Exploring functional metabolites in preterm infants

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Funding information
C. J. S. acknowledges funding from a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (grant number 221745/Z/20/Z) and the 2021 Lister Institute Prize Fellow Award. NEC-UK supports PhD studentship fees to V. L. R

Abstract
Aim: Metabolomics is the study of small molecules that represent the functional end points of cellular reactions that can impact health. Necrotising enterocolitis (NEC) and late onset sepsis (LOS) are the main cause of death in preterm infants surviving the initial days of life.

Methods: This review will explore and summarise the current literature exploring metabolomics in preterm infants.

Results: There are a relatively limited number of studies investigating metabolomics in preterm infants with NEC and/or LOS and matched controls. Nonetheless, it is evident across longitudinally age-related metabolomic studies that there are significant changes in metabolite profiles post-partum and over the first year of life. Existing studies have reported associations between the metabolite profiles of serum, urine and stool in health and disease in preterm infants. Although some studies have found selected metabolites are associated with disease, the specific metabolites vary from study to study, and larger studies are required. Excitingly, recent work has also begun to untangle how microbially produced metabolites can impact immunoregulation of the infant.

Conclusion: Metabolic exploration is an emerging research area with huge potential for developing novel biomarkers and better understanding disease processes in preterm infants.

Keywords
late onset sepsis, metabolites, metabolomics, necrotising enterocolitis, preterm neonates

1 | PRETERM DISEASE

Necrotising enterocolitis (NEC) is a serious inflammatory gut condition that primarily impacts preterm infants born under 32 weeks gestation. The end point of NEC includes variable intestinal injury that ranges from submucosal oedema and haemorrhage, infiltration of neutrophils in the intestinal wall, epithelial and intestinal villus injury, to necrosis, intestinal wall perforation and often bacterial invasion.1–3 However, the full pathophysiology of NEC is poorly understood. Low bacterial diversity has been reported to be a factor to the disease, but no single bacterial genera or species have been consistently identified as a causative microorganism.4 Visible clinical
signs of NEC are indistinct, but they may show as a medley of the following medical anomalies: decreased oxygenated blood levels, slowed heart rates, apnoea, temperature fluctuations, parental and troptic feed intolerance, vomiting, bloody stools, abdominal distention and/or discolouration and therefore often infant fatigue.  

Late onset sepsis (LOS) is neonatal sepsis that occurs in infants later than 72 h after birth.  

The gold standard method of diagnosis is blood culture, where the causative organism is isolated. However, this can lead to false negatives. Gram-positive organisms are primarily isolated as the causative pathogens for LOS in infants, largely resulting from Coagulase-negative staphylococci (CoNS) (54%) and, to a lesser extent, Enterobacteriaceae (Gram negative, 21%) and Staphylococcus aureus (Gram positive, 18%).

The most common cause of death in premature infants is NEC and LOS. Twenty-one per cent of all deaths in infants born 24–31 weeks gestation are caused by NEC and/or infection. One in 10 infants are born preterm, and this number is increasing every year. Preterm births is a major cause of death and long-term morbidity, including increased risk of cerebral palsy and costs for surgery for NEC and intensive care requirements, costing the public sector approximately £3 billion per year in the United Kingdom.  

1.1 What causes NEC and LOS

Necrotising enterocolitis can arise from several different risk factors, including abnormal bacterial colonisation, bacterial translocation and activation of the cytokine cascade, decrease in epidermal growth factor, increase in platelet activating factor and mucosal damage from free radical production. Infants who show intolerance to enteral feeding are likely to have higher parenterally administered calories, which has also been linked to a higher risk of developing NEC. Furthermore, a relationship between vitamin deficiency and the potential benefit of supplementation of vitamin D in pregnancy is thought to be important in preventing NEC in preterm infants.

There are a number of risk factors for developing NEC that include low birth weight, prematurity and formula rather than breast-feeding. The impact of antibiotics may predispose the neonate to NEC by delaying the colonisation of potentially beneficial bacteria and reducing the diversity of the intestinal microbiome. For instance, a correlation between antibiotic duration and risk of developing NEC among infants without culture-proven sepsis has been reported.  

Early onset sepsis, defined as sepsis onset within 72 h of birth, most likely occurs during birth, with group B Streptococcus (58%) and Escherichia coli (18%) from the mothers’ genital tract is leading to subsequent infection. LOS, on the other hand, is likely to be hospital acquired. As survival has improved in extreme preterm infants, so too has the length of time the preterm infants may spend in intensive care. This increases the use of central venous lines, parental nutrition, mechanical ventilation, use of catheters, endotracheal tubes and other invasive procedures which likely increase rates of LOS.

1.2 Current best practice treatment options for NEC and LOS

Current NEC treatment practices include bowel decompression, antibacterial therapy and haematological and electrolyte support. The provision of breast milk, trophic feeds and the advancement of enteric feeds can reduce the risk of NEC. Further preventative strategies include the use of antibacterial, antioxidants, epidermal growth factor and probiotics.

Prompt antibiotic treatment is needed for infants with LOS as infants can deteriorate rapidly. Diagnostic blood culture is a timely process and can produce false negatives due to the small blood samples taken from the preterm neonates. LOS is treated with an array of different antibiotic types which are typically commenced while awaiting results, which may contribute to immunosuppression and antibiotic resistance for those infants, but it is necessary to control the infection.

2 METABOLITES

The human gut contains a vast collection of metabolically active microbial organisms and a diverse array of biological molecules and cells which make up the human gut environment. This human gut environment also includes a vast number of immune cell types including T cells, phagocytes, lymphocytes, stroma cells and molecules including proteins, sugars and lipids, as well as the microbiome and metabolome. The gut microbiome is a microbial community that includes bacteria, bacteriophages, viruses, archaea and fungi. The metabolome is the collection of functional low molecular weight compounds (metabolites) that represents the functional end points of cellular reactions in a biological system, including carbohydrates, fatty acids, hormones and amino acids. Metabolomics is the study of small molecules, and metabolomic research aims to characterise metabolites produced by gut microbes and host cells and represent a large collective output from the different organs within the human body. For instance, short chain fatty acids (which improve tight
junction integrity within the gut) are produced by anaerobic gut bacteria including bifidobacteria. Metabolites that are observed through metabolomics are impacted by an array of variables such as lifestyle (nutritional and environmental), drug substance usage, disease, genetics and non-biological factors. Metabolomics is a developing technology that has the potential to inform the practice of precision medicine due to its ability to profile such large numbers of metabolites, providing widespread insights into biological processes.

2.1 Sample types

Metabolites can be found in human cells and a range of biological material including urine, blood and stool, providing multiple sample options for metabolomic research. Blood (including serum and plasma) has been the preferred sample type for metabolite work to date (Figure 1). Nonetheless, studies involving urine and stool are increasing, and there is a general rise in metabolite studies over the past 10 years.

Each biological sample choice has a unique biochemical composition which is determined by response to physiological stimuli, resulting in metabolic activity and metabolome profiles that are unique and specific to an individual. Metabolomic analysis of blood (or serum or plasma) provides a snapshot of absorbed luminal metabolites and host metabolic activity across the entire human body. Notably, preterm infants have limited circulating blood volumes and are at higher risk of developing sepsis; thus, invasive procedures are generally not permissible for solely research purposes in this population. For this reason, researchers have turned to salvaging samples that are collected for clinical procedures and where remaining sample would otherwise be discarded. This can limit the utility of the sample, where volume will be limited and samples may have been collected and stored in nonoptimal conditions for downstream metabolomic experiments. Stool as a sample option in comparison with blood/serum/plasma provides a non-invasive means of measuring functional small molecules derived from the gut lumen, including from microbes that colonise in the gut. Stool is a more direct sample obtained from the site of medical interest for NEC. Urine is a downstream sample in comparison and includes more water soluble and metabolic by products. Because urine contains concentrated waste products of many metabolic processes that give insight into the underlying metabolic activity, it is possible to analyse a large number of compounds.

For neonatal practice, non-invasive techniques are used to collect the urine samples, where a sterile ball of cotton is placed into the nappy/diaper and absorbed urine is transferred with a sterile syringe into a sterile cryovial. Stool sample collection in neonatal practice uses sterile sampling spoons to directly transfer the sample from the infants nappy/diaper into sterile sample containers. Where immediate analysis is not possible, samples are typically frozen at −80°C for long-term storage. Non-biological factors such as sample collection, transport, storage temperature, duration of storage and freeze–thaw cycles are known to impact the metabolomic output. This highlights the importance of standardising any sample handling practices before analysis so that the metabolic integrity is preserved. Although optimal sample collection and storage conditions are not always possible during clinical studies, it is usually appropriate to utilise samples that have been collected, stored and processed in the same way.

2.2 Microbiome and metabolome interplay

Most often the microbiome is studied for taxonomic purposes, to identify what bacteria are present within the human gut. At the taxonomic level, the gut microbiome is known to differ between individuals, yet many metabolic pathways are shared, suggesting there are multiple ways that different microbes may produce or modify the same metabolites. The microbiome and the metabolome are entwined through dietary, genetic and environmental

![Figure 1](image-url) Summary of the annual number of publications employing different sample types in metabolome studies between 1950 and 2020. The trend data from this graph were collated from the number of published papers measured yearly in PubMed containing the respective keywords: ‘Metabolite Blood OR Metabolite Serum OR Metabolite Plasma’, ‘Metabolite Urine’, and ‘Metabolite Stool OR Metabolite Faeces OR Metabolite Feces’
| Study (ref no.) | Year published | Metabolomic cohort size | Sample type | Metabolomic platform | Metabolomic instrument | Focus | Study metabolomic conclusion |
|----------------|----------------|-------------------------|-------------|----------------------|------------------------|-------|-------------------------------|
| Picaud J. et al.⁴⁵ | 2021           | 18 (12 NEC)            | Urine       | Untargeted 1H NMR    | Varian UNITY INOVA 500 spectrometer (Agilent Technologies) | Metabolomics: healthy (feed intolerance and good feed tolerance infants) vs. NEC (early and late onset) preterm infants | The urinary metabolome of infants with NEC were identified as significantly different from no-NEC controls. This was a pilot study with a small number of infants. |
| Brehin C. et al.⁴⁷ | 2020           | 32 (11 NEC)            | Stool       | Untargeted 1H NMR    | Bruker DRX-600 Avance NMR spectrometer | Microbiome and metabolomic: healthy vs. suspected NEC preterm infants | The stool metabolome of infants with suspected NEC were identified as significantly different from non-NEC controls, especially between Days 20 and 30. The study included microbiome data and longitudinal data but was based on a small number of suspected NEC infants, and no definite NEC were included. |
| Sinclair et al.¹² | 2020           | 995 (73 NEC)           | Blood       | Targeted MS-MS       | No details identified | Metabolomics: healthy vs. NEC preterm infants | Significant blood metabolite differences at birth, sampled at Day 1, were shown in infants who were extremely preterm and/or went on to develop NEC. Metabolic differences were also identified in infants prior to NEC onset and showed links to nutrients administered, feeding intolerances and calorie intake prior to NEC onset. In contrast to Becker et al. (2000) and Zamora et al. (1997), arginine was higher in infants that developed NEC on Days 1 and 7. This study was targeted, but included a large cohort and longitudinal data. |
| Thomaidou A. et al.⁴³ | 2019           | 30 (15 NEC)            | Urine       | Untargeted NMR and targeted LC-MS/MS | No details identified | Metabolomics: healthy vs. NEC preterm infants—seeking biomarkers | The urinary metabolome of infants with NEC was found to be significantly different from no-NEC controls. This study was small in cohort size and only included a small number of NEC infants. |
| Wang F. et al.⁵² | 2019           | 39 (19 NEC)            | Blood       | GC-MS                | 7890B-5977A GC/MSD GC-MS (Agilent Co.) | Metabolomics: preterm vs. term (NEC blind study) | The blood metabolome of preterm infants showed significant differences from term infants. Potential NEC diagnostic metabolites were identified as the following: O-phosphonothreonine, luteolin, gallic acid, monostearin, citraconic acid and D-fructose 1,6-bisphosphate. A larger study cohort and longitudinal sampling could help support the validation of the findings of this study. |

(Continues)
| Study (ref no.)   | Year published | Metabolomic cohort size | Sample type | Metabolomic platform | Metabolomic instrument | Focus                                              | Study metabolomic conclusion                                                                 |
|------------------|----------------|-------------------------|-------------|----------------------|------------------------|----------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Rusconi B. et al. | 2018           | 69 (23 NEC)             | Stool       | Targeted UPLC-MS/MS  | Thermo Scientific TSQ Quantum Ultra MS operated in SRM mode under ESI(+)| Metabolomics: healthy vs. NEC preterm infants Metabolomics: gut sphingolipid | This study employed targeted metabolomics. Significant changes in stool metabolomic sphingolipid metabolism components were identified in pre-NEC stools compared with no-NEC controls. |
| Wandro S. et al. | 2018           | 32 (3 NEC, 8 LOS)       | Stool       | Untargeted GCMS      | GC-time of flight-MS   | Microbiome and metabolomic: exploratory microbiota and healthy vs. NEC, LOS preterm infants | This study only included a small number of infants with NEC or LOS so conclusions of healthy infants in comparison with infants with NEC and LOS were not made. Despite these limitations, evidence of the preterm infant gut microbiome and metabolome being unique to the individual was shown. |
| Stewart C. J. et al. | 2017           | 35 (7 LOS)              | Stool       | Untargeted UPLC-MS/MS | Accucore C18 column. Q-Exactive (Thermo-Scientific), +/− switching | Microbiome and metabolomic: healthy vs. LOS preterm infants | The study reported 14 stool metabolites as significantly different between LOS and no-LOS controls. This study included longitudinal data; however, only four infants with LOS and 10 controls were included for the metabolomic part of this study, and a larger study is needed for verification of the findings. |
| Stewart C. J. et al. | 2016           | 19 (6 NEC/4 LOS)        | Blood       | Untargeted UPLC-MS/MS | Accucore C18 column. Q-Exactive (Thermo-Scientific), +/− switching | Metabolomic and proteomic: healthy vs. NEC, LOS preterm infants | Blood metabolomic analysis of NEC, LOS and no-disease infant controls identified no differing metabolites and suggests a single biomarker for NEC and LOS is not probable. This study included limited NEC and LOS cases. |
| Stewart C. J. et al. | 2016           | 16 (6 NEC)              | Stool       | Untargeted UPLC-MS/MS | C18 column. Q-Exactive (Thermo), +/− switching | Microbiome and metabolomic: healthy vs. NEC preterm infants | Stool metabolomic profiling identified significant pathways and related discriminatory metabolites associated with infants that developed NEC. However, only six infants with NEC were analysed. |
| Wilcock A. et al. | 2016           | 12 (5 NEC)              | Blood       | Untargeted GC-MS     | Pegasus HT, Leco Corporation, St Joseph, MI | Metabolomic: healthy preterm and term infants vs. NEC preterm infants | Significant differences were observed in the blood metabolites between healthy term and preterm infants that went on to develop NEC. The small cohort size and only five infants with NEC within the study prevented potential NEC biomarker identification. |
| Morrow A. L. et al. | 2013           | 32 (11 NEC)             | Stool and Urine | Untargeted NMR       | Bruker Avance™ III spectrometer | Microbiome and metabolomic: healthy vs. NEC preterm infants | Urine metabolomic output showed a high urinary alanine:histidine ratio that could predict NEC. This study couples microbiome and metabolomic analysis but was small in cohort size and only included a small number of NEC infants. |
exposures. Metabolite profiles linked to the microbiota can therefore offer more comprehensive assessment of the impact of lifestyle, dietary factors and disease.

Recent studies have underlined the importance of studying the microbial metabolic potential rather than focusing on taxonomy alone to find therapeutic biomarkers. To illustrate the connections between the microbiome and the metabolites in the human gut, Visconti et al. (2019) performed a study of over 2,000 samples collated from the TwinsUK registry. Metabolomic samples were obtained from 479 infants for stool samples and 859 individuals for blood samples, with 1,054 randomly selected stool samples for metagenomic sequencing. The study showed 82% of metabolic pathways were shared between individuals, in comparison with bacterial species in common identified at a lesser percentage of 43%. The study followed this with estimations that the microbiome was involved in metabolite microbiome interplay for 71% of faecal and 15% blood metabolites, demonstrating stool metabolic content are strongly linked.

To further this concept, Nguyen et al. (2021) recently investigated the taxonomic and functional metabolic phenotype connections across gut microbial communities in early human life. A total of 440 stool samples from infants enrolled in the New Hampshire Birth Cohort Study were collected at approximately 6 weeks and 12 months of age. The results showed the relationship between the microbiome and metabolome differs over time, being more integrated at 6 weeks in comparison with 12 months, possibly owing to the infants evolving diet.

The function of the metabolites present in a given stool sample can therefore often be deduced by determining which bacteria they are associated with and if they are produced by or stimulated by those bacteria within the gut. With this, the microbiome-metabolome crosstalk may be more visible earlier in life, perhaps due to an increase in complexity of the human gut environment over time. Studies are now producing evidence, with the use of stool samples, of microbiome-metabolome linkage. Such work demonstrates feasibility for multi-omic study design leading to identification of metabolites of interest that could be used as biomarkers of disease in preterm infants.

### 2.3 Metabolites and human health

The large number of different bacteria in the gut microbiome generates compounds that are part of the metabolic signalling network and can influence human health. The use of metabolites for disease diagnosis has been used for decades, for example, measuring phenylalanine in neonates to screen for phenylketonuria. It has also already been identified that microbially produced metabolites can impact immunoregulation. For example, varying types of short chain fatty acids, which are metabolic output from fermented undigested dietary fibre, have a vast range of functions within the human gut immune system. Short chain fatty acids support the gut epithelial barrier and are linked in production of anti-inflammatory...
cytokines.\textsuperscript{34} They can also act on antigen presenting cells in the brain or lungs which leads to a decreased inflammatory response.\textsuperscript{34}

Although the phases of maturation of metabolites from neonate to adult are not as clearly defined as the microbiome,\textsuperscript{35} there are defined changes related to age. First, Chiu et al. (2016) illustrates that the urine metabolome of infants from birth to 12 months of age changes composition, specifically trimethylamine N-oxide (TMAO), citrate, creatine, glycine, succinate, acetone and creatinines, suggesting these metabolites could be further studied to provide valuable nutrition and growth insights in early childhood.\textsuperscript{36} This further serves to highlight that understanding metabolites in healthy infants can inform on potential processes contributing to neonatal disease. Lopez-Hernandez et al. (2020) performed a targeted tandem mass spectrometry (MS/MS) metabolomic study using urine samples collected within 24 h of birth from 48 healthy full-term neonates.\textsuperscript{37} They found that urinary metabolites were fewer in number and less diverse in neonates compared with children or adults.\textsuperscript{37}

Interestingly, neonates had higher levels of particular amino acids including collagen-associated amino acids and acylcarnitines, which are needed to support rapid growth early in life.\textsuperscript{37} Other work has used nuclear magnetic resonance (NMR)-based metabolomics of 253 healthy newborns to explore changes in the urinary metabolite profiles from the first and third days of life.\textsuperscript{38} This work showed clear changes in the metabolite profiles with the first 72 h after birth and distinctions between late preterm and term newborn infants.\textsuperscript{38} In a different NMR spectroscopy study of urinary metabolites, Diaz et al. (2016) looked at prematurity in newborns.\textsuperscript{39} This study included 46 control infants with an average gestational age of 39 weeks and investigated the urine metabolite profiles in comparison with 17 premature infants with an average gestational age of 35 weeks.\textsuperscript{39} The majority of the samples taken within this study were taken on the second day after the infants birth.\textsuperscript{39} Comparisons were made between Day 1 and 2 of life, which did not show evidence of metabolic change; however, there were limited samples taken on Day 1, so this finding is inconclusive.\textsuperscript{39} This study did highlight variations between preterm and term infants observed in NMR spectral output, partial least squares-discriminant analysis (PLS-DA) statistical assessment, and 25 identical metabolite changes were visible in comparison Volcano plot analysis.\textsuperscript{39} There were gender ratio inconsistencies, however, between the preterm and term control groups, so perhaps, a more balanced cohort group in future studies could help verify these findings.\textsuperscript{39}

2.4 Techniques for measuring metabolites

No one analytical technique has been assigned as the gold standard for metabolomics, and each technique has benefits and limitations.\textsuperscript{40} Selection also considers if the study is for targeted or untargeted metabolite exploration. With predefined targeted metabolites of interest, targeted mass spectrometry (MS) is typically used. Targeted MS has high sensitivity, selectivity, accuracy and precision. When the target metabolites or pathways are unknown or when a completely unbiased approach is preferred, liquid chromatography mass spectrometry (LC-MS) or nuclear magnetic resonance is typically employed.

3 NEC AND LOS ASSOCIATED METABOLITES

Metabolomic studies of preterm infant populations in relation to NEC and LOS are summarised in Table 1. LOS studies were limited, with no specific metabolites being identified across studies as associated with disease onset or protection. Stewart et al. (2016) did not find any significant metabolites in NEC or LOS in comparison with the control infants that could be used for biomarkers. However, this study cohort was cross-sectional and small (only 19 infants; 6 NEC and 4 LOS), and more work in larger cohorts with longitudinal sampling is required.\textsuperscript{41} In more recent work, Stewart et al. (2017) showed that 14 stool metabolites were identified as significantly different between LOS and no-LOS controls.\textsuperscript{42} This study included longitudinal data; however, there were only four infants with LOS and 10 controls analysed for the metabolomic part of this study,\textsuperscript{42} and again, a larger study would be needed for verification of the findings.

Metabolomic studies investigating NEC were more numerous than LOS, with most studies finding a metabolic difference between NEC and control infants. Sinclair et al. (2020) enrolled the largest cohort study involving targeted metabolomics for investigating NEC, with 995 preterm infants of whom 73 were diagnosed with NEC. Significant blood metabolite differences at birth, sampled at Day 1, were shown in infants who were extremely preterm and/or went on to develop NEC.\textsuperscript{12} Metabolic differences were also identified in infants prior to NEC onset and showed links to nutrients administered, feeding intolerances and calorie intake prior to NEC onset.\textsuperscript{12} On the first day of life, several metabolites and metabolite ratios were identified in infants that went on to develop NEC including alanine, phenylalanine, arginine and citrulline/phenylalanine.\textsuperscript{12} This study holds promise due to cohort size and longitudinal sampling (Days 1, 7, 28 and 42); however, limitations would include singular sample types and no metabolic coupled analysis. Unfortunately, to date, there were no other similar sized studies to support these findings. As highlighted within Table 1, existing studies are typically preliminary or pilot in nature. Thus, larger longitudinal studies combining samples from multiple sites are required for validation.\textsuperscript{43–47}

Despite the differing cohort characteristics, sizes, sample types and analytical methods, several metabolites were consistently found to differ in abundance in NEC infants compared with controls, including alanine, arginine, proline, glutamine, histamine, creatinine and betaine.\textsuperscript{12,43–45,48,49} Alanine, arginine, proline and glutamine are amino acids that are building blocks of proteins used in the body as a source of energy for muscles and the central nervous system. Glutamine has been specifically used in supplementation studies in the past to help treat immunosuppressed individuals.\textsuperscript{50} Histamine is a chemical that is also connected to immunity, and it is thought that histamine intolerance is a disorder connected to gut health.\textsuperscript{51}
Arginine has been reviewed in previous literature as arginine is a substrate for nitric oxide synthesis. It has been speculated that nitric oxide synthesis is connected to NEC; however, this remains uncertain.

To summarise, there are minimum metabolite studies looking at urine samples or combining multiple sample options within the same study (Table 1). Differing biofluids provide their own metabolic ‘fingerprint,’ and the connections between the microbiome and the metabolites in the human gut show high levels of metabolite microbiome interplay shown for faecal metabolites. This highlights the importance for metabolomic studies investigating NEC and LOS in preterm infants should be performed with multiple different biofluid samples, combined with microbiome data, to either deduce new or validate already identified insights.

4 CONCLUSIONS

Necrotising enterocolitis and LOS are the most common cause of death in premature infants, and understanding metabolite fluctuations/presence could give insights to hospital best practice treatments or present biomarkers to identify disease. However, compared with other areas such as the gut microbiome, there are limited numbers of studies investigating metabolomics in preterm infants with NEC and/or LOS and matched controls. It is evident that across longitudinally age-related metabolomic studies, there are significant changes in metabolite profiles in the first days and years of life from birth.

Existing studies have reported associations between the metabolite profiles of serum, urine and stool in health and disease in preterm infants. Some studies have found selected metabolites are associated with disease; however, additional or larger studies would be required to validate. Furthermore, recent work has begun to untangle how microbiobly produced metabolites can impact immunomodulation of the infant. Although metabolic exploration in infants is still an underutilised technology, there is clearly huge potential for this field in developing novel biomarkers for early detection of NEC and LOS in preterm infants, which could reduce unnecessarily treating or not treating preterm infants accurately.

CONFLICT OF INTEREST

C. J. S. declares receiving lecture honoraria from Danone Early Life Nutrition and Nestle Nutrition Institute but has no share options or other conflicts.

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How to cite this article: Renwick VL, Stewart CJ. Exploring functional metabolites in preterm infants. Acta Paediatr. 2022;111:45–53. https://doi.org/10.1111/apa.16146