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Invited Commentary

Guts, germs and glucose: understanding the effects of prematurity on the interaction between bacteria and nutrient absorption across the intestine

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It is well known that necrotising enterocolitis (NEC) is one of the leading causes of death in preterm infants, and is by far the leading cause of long-term morbidity and mortality in infants from gastrointestinal causes[1]. However, despite numerous theories that have been advanced in order to define the causes of NEC, the precise underpinnings of this disease remain incompletely understood[1–3]. One consistent feature in infants who develop NEC is the observation that this devastating disease develops almost exclusively after feeds have been initiated and in the setting of microbial colonisation of the intestine, raising the distinct possibility that an underlying inability of the premature infant to tolerate bacterial products and feeds may be central in NEC pathogenesis[4]. In this issue, Bering *et al.*[5] seek to test this possibility directly, and in particular have evaluated the novel hypothesis that preterm birth increases the sensitivity of intestinal nutrient absorption to bacterial endotoxins – lipopolysaccharides – that are integral constituents of the cell wall of certain bacteria that are present within the intestinal tract – and that feeding after birth reduces this response. From *ex vivo* studies, Bering *et al.* describe that the preterm piglet intestine displays reduced absorption of feeds compared to term intestine, and that the administration of feeds to the piglet restored absorption to the levels seen in full-term animals. Interestingly, the exposure of bacteria to the intestinal samples resulted in a marked reduction in the absorption of nutrients in both term and preterm piglets. It is noteworthy that the greatest reduction in the extent of nutrient absorption was observed after stimulation of intestine with bacteria that had been obtained from pigs with NEC, providing insights into the physiological relevance of the present findings. And while prematurity was not found to influence the ability of the intestine to respond to bacteria or nutrient absorption, these findings raise the possibility that bacteria may exert previously unrecognised effects on the ability of the host to absorb nutrients, and may indeed provide a link between the seemingly unrelated risk factors for NEC in feeds and bacterial exposure.

It is useful to place the present findings in the context of what is generally known to occur with respect to the interaction between nutrient absorption and bacterial exposure in the intestine. Previous authors have shown that infants and adults with systemic infections and with gastrointestinal disease exhibit impaired nutrient absorption, although the mechanisms involved remain incompletely understood[6,7]. However, previous authors have not fully assessed the relationship between prematurity and nutrient absorption in the presence or absence of NEC-related microbes as the authors now accomplish. Secondly, while it is known that the expression of the membrane proteins that mediate the transport of nutrients across the intestinal epithelium is initially low at birth and increases with age[8–10], the specific effects of bacterial exposure on these processes, and the contribution of prematurity to the degree of acquisition of absorption capacity have not been explored in great detail. Moreover, by using a large animal model system that shares features with the human infant intestine, and by utilising a robust *ex vivo* experimental system, the authors are now able to take a unique reductionist approach to address these questions.

So how do the present findings fit within the conceptual framework of factors that lead to the development of NEC? Much interest in the field has focused broadly on how the premature host fails to adapt appropriately to its indigenous flora, and instead mounts a deleterious pro-inflammatory response first within the intestine and then systemically, leading to NEC. In determining the individual steps which lead to the cascade that culminates in NEC, investigators have shown that the release of pro-inflammatory Molecules such as platelet activating factor plays a role in NEC pathogenesis[11], while signalling through heparin-binding epidermal growth factor may play a protective role in this disease[12]. Others[13,14] have shown that the intestinal epithelium in the premature host is more apt to releasing pro-inflammatory cytokines when compared with post-natal intestine, while a causative role for an underdeveloped intestinal microcirculation that predisposes to impaired perfusion has also been proposed[15,16]. Finally, we and others[17–19] have identified an important role for aberrant activation of the innate immune system of the intestinal epithelium in disease pathogenesis. It is therefore possible that each of these aetiological factors is influenced variably in the premature intestine by the presence of nutrients in the gut and by exposure to bacteria. Further studies along the lines of those that have been performed by Bering *et al.* will need to be completed in order to fully clarify how

each of these factors may act in concert in the steps that lead to NEC development.

It is noteworthy that the present study sought to evaluate a potential role for the lipopolysaccharide receptor, Toll-like receptor (TLR)-4, in the present model. Such a role may indeed have been predicted, given that the authors do demonstrate that bacteria and lipopolysaccharide affect intestinal function within the piglet intestine *ex vivo*. However, the authors did not demonstrate any differences in TLR-4 expression between premature and full-term piglets, despite observing an effect of bacterial exposure on nutrient absorption. These findings are difficult to reconcile in view of an abundance of studies showing the importance of TLR-4 signalling in the gut to the pathogenesis of NEC[17,19–22], as well as studies that have shown that TLR-4 expression is elevated in the premature intestine under conditions that lead to NEC in a variety of species including humans[18,23]. It is possible therefore that the findings in the present study in which changes in TLR-4 expression between premature and postnatal piglet intestine were not detected may simply reflect differences between piglets and other species. Additional investigations in which the piglet intestine is examined from various regions of the bowel and at varying gestational ages may be required in order to fully determine the precise role – if any – of enterocyte TLR-4 in the steps by which bacteria may affect nutrient absorption using the present *ex vivo* system.

In summary, the present findings provide useful information regarding the role of prematurity and bacteria on nutritional absorption across the intestine. While the findings do not provide a definitive link between these factors in a model of NEC, they clearly offer an additional piece to the vast and complex puzzle that characterises the development of NEC.

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