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REVIEW ARTICLE

The Role of Metabolomics in Assessing the Nutritional Intervention in Preterm Infant Feeding: A Scoping Review

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ABSTRAK

Pemakanan parenteral (PN) telah menjadi standard penjagaan bayi pramatang untuk merapatkan jurang pemakanan sebelum pemakanan enteral (EN) dimulakan. Cadangan semasa untuk PN adalah berdasarkan pertambahan nutrien intrauterin dan kandungan susu ibu. Sama ada anggaran ini mencukupi untuk keperluan metabolik luar rahim pada bayi pramatang masih tidak jelas. Ulasan ini mengkaji bukti yang ada mengenai hasil metabolomik yang membandingkan kedua-dua PN dan EN dalam bayi pramatang. Hanya artikel asal yang melaporkan hasil metabolomik dalam bayi pramatang yang menerima kedua-dua PN dan EN dimasukkan. Selepas proses saringan, 6 artikel asal [China (n=1), Norway (n=1), Sweden (n=2), Mexico (n=1) dan Amerika Syarikat (n=1)] dipilih untuk ulasan ini. Kajian terlibat memperihalkan profil metabolomik dalam kalangan 525 bayi yang menerima PN dan EN dalam sampel serum (n=2), air kencing (n=3) dan sampel darah kering (n=1) menggunakan ^{1}H-NMR (n=4), GC-MS (n=1), UHPLC-MS/MS (n=1) sebagai kaedah analisis. Metabolit termasuk glukosa, asid amino dan 'acylcarnitine' rantai-pendek diperkaya dalam biobendalir bayi menerima PN, mencadangkan lebihan bekalan. Secara perbandingan pelbagai jenis kolina, asid amino dan metabolit lipid yang penting untuk pertumbuhan dan perkembangan lebih banyak terdapat dalam bayi yang diberi EN secara eksklusif. Semua kajian terlibat meringkaskan perbezaan metabolomik yang ketara yang diperhatikan pada bayi pramatang semasa mereka beralih daripada PN kepada EN penuh. Ia meningkatkan pemahaman kita tentang bagaimana formulasi pemakanan semasa menyumbang kepada kecukupan, lebihan atau kekurangan penghadaman nutrien.

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Penyelidikan masa depan harus mengutamakan usaha kolaboratif untuk mewujudkan pangkalan data julat rujukan metabolit penting berdasarkan umur. Ini akan membantu dalam membangunkan pelan pemakanan yang diperibadikan mengikut keperluan khusus bayi pramatang, berpotensi meningkatkan pertumbuhan dan perkembangan mereka dalam jangka panjang.

Kata kunci: Metabolomik serum; intervensi pemakanan; metabolit; pemakanan enteral; pemakanan parenteral; pramatang

ABSTRACT

Parenteral nutrition (PN) is the standard of care for preterm infants to bridge the nutritional gap before enteral nutrition (EN) is established. Current recommendations for PN are largely based on intrauterine nutrient accretion and breast milk constituents. Whether this estimation sufficiently meets the extrauterine metabolic needs of preterm infants remains unclear. This review examines the available evidence on metabolomic outcomes comparing both PN and EN in preterm infants. Only original articles reporting metabolomic outcomes in preterm infants receiving PN and EN were included. After the screening process, six original articles [China (n=1), Norway (n=1), Sweden (n=2), Mexico (n=1), and the USA (n=1)] were selected for this review. The studies examined the metabolomic profiles of 525 infants receiving PN and EN from their serum (n=2), urine (n=3), and dried blood spots (n=1) samples using ^{1}H-NMR (n=4), GC-MS (n=1), UHPLC-MS/MS (n=1) as analytical platforms. Metabolites including glucose, amino acids and short-chain acylcarnitine were upregulated in the biofluids of PN-fed infants, suggesting an oversupply. In contrast, a diverse range of choline, amino acids and lipid metabolites essential for growth and development were enriched in the exclusively EN-fed infants. This scoping review summarised the significant metabolomic differences observed in preterm infants transitioning from parenteral to full enteral feeding. It enhances our understanding of how current nutritional formulations contribute to sufficiency, excess or lack of nutrient assimilation. Future collaborative research should aim to establish a database of age-related reference ranges for essential metabolites. This will aid in developing personalised nutrition tailored to the specific needs of preterm infants, potentially improving their long-term growth and development outcomes.

Keywords: Enteral nutrition; metabolites; parenteral nutrition; prematurity; serum metabolomics; nutritional intervention

INTRODUCTION

Metabolomics is an emerging field within the "omics" sciences that has garnered significant interest in neonatology due to its capacity to track dynamic changes over the extrauterine transition using minimal biofluids (Fanos et al. 2018; Nicholson & Lindon 2008). The application of cutting-

edge analytics has significantly enhanced precision in neonatal care through the use of advanced computational techniques such as proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and mass spectrometry (MS), in conjunction with separation techniques including liquid chromatography (LC) and gas chromatography (GC). These combined techniques effectively mitigate the matrix effects of biospecimens and increase sensitivity, enabling comprehensive analysis of the entire metabolome, namely the small molecules (typically < 1500 Da) or metabolites that make up a biological system (Munjal et al. 2022; Naz et al. 2014). Over the past decade, researchers have characterised and quantified an array of metabolites in neonatal biofluids, such as urine, blood, faeces and saliva. Such knowledge provides valuable insights into the intricate physiological interplay with nutrition, pharmacological treatment, environmental influences and genetic effects. It supports associations between metabolites and various morbidities, thereby enabling the development of precise intervention and treatment strategies to enhance outcomes for newborns and premature infants.

Preterm birth is defined as infants born alive before 37 completed weeks of gestation and one in ten infants is born preterm globally (Blencowe et al. 2013; Liu et al. 2012; WHO 2023). The gestation of borderline viability is progressively lowered to 22 and 24 weeks (Isayama et al. 2024; Kim et al. 2024). The increased extreme prematurity results in specialised nutritional intervention requirements for these infants. While early nutritional interventions aim to simulate intrauterine nutrient accretion, the complex metabolic needs of the extremely or the "fetal" neonate may not be sufficiently met because of extrauterine energy losses from transepidermal water loss and compounded by extra energy requirements for essential bodily functions such as respiration.

Parenteral nutrition (PN) is considered as a nutritional emergency for these extremely preterm infants who cannot feed enterally due to gut immaturity. These infants are often in a catabolic state due to their critically ill conditions while receiving intensive care support. Although PN is crucial and life-saving, it remains unclear whether the composition of PN formulation can serve as a surrogate for what enteral nutrition (EN) assimilates even for a short period in the first weeks of life.

Numerous researchers have described metabolomic findings in newborns who received enteral feeding but information from preterm infants receiving PN is scarce. Nevertheless, the metabolomic profile of very preterm infants receiving EN exclusively in the first week of life is impossible to characterise as PN with minimal EN is the standard of care for this gestational age group. A previous scoping review discussed metabolomics maturation relative to gestational age, with changes over time in relation to nutrition and growth (Marino et al. 2022). Another non-systematic review discussed metabolomics in perinatal nutrition from the perspectives of gestational diabetes, intrauterine growth restriction and breast milk feeding (Pintus et al. 2023). However, the metabolomic profile in preterm infants exposed to PN in the early days as opposed to those who are not has not been highlighted. In light of the growing

body of scientific evidence in recent nutri-metabolomics (an integration between nutrition and metabolomics) research in neonates, this scoping review focuses on the research question: "What is the metabolomic difference in preterm infants receiving parenteral nutrition intervention compared to when they are on exclusive enteral feeding?"

MATERIALS AND METHODS

Protocol & Registration

Our protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocols for Scoping Reviews (PRISMA-ScR) (http://www.prisma-statement.org/Extensions/ScopingReviews).

Study Eligibility

Original articles of human studies written in English were eligible for potential inclusion in this review. Only studies comparing parenteral and enteral nutrition using metabolomics as an analytical approach were selected for this review. Any papers that did not fit the conceptual framework of the study were excluded.

Search Strategy

A literature search was conducted via PubMed, Scopus and Web of Science to identify studies relevant to metabolomics and neonatal nutrition (parenteral and enteral) in infants/neonates based on the search string: "term OR preterm OR prematur* OR baby OR babies OR infant* OR neonat* OR "preterm infants" AND "parenteral nutrition" OR intravenous AND "enteral nutrition" OR enteral OR milk OR feed* OR oral AND metabolomic OR metabol* combined keywords. The reference list of relevant review papers was manually screened to identify potential articles that may have been missed in the database literature search. The final search results were then exported into EndNote and screened for duplicates, relevant research areas and keywords.

Study Selection

Two reviewers jointly developed a form for data charting to determine which variable to extract and independently read all abstracts to classify them as potentially included or rejected. The primary reviewer conducted data extraction independently and the summary of evidence table was cross-checked by the second reviewer. Extracted data were iteratively discussed with the team to ensure consistency in results synthesis.

Data Extraction

The extraction of data was carried out by two researchers (SKC and FCC). We abstracted data based on article characteristics, including authors and study publication year, study characteristics (eg. country of study site), characteristics of study participants (e.g. sample size, mean gestational age), study designs (analytical platform, biofluid sampling, nutritional types, intervention, comparison), metabolomic findings (eg. relative abundance of metabolites, impacted pathways) and study limitations. If there were disagreements between the two reviewers, a third reviewer was consulted for the final decision.

Data Synthesis

The summary of findings was presented in an evidence table and narrative format to meet our research goals. The studies were comprehensively summarised based on authors, year of publication, journal of publication, study location, study designs, patient characteristics, analytical platforms, biofluid sampling, sampling schedule, description of parenteral and enteral nutrition regime and the corresponding metabolomic findings. Outcomes were categorised based on the significant metabolic pathways affected and the upregulation and downregulation of metabolites in neonates receiving parenteral versus full enteral nutrition (EN).

RESULTS

Selection of Articles Including Both Parenteral and Enteral Nutrition as Nutritional Types

Based on the PRISMA-ScR algorithm, we identified 3,866 studies on metabolomics, parenteral nutrition and enteral nutrition in newborns from searches conducted on PubMed, Scopus and Web of Science, as of 16 July 2024 (Figure 1). A total of 1,894 papers were subjected to title or abstract review for inclusion after removing duplicates. Eleven full-text articles were evaluated for eligibility. Six articles discussing metabolomics in PN and EN in animals were excluded because

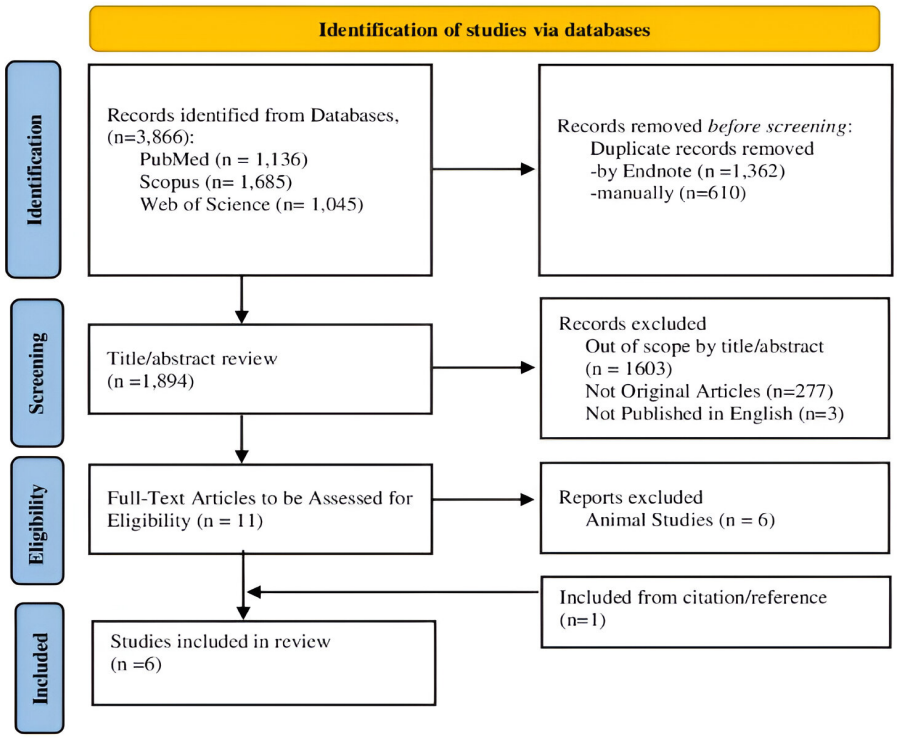

FIGURE 1: PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

it was uncertain if the animal species' metabolism was comparable to that of human neonates. One original article was included from hand-searching in the reference list of a review paper (Marino et al. 2022; Moltu et al. 2014). Finally, six studies conducted in preterm infants [China (n=1), Norway (n=1), Sweden (n=2), Mexico (n=1), and the USA (n=1)] were included in this review, as summarised in Table 1.

Characteristics of Articles

(i) Study population and sampling methods

The number of infants in each study ranged from 34 to 314 (Esturau-Escofet et al. 2022; Guardado et al. 2023). Two studies sampled infants from more than one centre (Guardado et al. 2023; Moltu et al. 2014). Across all included studies, the infant metabolome was profiled in the biofluids of 525 preterm infants (two studies using samples from the same cohort were considered once) (Nilsson et al. 2021; Nilsson et al. 2022). Urine was used as the sampling method (n=3) as its collection is simple and non-invasive. Blood collection was also used in metabolomics research (serum, n=2; dried blood spot, n=1) as it is minimally invasive compared to the collection of other body fluids like cerebrospinal fluid and tissues.

(ii) Nutritional intervention

Studies selected for this scoping review included subjects receiving parenteral and enteral feeds with metabolomic outcomes. However, one study compared the metabolite profile between conventional and enhanced regimens in both parenteral and enteral nutrition with higher energy and protein supply (Moltu et al. 2014). Another compared metabolomics in preterm infants receiving both PN and EN with extrauterine growth restriction (EUGR) versus non-EUGR (Wang et al. 2020). Hence, only four observational studies shared the same research question of characterising metabolomic profiles comparing parenteral and enteral feeding modes. Three of the studies followed-up the preterm infants longitudinally from PN transitioning to full EN (Esturau-Escofet et al. 2022; Nilsson et al. 2021; Nilsson et al. 2022). One study had a cross-sectional design with a subgroup monitored longitudinally through the transition from PN to EN (Guardado et al. 2023).

(iii) Metabolomic approach

Four of the six studies utilised an untargeted metabolomic approach (Esturau-Escofet et al. 2022; Guardado et al. 2023; Moltu et al. 2014; Nilsson et al. 2022). Two studies used a targeted approach to study the profile of amino acids (Nilsson et al. 2021; Wang et al. 2020). Among these studies, four employed ^{1}H-NMR as the analytical platform (Esturau-Escofet et al. 2022; Moltu et al. 2014; Nilsson et al. 2021; Nilsson et al. 2022) while two used ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), (Guardado et al. 2023; Kindt et al. 2022) and one used gas chromatography-mass spectrometry (GC-MS) (Wang et al. 2020). The recent development of UHPLC-MS/MS is considered the best analytical technique for wide metabolome coverage, complemented by extensive MS spectral libraries, making MS-based analysis a reliable and preferred metabolomics

TABLE 1: Studies describing metabolite profile in preterm infants receiving parenteral nutrition (PN) and enteral nutrition (EN)

Author, Year, Location, Published	Methodology, Analytics	Study Numbers	Inclusion Criteria, Patient Characteristics	Study Aim	Type of Sample, Sampling Time	Description of PN	Description of EN	Metabolomic Findings
Moltu et al. (2014), Norway, Nutrients	Observational open randomised controlled trial, two-centred; untargeted, 1H-NMR	50 (24 intervention, 26 control; 2 died, 48 analysed)	Preterm infants born with BW < 1500 g. Intervention (n=24): GA 28.1 weeks (25.0-33.6), BW 940 g (460-1311), SGA 11/24 (46%); Control (n=26): GA 28.5 weeks (24.0-32.6), BW 1083 g (571-1414), SGA 5/24 (21%)	To analyse urine samples from premature infants about two different nutritional exposures and to assess the infants' postnatal metabolic maturation.	Urine; 1st week of life, then every other week until discharge	Intervention: AA 3.5 g/kg/day; Lipids 2.0 g/kg/day (*SMOF®, Fresenius Kabi Norge AS, Oslo, Norway)* Control: AA 2.0 g/kg/day; Lipids 0.5 g/kg/day (*Clinoleic®, Baxter AS, Oslo, Norway*)	Both Groups: Human milk, equal increment, standard fortification initiated with 4.2 g Nutriprem® (*Nutricia Norge AS, Oslo, Norway*)/100 mL human milk when 110 mL/kg/day enteral supply was tolerated. Intervention: Additional fortification with 0.6 g Complete Amino Acid Mix® (*Nutricia Norge AS, Oslo, Norway*)/100 mL human milk, 60 mg/kg/day of docosahexaenoic acid (22:6, n-3), arachidonic acid (20:4, n-6), and 1500 g/kg/day vitamin A (*Ås Laboratory, Ås, Norway*) On average, the intervention group received approximately 10% higher energy and 25% higher protein than the control group.	· Metabolite changes correlated with PMA. · Nutritional intervention, infections, and infants' sex did not influence the urinary metabolite profile. ·1st week, SGA vs AGA: ↑ glycine, threonine. · Early postnatal period: ↑ glycine, threonine, hydroxyproline, tyrosine; ↑ succinate, oxoglutarate, fumarate and citrate

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Wang et al. (2020), China, Frontiers in Pediatrics	Observational, single-centre; targeted (AA metabolites), GC-MS	40 (20 EUGR, 20 non-EUGR)	Preterm infants born GA < 32 weeks and BW < 1500g. EUGR (n=20): GA 29.89 1.90 weeks; BW 1188 ± 177 g; Non-EUGR (n=20): GA 28.93 ± 1.58 weeks; BW 1287 ± 191 g.	To identify AA metabolites most likely associated with poor growth and the association between AA metabolites and nutrition regimens in preterm infants during the transition from PN to EN.	Dried blood Spot; T_1: <24 H of life; T_2: stable PN (amino acids 3.5-4.0 g/kg/d), before EN started T_3: Enteral energy reached 50% of total energy intake; T_4: Full EN	Individualised PN; Started < 24 H of life at 80 mL/kg/day, ↑ by 20mL/kg/day; AA 1.5-2 g/kg/day, ↑ by 0.5-1.0 g/kg/day to 3.5-4.0 g/kg/day *(Pediatric Compound Amino Acid injection 6%, Treeful, Shanghai, China);* Lipids 1 g/kg/day, then ↑ by 0.5-1 g/kg/day to 3 g/kg/day (*Lipofundin MCT/LCT 20%, Braun Medical, Melsungen, Germany);* Dextrose: 4-8 mg/kg/min.	Start at birth as tolerated as trophic feeding < 20 mL/kg/day, gradually ↑ to 160-180 mL/kg/day. Human milk, preterm infant formula, partially hydrolysed formula, extensively hydrolysed formula, amino acid-based formula.	· 18 AA metabolites · Associated with risk of EUGR: T3: ↓ citrulline, threonine T4: ↓ threonine, arginine, methionine, phenylalanine, tyrosine, leucine, valine, proline · Citrulline at T_3 positively correlated with average enteral energy (r=0.39, p=0.02) and protein intake (r=0.37, p=0.03).
Esturau-Escofet et al. (2022), Mexico, Metabolites	Observational, single-centre; untargeted, ^{1}H-NMR.	34 (107 samples: 48 PN, 59 EN; 22 AGA, 12 SGA)	Preterm infants born with GA 31 weeks (26-36); BW 1587 g (740-2945); height 40.5 cm (32-48); average age at initial sampling 12 days of life; body weight at sampling 1601 g (540-2840); height 41 cm (31.5-51)	To analyse urine samples from hospitalised preterm newborns with critically ill conditions who received PN or EN to determine whether there are metabolomic differences.	Urine; upon admission, then weekly until discharge.	AA 4 g/kg (*LEVAMIN® 10%*); lipids 3.5 g/kg (*LIPOFUNDIN ®MCT/LCT or SMOFlipid®*); carbohydrates 17.2 g/kg; glutamine 0.3 g/kg; carnitine 5 mg; trace elements 0.3 ml/kg; zinc 100 mcg/kg; magnesium sulfate 50 mg/kg/day; calcium gluconate 10% 100-200 mg/kg/day.	Mother's own milk, donor human milk (as supplement from birth to PMA 34 weeks), preterm formula (*Enfamil Prematuros® Mead Johnson®*), up to 180 mL/kg/day.	14 metabolites · PN:↑ glucose, gluconate, N-acetyltyrosine, 3-aminoisobutyrate; · EN: ↑ succinate, galactose, citrate, lactose Pathway analysis (p<0.05, i>0.1): The most impacted pathways related to PN treatment were tricarboxylic acid cycle and galactose metabolism, indicating an energetic dysfunction that must be considered for better nutritional management.

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Guardado et al. (2023), USA, Metabolites	Observational; TOLSURF (2010-2013): 25 centres, PROP (2010-2013): 8 centres; untargeted, UHPLC-MS/MS	314 (TOLSURF: 108 PN, 63 EN, 28 with longitudinal profiles between 7-50 days of life, 5 subjects were excluded from the analysis; PROP: 80 PN, 63 EN).	TOLSURF: (PN) GA 25.2 ± 1.2 weeks, BW 698 ± 164 g, Age of sampling 27.3 ± 2.2 days; (EN) GA 25.6 ± 1.2 weeks, BW 778 ± 181 g, Age of sampling 28.1 ± 2.0 days. PROP: (PN) GA 25.7 ± 1.0 weeks, BW 770 ± 161 g, Age of Sampling 28.3 ± 0.9 days; (EN) GA 26.0 ± 1.3 weeks, BW 804 ± 131 g, Age of Sampling 28.0 ± 0.9 days.	To perform untargeted global metabolomics on urine samples from extremely premature infants on TPN vs enteral feeds from two independent clinical cohorts.	Urine; Cross-sectional: 1 sample (day 23-30 of life) Longitudinal cohort (2-time points): ≥ 5 days before PN was stopped and > 0 to 2 days after full EN	Started <2 days of birth, details not specified.	TOLSURF: exclusive breast milk (longitudinal cohort); PROP (86.9% full EN at weeks 4-5): 70.2% exclusive breast milk; 6.7% mixed breast milk/formula feeding.	· 309/609 (51%) metabolites associated with feeding status ($p<0.05$) · 88% of metabolites were more enriched in EN · 8 of the 20 top metabolites associated with feeding status were cofactors and vitamins, of which 6 of these were higher in EN-fed · 11 of the 20 AA significantly associated with feeding status were higher in infants on EN, 4 of these were essential AA (lysine, leucine, threonine, tryptophan) · Only 1 (cysteine) out of 20 AA significantly associated with feeding status were higher in PN · PN: ↑ dexpanthenol, gluconate, N-acetyltyrosine, N-acetylphenylalanine, C5 acylcarnitine; · EN: ↑ ferulic acid 4-sulfate, 2,6-dihydroxybenzoic acid, ascorbic acid 3-sulfate, 3-methyladipate, N-acetyl asparagine, acetylhydroquinone sulfate, N-succinyl-phenylalanine, lyxonate, arabonate/xylonate, isocitric lactone, threonate, tartronate, 3-hydroxy-2-methylpyridine sulfate, N-delta-acetylornithine, pantothenate, C12 acylcarnitine · Pathway analysis: 2 major pathways (energy & nucleotide) and 8 subpathways significantly enriched in enteral feeds (3 acylcarnitines: long-chain saturated, dicarboxylate, dihydroxy fatty acids)

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Nilsson et al. (2022), Sweden, Frontiers in Neuroscience	Observational (Part of Donna Mega Study, a randomised open-label trial), single centre; untargeted, ^{1}H-NMR	87 (GA < 25 weeks, n=33, GA 25-26 weeks, n=36; GA 27 weeks, n=18; 503 samples)	Preterm infants born with GA 25.4 ± 1.4 weeks, BW 780 ± 224 g	To assess the serum metabolite profiles of extremely preterm infants from birth to term equivalent age and analyse the metabolome in regards to GA, postnatal age, nutrition and morbidities.	Serum; PND 1, 7, 14, 28 and PMA 32, 36, 40 weeks.	Standardised PN (contain *Vaminolac* and Glucose 10%); started < 24 H of life at 80 - 90 mL/kg/day; lipids: subjects were randomised to *ClinOleic* [80% olive oil and 20% soybean oil (*Baxter Medical AB, Kista, Sweden*)] or *SMOFlipid* [30% soybean oil, 30% medium-chain triglycerides, 25% olive oil, and 15% fish oil (*Fresenius Kabi AB, Uppsala, Sweden*)] at 6-12H of life at 1 g/kg/day, then ↑ to 2 g/kg/day.	Trophic feeds started at < 3H of life with 1-2 mL/feed human milk, by 10-20 mL/kg/day to 160-180 mL/kg/day. Human milk, pasteurised donor milk (as supplement up to PMA 34 weeks), preterm formula, individualised fortification based on milk analysis for both maternal and donor milk.	·31 metabolites. ·PN: ↑ Glycerol, Threonine, ornithine ·EN: ↑ Choline, Tyrosine ·% enteral energy intake positively correlated with isoleucine & tyrosine, negatively correlated with glycerol, threonine, creatinine ·Low (<median) enteral energy intake PND 1-28 had two-fold higher daily mean glycerol and threonine than high (>median) enteral energy intake
Nilsson et al. (2021), Sweden, European Journal of Nutrition	Observational (Part of Donna Mega Study, a randomised controlled trial), single-centre; targeted (free choline, betaine, methionine), ^{1}H-NMR	87	Preterm infants born with GA 25.4 ± 1.4 weeks; BW 780 224 g; 14.0 days (10.8-19.3) to full enteral intake	To examine the longitudinal serum levels of free choline, betaine and methionine in extremely preterm infants.	Serum; PND 1, 7, 14, 28, and PMA 32, 36, 40 weeks.	Standardised PN (contain *Vaminolac* and Glucose 10%); started < 24 H of life at 80 - 90 mL/kg/day; lipids: subjects were randomised to *ClinOleic* [80% olive oil and 20% soybean oil (*Baxter Medical AB, Kista, Sweden*)] or *SMOFlipid* [30% soybean oil, 30% medium-chain	Trophic feeds started at < 3H of life with 1-2 mL/feed human milk, ↑ by 10-20 mL/kg/day to 160-180 mL/kg/day. Human milk, pasteurised donor milk (as supplement up to PMA 34 weeks), preterm formula, individualised fortification	·Infants receiving above median parenteral/total fluid (%) had significant ↓ serum choline at PND 7 and 14, ↑ serum betaine at PND 28 and methionine levels at PND 7 ·every 1% ↑ in parenteral fluids, serum choline at PND 7 would ↓ by 0.33 µM (p=0.0003);

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	triglycerides, 25% olive oil, and 15% fish oil (*Fresenius Kabi AB, Uppsala, Sweden*)] at 6-12H of life at 1 g/kg/day, then to 2 g/kg/day.	based on milk analysis for both maternal and donor milk.	·Choline: highest on PND 1 at 33.7 (26.2-41.2) μM, lowest on PND 7 at 18.4 (14.1-26.4) μM, and slowly ↑ after PND 7; ·Betaine: higher at PND 1 at 71.2 (53.2-100.8) μM than later time points then declined to 34.2 (24.5-48.7) μM between PND 33-74 before ↑ again; ·Methionine: 25.6 (16.4-35.3 μM at PND 1, 21.0 (16.0-29.1) μM at PND 7, then ↑ throughout the study period.

Abbreviations: BW: birth weight; GA: gestational age; PMA: postmenstrual age; PND: postnatal day; ^{1}H-NMR: Proton nuclear magnetic resonance; UHPLC-MS/MS: ultrahigh performance liquid chromatography-tandem mass spectrometry; SGA: small-for-gestational age; AGA: appropriate-for-gestational age; TCA: tricarboxylic acid; PMA: postmenstrual age; AA: amino acid; EUGR: extrauterine growth restriction; EN: enteral nutrition; PN: parenteral nutrition; GC-MS: gas chromatography-mass spectrometry; H: hour

research method for biological samples.

(iv) Parenteral and enteral feeding regimen

The nutritional regimen for infants receiving PN was outlined in five studies. The remaining study, which utilised samples from trials conducted ten years ago, did not define the PN regimen (Guardado et al. 2023). In all studies, enteral feeding commenced at birth with minimal enteral feeding of less than 20 mL/kg/day. The EN was gradually increased to a maximum of 180 mL/kg/day as tolerated. All infants primarily received human milk, either their mother's own milk with or without individualised fortification or supplemented with pasteurised donor milk until the gestational age of 34 weeks. Specialised formula milk for preterm infants was used as an alternative when human milk was not available.

Infants receiving PN demonstrated a significant shift in the metabolomic profile as they transitioned from PN to EN. The variation in infant metabolome was distinguished by unique metabolic signatures derived from amino acids, lipids, carbohydrates, carboxylic acids and vitamins (Table 2; Figure 2).

Metabolomic Alterations in Preterm Infants Receiving Parenteral Nutrition

Four studies characterised the upregulation of metabolites in preterm infants receiving PN in the first weeks of life based on serum (Nilsson et al. 2021; Nilsson et al. 2022) and urine samples (Esturau-Escofet et al. 2022; Guardado et al. 2023). They identified changes in (i) amino acids and derivatives such as the acetylated

TABLE 2: Altered metabolites in preterm infants on parenteral nutrition (PN) and enteral nutrition (EN)

HMDB Superclass/Class/Sub-Class	PN ↑	EN ↑
Alcohol & Polyol	dexpanthenol[1]	pantothenate[1]
Amino Acids, peptides and analogues	N-acetyltyrosine[1,2] N-acetyl phenylalanine[1] threonine[3] ornithine[3] cysteine[1] methionine[5] 3-amino isobutyrate[2]	citrulline[4] tyrosine[3] N-acetylasparagine[1] N-succinyl-phenylalanine[1] N-delta-acetylornithine[1]
Benzenoids		2,6-dihydroxybenzoic acid[1]
Carbohydrates and carbohydrate conjugates	glucose[2] gluconate[1,2] glycerol[3]	threonate[1] lyxonate[1] arabonate/xylonate[1] tartronate[1] lactose[2] galactose[2]
Carboxylic acids and derivatives		isocitric lactone[1] succinate[2] citrate[2]
Cinnamic acids and derivatives		ferulic acid 4-sulfate[1]
Furanones		ascorbic acid 3-sulfate[1]
Lipids and Lipid-like molecules	C5 acylcarnitine[1]	C12 acylcarnitine[1] methylsuccinate[1] 3-methyladipate[1]
Organic sulfuric acids and derivatives		acetyl hydroquinone sulfate[1]
Organonitrogen compounds		choline[3,5]
Pyridine carboxylic and derivatives		3-hydroxy-2-methylpyridine sulfate[1]

[1](Guardado et al. 2023), [2](Esturau-Escofet et al. 2022), [3](Nilsson et al. 2022), [4](Wang et al. 2020), [5](Nilsson et al. 2021)

form of aromatic amino acids (N-acetyl tyrosine, N-acetyl phenylalanine), cysteine, 3-aminoisobutyrate, threonine and ornithine; (ii) carbohydrates and conjugates such as glucose, gluconate, glycerol; and (iii) lipid molecules, specifically the 5-carbon acylcarnitine (isovalerylcarnitine, pivaloylcarnitine, 2-methylbutyrylcarnitine), and (iv) dexpanthenol (alcohol metabolites, a derivative of vitamin B5) (Table 2). These metabolites typically resembled the composition of PN ingredients and the upregulation may suggest the amount was in excess relative to those who were enterally-fed exclusively.

Metabolomic Alterations in Preterm Infants Receiving Enteral Nutrition

Three studies revealed a diverse spectrum of enriched metabolites from preterm infants as they transitioned from PN to EN in the serum (Nilsson et al. 2022), dried blood spot (Wang et al. 2020) and urine (Guardado et al. 2023). The upregulated

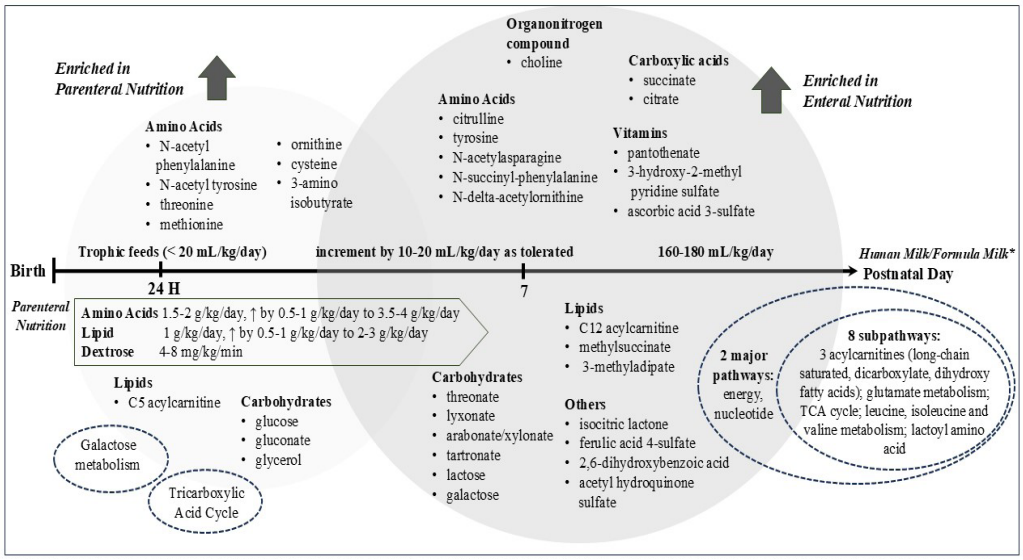

FIGURE 2: Enrichment in the metabolite profile in preterm infants receiving parenteral nutrition (PN) and enteral nutrition (EN). Infants receiving PN demonstrated a significant shift in the metabolomic profile as feeding transitioned from PN to EN. The variation in infant metabolome was distinguished by unique metabolic signatures derived from amino acids, lipids, carbohydrates, carboxylic acids, and vitamins. *Human milk: mother's own milk +/-fortification, pasteurised donor milk; formula milk: preterm, partially/extensively/hydrolysed, amino acid-based (Esturau-Escofet et al. 2022; Guardado et al. 2023; Liu et al. 2020; Nilsson et al. 2021; Nilsson et al. 2022)

metabolites in EN include (i) amino acids and derivatives such as citrulline, tyrosine, N-acetylasparagine, N-succinyl-phenylalanine, N-delta acetylornithine; (ii) carbohydrates, carboxylic acids and derivatives including threonic acid, lyxonate, arabonate/xylonate, tartronate, lactose, galactose, isocitric lactone, succinate, and citrate; (iii) lipid molecules including 12-carbon acylcarnitine, methylsuccinate, 3-methyladipate; and (iv) vitamin and derivatives such as ascorbic acid, pantothenate, 3-hydroxy-2-methylpyridine sulfate; and (v) micronutrients and derivatives such as choline, benzenoid (2,6-dihydroxybenzoic acid), cinnamic acid (ferulic acid 4-sulfate) and organic sulfuric acid (acetyl hydroquinone sulfate). A higher concentration of sugar metabolites, including lactose, galactose, succinate and citrate, were observed in urine samples, as reported by Esturau and colleagues (2022). Infants who received a higher proportion of PN were shown to experience a non-physiological decline in serum choline level over time, corresponding to the lack of choline supplementation in the PN regime. Each 1% increase in PN fluids was associated with a reduction in serum choline by 0.33 μM at postnatal day 7 (Nilsson et al. 2021) due to limited choline supplementation when the infant was parenterally-fed.

Metabolomic Patterns from Perturbations in Metabolic Pathways

Only two studies conducted pathway analysis to elucidate the impact of metabolic pathways on the observed metabolomic differences within biological systems (Esturau-Escofet et al. 2022; Guardado et al. 2023). Using Parallel Factor Analysis (PARAFAC-2), Esturau and co-workers attributed these metabolomic differences to the alterations in the tricarboxylic acid (TCA) cycle and galactose metabolic pathways (Esturau-Escofet et al. 2022). The mitochondrial TCA cycle has a central role in cellular respiration and energy production. Galactose metabolism enables galactose to enter glycolysis that contributes to energy production. The findings indicate the disruption of the TCA cycle in preterm infants receiving PN was due to relatively lower concentrations of citrate and succinate, which are important intermediates of the TCA cycle. Similarly, a relatively lower concentration of galactose and lactose was observed in parenterally-fed preterm infants as they were not sufficiently fed with breast milk or formula that are sources rich with these nutrients.

Using pathway enrichment analysis with the metabolon platform, Guardado et al. (2023) identified the perturbations of all nine metabolomic major pathways when comparing infants on PN versus EN. These included energy and nucleotide major pathways, along with eight subpathways: three acylcarnitines (long-chain saturated, dicarboxylate and dihydroxy fatty acids); glutamate metabolism; TCA cycle; leucine, isoleucine and valine metabolism; and lactoyl amino acid. This indicates metabolic alterations with a gradual reduction of the PN dose and volume when transitioning to full EN. The enrichment of serum metabolites in infants after initiation of EN was thought to be attributed to the diverse nutritional content present in breast and formula milk, rather than the limited composition of PN. Additionally, this process was influenced by the intestinal degradation and absorption of nutrients (Guardado et al. 2023).

DISCUSSION

This scoping review described the metabolomic signatures profiled in preterm infants exposed to parenteral nutritional intervention before full enteral feeding can be established, versus those more mature, exclusively enterally-fed in the first weeks of life. There are no very preterm infants who are fully on EN to be compared, as such more mature infants on EN become the reference with a view that very preterm will mature to this stage later. PN-exposed preterm infants demonstrated upregulation of amino acid metabolites, short-chain acylcarnitine, glucose, and glycerol; as well as downregulation of lactose and its monosaccharide, tricarboxylic acid derivatives and choline.

As the infants transitioned from full parenteral feeding to full enteral feeding, a significant metabolomic shift was observed, with a wide range of metabolites upregulated in the amino acid, lipid, carbohydrate, and vitamin groups. Guardado et. al. (2023) found that 88% of these metabolites were more abundant in the urine of infants who had transitioned to enteral feeding compared to those solely on PN. This suggests a more robust and diverse metabolic response occurring during EN than PN (Guardado et al. 2023). Digestive processes and enzymatic breakdown of dietary nutrients begin once the food is

orally ingested along the gastrointestinal tract. The enzymatic maturation in infants differs between preterm and term infants. In addition, a recent study by Henderickx et. al. (2021) associated gastrointestinal and beneficial microbial proteins essential in gut maturation with gestational and postnatal age using the metaproteomics approach in gastric and fecal samples. In the extreme preterm, gut immaturity was characterised by reduced proteolytic enzymes, diminished gastrointestinal barrier proteins and *Bifidobacterium*-derived proteins, and higher oxidative stress proteins, as opposed to the term counterparts (Dallas et al. 2012; Nielsen et al. 2020; Henderickx et al. 2021; Rogido & Griffin 2019) (Figure 3). This could explain why preterm infants who are exclusively enterally fed show up- or down-regulation of a variety of serum metabolites due to the differences in mechanical and enzymatic processes involved in digestion, absorption in the intestines, and also breakdown products from colonic protein-fermenting microbiota.

In parenterally-fed preterm infants, nutrients are directly administered into the blood circulation bypassing the gastrointestinal tract. Hence, this bypasses brush-border enzymes at the intestinal wall and receptor uptake of nutrients into the bloodstream, as well as the complex processing by gut microbiota. While PN compounding is designed to provide the essential nutrients intravenously, it

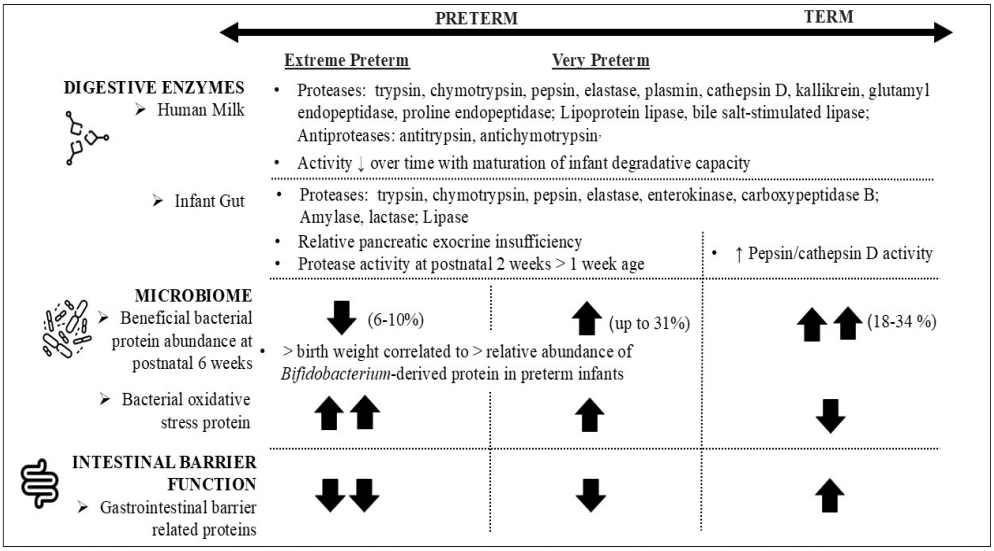

FIGURE 3: Digestive and functional differences in the gastrointestinal tract between preterm and term infants on enteral feeding. The enzymatic maturation in infants differs between preterm and term infants. Using the metaproteomics approach in gastric and fecal samples, gastrointestinal and beneficial microbial proteins essential in gut maturation are associated with gestational and postnatal age. In the extreme preterm, gut immaturity was characterised by reduced proteolytic enzymes, diminished beneficial bacterial and gastrointestinal protein abundance and higher oxidative stress proteins (Dallas et al. 2012; Henderickx et al. 2021; Nielsen et al. 2020; Rogido & Griffin 2019)

may lack the diverse amino acids, lipids, carbohydrates, and vitamins important for optimal metabolic health. PN will be deficient compared to intrauterine transfer of nutrients through the placenta and also lacking in the various nutrients and metabolites from enteral feeding. These findings highlight a substantial knowledge gap regarding the optimal parenteral nutritional regimen for preterm infants to narrow the metabolomic gap and meet actual metabolic requirements, which may potentially impact growth and development outcomes, as well as cardiometabolic sequelae in later life (Lurbe et al. 2014). This becomes more concerning if a preterm infant is on PN over a protracted period.

In addition to the nutritional content, factors such as gestational age, anthropometrics differences, maternal factors and clinical conditions like jaundice, intrauterine growth restriction, sepsis, retinopathy of prematurity (ROP) and necrotising enterocolitis (NEC) are also known factors that are associated with metabolomic profiles with high inter-individual variability (Cai et al. 2016; Clark et al. 2014; Dudzik et al. 2020; Embleton et al. 2017; Mardegan et al. 2021; Overgaard et al. 2018; Priante et al. 2022; Sarafidis et al. 2017; Sarafidis et al. 2019; Scalabre et al. 2017; Sinclair et al. 2020; Thomaidou et al. 2019; Thomaidou et al. 2022; Vidarsdottir et al. 2021; Wilson et al. 2014; Wilcock et al. 2016; Yang et al. 2022; Yap et al. 2021; Younge et al. 2019). However, a detailed analysis of these factors is outside the scope of this review.

The Amino acids in Metabolomics

The upregulation of amino acid metabolites such as N-acetyl tyrosine, N-acetyl phenylalanine, cysteine, methionine, and threonine is expected in preterm infants that receive the parenteral amino acid composition directly into the bloodstream (Esturau-Escofet et al. 2022; Guardado et al. 2023; Nilsson et al. 2021; Nilsson et al. 2022). The acetylated amino acids in newer formulations enhance the stability and solubility of amino acids in the PN solution (Van Goudoever et al. 1994). However, the upregulation of urinary tyrosine, phenylalanine, and cysteine metabolites suggests an oversupply, resulting in increased excretion (Esturau-Escofet et al. 2022; Guardado et al. 2023). Increased serum methionine, threonine, and ornithine levels reflect physiological homeostasis, which may result from various factors. Methionine is a key methyl donor in numerous metabolic pathways, especially in newborns with a high methylation demand for growth, resulting in increased transmethylation rates of methionine (Thomas et al. 2008). Following a high influx of methionine from PN, the transsulfuration of methionine to cysteine is activated to maintain an adequate level of glutathione, an important antioxidant (Prolla et al. 2021; Thomas et al. 2008). High protein catabolism produces energy in early life as part of the extrauterine metabolic adaptations that could contribute to the rise in serum amino acid levels like methionine and ornithine (Beken et al. 2021; Liu et al. 2020). A vital urea cycle intermediary is ornithine, a non-essential amino acid. Its accumulation in the serum could result from an immature urea cycle due to key enzyme deficiencies such as ornithine transcarbamylase and arginase. This may directly lead to inefficient conversion

to citrulline and arginine (Boehm et al. 1988). Parenteral supplementation of arginine, proline and glutamic acid may further increase ornithine synthesis as a downstream product.

Nevertheless, data on recommended parenteral requirements of individual amino acid as determined by the indicator amino acid oxidation method is limited to methionine/cysteine (47 mg/kg/day), tyrosine (74 mg/kg/day), threonine (38 mg/kg/day) and lysine (105 mg/kg/day) (Van Goudoever et al. 2018). PN compounding based on the total protein recommendation is restricted by fixed compositions of individual amino acids in the PN solution. A recent review by Prolla and colleagues unveiled commercially available PN amino acid solutions provide methionine, tyrosine, threonine and lysine in amounts varying from current recommendations by 90% (Prolla et al. 2021; Van Goudoever et al. 2018). Although protein is essential as a building block in newborns to facilitate muscle and tissue growth and development, further research is required to investigate the potential impact of supratherapeutic amino acid delivery, especially in infants who require prolonged PN. This suggests a need to review commercial PN amino acid mixtures in the context of supply, degradation and assimilation to ensure nutritional balance as "more is not always good". In addition, Sung et al. (2019) demonstrated an increased risk of refeeding-syndrome like electrolyte imbalances with severe hypophosphatemia (<2.5 mg/dL) even with a low amino acid initiation rate at 1.5 g/kg/day in infants born with small-for-gestational-age (<10th percentile) and extremely low birth weight (born with birth weight <1000 g). This shows a necessity to consider tailored and precise nutritional approach in a single infant, especially those with high-risks (De Rose et al. 2024). The metabolomic approach enables a quantitative analysis of the metabolite profile whether, there is a state of excess or insufficiency, thus helping to navigate adaptive measures to be undertaken to attain optimal clinical outcomes. This may also guide in pharmaceutical production of more refined PN amino acid mixtures.

In enterally-fed infants, an increase in several amino acids, including citrulline, tyrosine, and derivatives of phenylalanine, ornithine, and asparagine in the biofluids have been reported (Guardado et al. 2023; Nilsson et al. 2022; Wang et al. 2020). The immature enzymatic activity in the premature liver and kidney may lead to metabolite accumulation, especially when using specific formulations with enriched selective amino acids (Prolla et al. 2021). Interestingly, a paradoxical relationship was observed between citrulline and EN, with a negative correlation i.e. greater gestational age, birth weight and volume of EN are associated with lower citrulline levels during the first week of life, but higher citrulline levels after a few weeks of life. This suggests fetal maturation and growth may increase intrauterine citrulline catabolism initially, and its synthesis postnatally (Obayashi et al. 2024). Therefore, low citrulline levels after the first week of life and high citrulline levels soon after birth may be markers for neonates requiring adaptation to their feeding regimen. During the transitional phase at approximately 2 weeks of life, a lower citrulline level was associated with low energy and protein intake (Wang et al. 2020). As citrulline is synthesised from glutamine in the intestines and then

converted to arginine, low citrulline levels could be a risk factor for NEC (Becker et al. 2000; Feenstra et al. 2021; Ioannou et al. 2012; Jawale et al. 2021; Zamora et al. 1997). In preterm infants, tyrosine is a conditionally essential amino acid due to limited phenylalanine hydroxylation. In a longitudinal study, serum tyrosine was positively associated with the amount of enteral energy intake, while in another study, the N-acetylated form of tyrosine had a significantly higher relative concentration in the urine samples of the PN-fed (Esturau-Escofet et al. 2022; Nilsson et al. 2022). Enterally-fed individuals show enhanced protein synthesis that may be due to a more diverse nutrient composition in breast milk or formula milk, together with gut microbiota-mediated metabolic responses. This may contribute to the elevated levels of amino acid derivatives such as N-succinyl-phenylalanine, N-acetylornithine, and N-succinylornithine. These metabolites may be involved in various metabolic pathways related to protein synthesis and positive nitrogen balance.

The Lipids in Metabolomics

A distinctive trend was observed for the lipid metabolites with acylcarnitines (AC), the ester formed between the conjugation of fatty acids and carnitine. AC acts as a transporter for the activated long-chain fatty acids (LCFA) from the cytosol to cross the inner mitochondrial membrane for beta-oxidation to produce energy as adenosine triphosphate (ATP). While the short-chained ACs were elevated in the parenterally fed, long-chained variants were elevated in the enterally fed (Guardado et al. 2023) (Table 2). Carnitine supplementation is not routinely included in parenterally-fed infants. Only one study in this review listed the addition of 5 mg carnitine in the PN solution (Esturau-Escofet et al. 2022). Preterm infants have limited intrauterine carnitine accumulation and also its endogenous synthesis. Its absence in PN deprives these infants from acquiring adequate dietary carnitine intake if they were to be enterally fed with breast milk or formula (Cerdó et al. 2022). Carnitine deficiency may cause irregularities in the fatty acid oxidation process. This affects the infant's ability to utilise parenteral lipids effectively and may result in acylcarnitine accumulation (Ramaswamy et al. 2019; Sylvester et al. 2017). Such metabolic vulnerability may impact especially the preterm infant due to an increased metabolic need for rapid growth.

The diverse fatty acid profile in human milk enriches the infant metabolome with various lipid metabolites, in the form of C12 acylcarnitine and intermediates such as methyl succinate and 3-methyl adipate. Breast milk lipid composition comprises 50% saturated fatty acids, 44% polyunsaturated fatty acids (PUFAs), and 6% monounsaturated fatty acids (MUFAs) (Simon Sarkadi et al. 2022). The main long-chain polyunsaturated fatty acids (LCPUFA) in breast milk include 26 to 28% palmitic acid (C16:0), 23 to 28% linoleic acid (C18:2) and 15 to 17% α-linolenic acid (C18:3). Other fatty acids include 5 to 8% myristic acid (C14:0) and 4 to 6% lauric acid (C12:0) (Simon Sarkadi et al. 2022). Although formula milk can be fortified with essential LCPUFAs like docosahexaenoic acid and arachidonic acid, the overall quality of these fatty acids may not be equivalent to that in

breast milk, which also varies among individuals. As a source of dense energy (9 kcal/g), lipid plays an important role in brain myelination, neurodevelopment and visual outcome. Altered lipid profiles during infancy have been associated with increased future cardiometabolic risks later in life (Mansell et al. 2022). The specialised field of metabolomics, known as lipidomics, may offer insights into the landscape of lipid metabolites, thus enabling strategies to refine future parenteral lipid formulations and breast milk substitutes for improved preterm infant health outcomes.

The Carbohydrates in Metabolomics

Dextrose is the main carbohydrate source in PN formulations. The direct infusion of glucose into the bloodstream in overwhelming quantities may exceed the infant's ability to utilise glucose effectively. The elevated urinary gluconate, which is the oxidised form of glucose, is the metabolomic evidence of glucose overload (Esturau-Escofet et al. 2022; Guardado et al. 2023). Calcium gluconate in PN may also contribute to urinary gluconate excretion (Esturau-Escofet et al. 2022). Additionally, preterm infants are often subject to stressors such as infections or critical illness that may increase gluconeogenesis and decrease peripheral glucose utilisation (Angelika et al. 2023). Furthermore, parenteral glycerol administration in lipid emulsions is also substrate for gluconeogenesis in premature infants (Sunehag 2003). Collectively, these factors may exacerbate hyperglycaemia, a common metabolic disturbance that affects up to 80% of very low birth weight infants (Beardsall et al. 2010). The parenteral glucose infusion rate is generally accepted to be in the range of 4 to 8 mg/kg/min. However, little is known about the ideal glucose requirement, especially for extremely preterm infants who are at risk of insulin resistance. Administering excess glucose than required may introduce an excess of precursors of cellular damage and glycation of albumin. There have been reported strong associations between hyperglycemia and the incidence of ROP (Almeida et al. 2021; Blanco et al. 2006; Kermorvant-Duchemin et al. 2013) The risk of ROP is elevated by 7% with each additional day of hyperglycemia and 2.7-fold with every 10 mg/dL increase in mean serum glucose (Garg et al. 2003; Mohamed et al. 2013). Moreover, an excess of glucose entering the polyol pathway induce oxidative and osmotic stress that predisposes to pathological retinal neovascularisation (Lorenzi 2007; Tomita et al. 2021). By targeting glucose metabolism, metabolomics can potentially personalise the needs of these preterm infants with varying glucose tolerance to mitigate the associated complications and improve health outcomes.

Urinary metabolomics in PN-fed infants showed downregulated lactose, the major disaccharide found in milk, as well as galactose, the monosaccharide product catalysed by the enzyme lactase (Esturau-Escofet et al. 2022). In the same study, downregulations of two important TCA cycle intermediates, succinate and citrate, suggest an alteration to the energy production pathway. This is likely due to the absence of lactose as substrate in PN fluids, leading to a lower metabolic turnover reflected in the reduced urinary levels of these compounds (Guardado et al. 2023; Marino et al. 2022). Upon

transitioning from PN to enteral feeds, whereby lactose makes up the major carbohydrate source in breast milk, the metabolomic shift is associated with a higher abundance of metabolites linked to carbohydrate metabolism, including lyxonate, arabonate/xylonate and tartronate (Guardado et al. 2023). The elevated levels may reflect the infant's adaptation to the predominant lactose content in EN and perhaps gut maturation stimulated by enteral feeding.

The Micronutrients in Metabolomics

Two studies showed downregulation of serum choline in the parenterally-fed and upregulation in the enterally-fed preterm infants (Nilsson et al. 2021; Nilsson et al. 2022). Choline is an organonitrogen compound that plays a critical role in the development of the central nervous system, cognitive function and overall growth in newborns. It is involved in various physiological functions, including cell membrane integrity and neurotransmitter synthesis (Sanders & Zeisel 2007). The prematurely interrupted placental transfer, lack of adequate parenteral choline supplementation, and insufficient enteral feeding led to a rapid decline in the postnatal serum choline concentration in infants receiving high volumes of PN (Nilsson et al. 2021). In contrast, enterally-fed infants receive choline-rich feeding sources from breast milk, formula milk and human milk fortifiers. During the postnatal metabolic adaptation, the utilisation and conversion of dietary choline into compounds like phosphatidylcholine, sphingomyelin, acetylcholine and PUFA are enhanced. These processes are crucial for cellular membrane formation, and brain and visual development. The enhanced turnover may exacerbate the postnatal choline deficit (Goss et al. 2020). Inadequate choline intake has been associated with poorer head growth, smaller brain size, adverse neurocognitive outcomes and growth failure. Metabolomics holds potential in the nutritional assessment of choline adequacy and may guide individualised nutrition regimes to optimise growth and neurodevelopment outcomes (Bernhard et al. 2020).

The Vitamins in Metabolomics

Only one study on metabolomics associated vitamins and cofactors with nutritional intake (Guardado et al. 2023). Pantothenate (vitamin B5), ascorbic-acid 3-sulfate and threonate were enriched in infants on EN and dexpanthenol (pro-vitamin B5) was enriched in infants on PN. The optimal requirements for vitamins and micronutrients for preterm infants are largely unknown. Supplementation is also dependent on overall health and well-being. Although information on vitamin and cofactor metabolism is very lacking, metabolomic characterisation of the vitamin and cofactor metabolites may unravel further insights into their physiological roles in the neonatal population and guide supplementation.

This review provides an overview of the current knowledge about the metabolomic differences in preterm infants on PN as compared to more mature preterm infants on exclusive EN. As the preterm infant matures, they are expected to transition to full EN. There are some limitations to this scoping review, such as the limited number of studies including both

parenteral and enteral nutritional types in the neonatal population especially the very preterm. Studies included vary in study populations, study designs, methodology, analytical platforms, biospecimens, and data interpretation, rendering the results inhomogeneous and not generalisable. The regulation of metabolites is mapped to the known biological pathways to elucidate the intricate relationships between metabolites and biological processes, the lack of standardised methodologies and pathway databases used across different studies may lead to inconsistencies in interpretations and reproducibility.

CONCLUSION

This scoping review summarises the substantial differences in the metabolomic profile when preterm infants transition from parenteral nutrition to enteral feeding. Metabolomics is an emerging scientific platform to study nutritional practice in neonatology. Metabolomics incorporation into nutritional science can explore how dietary components influence metabolic processes and potentially revolutionise future approaches to personalised nutritional interventions. Knowledge of metabolite excesses and insufficiencies facilitate a better understanding of how current nutritional formulations meet the needs of this highly vulnerable population. Future research should aim at establishing a database of gestational age-related reference ranges for different metabolites in newborn infants. Additionally, longitudinal studies to track the metabolic changes in preterm infants over time as they transition from PN to full EN may provide a better understanding of the dynamics involved. Such studies could help in formulating the ideal composition and strategy in nutritional therapies applied at critical windows of opportunities for improved infant health. By leveraging metabolomics, researchers can develop individualised nutrition plans that cater to the unique needs of each preterm infant, potentially improving their short- and long-term health outcomes.

AUTHOR CONTRIBUTIONS

F.C.C., J.K.T. and S.K.C. - conceptualisation; S.K.C., F.C.C. - methodology; S.K.C. - writing-original draft preparation; F.C.C., J.K.T - writing-review and editing; F.C.C. - supervision. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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