretion, as shown before for secreting rat intestine

(Cunha Ferreira et al., 1992; Pillai et al., 1994; Thillainayagam et al., 1993). Most probably an extremely high osmotic gradient between villous interior and intestinal lumen was created. This could be a more critical factor than fluid absorption depending on sodium (solute) absorption.

Although it is shown in this and several studies on rats (Farthing, 1990; Hunt et al., 1992, 1994; Pillai et al., 1994; Thillainayagam et al., 1993, 1998; Wall et al., 1997), that low osmolality promotes net fluid absorption in normal and (ETEC-induced) secreting intestine, we clearly showed for the first time in piglets that hypotonic solutions do not diminish the decreased net fluid absorption due to ETEC infection. The difference between the net fluid absorption in non-infected and ETEC-infected segments is fairly independent of the osmolality of the perfusion solution. The sodium and chloride concentrations as well as the osmolality of the outflow of the ETEC-infected segments did not differ from that in the non-infected segments, reflecting the capacity of the intestine to react to the infection and maintain homeostasis by rapidly returning luminal osmolality to isotonicity. Maintaining luminal isotonicity probably has a higher priority than keeping a positive fluid and electrolyte balance.

Substitution of sodium chloride with mannitol considerably decreased net fluid absorption both in non-infected and ETEC-infected segments. Similar findings were observed in secreting rat small intestine when a basic perfusion solution was supplemented with mannitol (Cunha Ferreira et al., 1992). Mannitol can not be actively absorbed and therefore will have a negative effect on fluid absorption, because the unabsorbed molecules contribute to luminal osmotic load. The significantly increased net fluid absorption in ETEC-infected segments perfused with ORS compared to a sodium chloride solution of similar osmolality was probably the result of the presence of a high glucose concentration in the ORS perfusion solution. Because of the ETEC infection neutral sodium chloride absorption is inhibited whereas the glucose–sodium co-transporter system remains intact resulting in fluid absorption.

All findings for piglets in the SISP model are in accordance with, confirm and elaborate on previous studies on rat and human intestine and can be summarized as follows. Perfusion of non-infected piglet small intestinal segments with hypotonic solutions resulted in an increased net fluid absorption. Fluid absorption from hypotonic solutions occurs in response to a large osmotic gradient and occurs even in the presence of net sodium secretion. Net fluid absorption was reduced in ETEC-infection, and this reduction was independent of the osmolality of the perfusion solution. Although hypotonicity of intestinal lumen by itself has no direct curative effect on ETEC infection, supplying hypotonic sodium chloride fluid or oral rehydration solutions can be beneficial in replenishing water and electrolyte losses in case of infectious diarrhoea. In the future, we can use the SISP model in piglets to test anti-diarrhoea agents on their capability to decrease the observed differ-

ence in net fluid absorption between uninfected and ETEC-infected segments.

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