

## REVIEW ARTICLE

# Metabolomics can provide new insights into perinatal nutrition

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**Abstract**

Perinatal nutrition is a key factor related to the Developmental Origin of Health and Disease hypothesis, which states that each and every event that happens during the periconceptional period and pregnancy can affect the health status of an individual. Metabolomics can be a very useful tool for gathering information about the effect of perinatal nutrition on both mothers and newborn infants. This non-systematic review focuses on the main metabolites detected by this technique, with regard to gestational diabetes, intrauterine growth restriction and breast milk. Conclusion. Nutrition, metabolome and microbiome interactions are gaining interest in the scientific community.

**KEYWORDS**

breast milk, gestational diabetes, intrauterine growth restriction, metabolomics, perinatal nutrition

## 1 | INTRODUCTION

The Developmental Origin of Health and Disease (DOHaD), or the foetal programming of future health, is widely accepted by the scientific community.<sup>1</sup> This hypothesis implies that every event that happens during the perinatal period may affect the health status of an individual up to their adult life. Several factors can affect the trajectory of the well-being of the foetus even before conception, such as their mother's weight or medical history. Furthermore, it is well known that some drugs taken during pregnancy may be harmful to the foetus or may compromise the development and maturation of several organs. For instance, some antidepressants, especially selective serotonin reuptake inhibitors, can affect the foetal brain and heart development.

Another important factor that can affect the health status of an individual is the perinatal nutrition of the mother and the newborn infant. On the one hand, the great obstetrical syndromes such as gestational diabetes mellitus (GDM) and obesity may be detrimental to the health of both the mother and her newborn infant. Mothers

can subsequently develop type 2 diabetes, while their offspring are at high risk of preterm birth, foetal mortality, intrauterine growth restriction (IUGR), macrosomia, adverse cardiometabolic phenotypes and metabolic disorders.<sup>2</sup> IUGR is a further adverse event that can compromise the health status of an individual, even in the long term. Indeed, these newborn infants are at high risk of neurodevelopmental disorders that can lead to psychiatric disease and neurocognitive abnormalities or delay. Foetal growth restriction may also cause abnormalities in several organs, resulting in several pathologies, such as kidney, lung, liver disease and cardiometabolic disorders.<sup>3</sup>

The Mediterranean diet is the gold standard of nutrition for mothers, and it has been demonstrated that adherence to this diet is beneficial and may reduce pregnancy complications and improve birth outcomes.<sup>4</sup> The nutritional gold standard for the newborn infant is breast milk and that is why it is important to promote breastfeeding.<sup>5</sup> Human milk is tailored to the nutritional and developmental needs of the neonate. It is unique for each mother and changes in its nutritional composition the same feed, the day and the whole course of lactation. Breast milk promotes the optimal growth of newborn infants thanks

**Abbreviations:** <sup>1</sup>H-NMR, proton nuclear magnetic resonance; DOHaD, Developmental Origins of Health and Disease; GC, gas chromatography; GDM, gestational diabetes mellitus; HMO, human milk oligosaccharides; IUGR, intrauterine growth restriction; LC, liquid chromatography; MS, mass spectrometry; SCFAs, short chain fatty acids.

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to its peculiar components, such as human milk oligosaccharides (HMOs), stem cells and microbiota that colonizes the infant's guts.

An in-depth investigation of perinatal nutrition is a very useful tool, especially when the omics sciences are used, including metabolomics.<sup>6</sup> Metabolomics is based on various high-throughput techniques, such as liquid chromatography (LC) or gas chromatography (GC) in conjunction with mass spectrometry (LC-MS or GC-MS) and proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy. Metabolomics can immediately identify, and quantify, the metabolites present in a biological fluid, such as saliva, urine, sweat, stool, blood, breast milk, tissues and cells. This provides a molecular snapshot of an individual during physiological and pathological conditions. For example, metabolomics can help to investigate the effects of different nutritional regimens. Our research group was the first to perform a metabolomic analysis of breast milk.<sup>7</sup> Our aim was to carry out a non-systematic review to discuss how metabolomics can be used to investigate GDM, IUGR and breast milk, focusing on the metabolites found most frequently in previous studies.

## 1.1 | Metabolomics studies about GDM, IUGR and breastmilk

We used PubMed to search the literature for metabolomics and GDM, IUGR and breast milk, and we identified a total of 71 studies. The results are summarised in Table 1.

The search for GDM and metabolomics identified 32 studies published between 2011 and 2020, and it was the most studied topic. It appeared in 32 studies published from 2010–2021 and comprised a total of 6814 mothers and 1174 infants. Some studies analysed more than one type of biological sample and used more than one analytical technique (Table 1). The most frequently used technique was LC-MS, in 45% of the studies, and the most analysed sample was urine, in 28% of the studies.

When we used the keywords IUGR and metabolomics with the same database, this identified 18 studies from 2011–2020 with a total of 1363 mothers and 675 infants. Even in this context, the most used analysis technique was LC-MS, in 42% of the studies, and umbilical cord blood was the most analysed sample, in 37% of the studies. Again, some studies used more than one technique and analysed more than one type of sample.

Finally, when we used breast milk and metabolomics as keywords on PubMed, we found 21 studies from 2014–2020, with a total of 1497 mothers and 781 infants. The most used analytical technique was <sup>1</sup>H-NMR in 62.5% of the studies, and the most frequently analysed sample was breast milk, in 69.5% of the studies.

## 1.2 | Gestational diabetes

Authors defined GDM as glucose intolerance resulting in different severities of hyperglycaemia during pregnancy.<sup>8</sup> The most relevant,

### Key notes

- Perinatal nutrition can affect the health status of individuals for the rest of their lives and metabolomics is a very promising tool for investigating the effects of perinatal nutrition on mothers and their offspring.
- This non-systematic review focused on how metabolomics can be applied to gestational diabetes, intrauterine growth restriction and breast milk.
- It showed that nutrition, metabolome and microbiome interactions are gaining interest in the scientific community.

and frequent, metabolites in the 32 GDM studies, and the number of studies they were mentioned in, were as follows: choline and its derivatives (n=7), lipids and phospholipids (n=7), glycine (n=5), sphingomyelins (n=4), carnitine and acylcarnitine (n=4) and alanine, betaine creatinine and histidine (n=3). The metabolite results and their biological significance are shown in Table 2.

Two papers found decreased levels of phosphocholine and diacyl-phosphatidylcholine in the infant's umbilical cord blood and mother's blood plasma when the woman had GDM, compared to the controls.<sup>9,10</sup> The first study suggested that these reductions may have been due to high foetal demand for these metabolites, due to their key role in brain development.<sup>9</sup> The second study showed that metabolomics may provide a more precise diagnosis of GDM than the oral glucose tolerance test.<sup>10</sup> Furthermore, another study found decreased levels of phosphocholine in the umbilical cord blood of foetuses if their mothers had GDM, confirming a close relationship between this phospholipid and perturbations in lipid metabolism.<sup>11</sup>

Conversely, Lu et al found increased levels of these metabolites in the umbilical cord blood of foetuses when their mothers had GDM. These metabolites have also been associated with type 2 diabetes and may play a putative role in preterm births associated with GDM. However, further studies are needed.<sup>12</sup>

Women with GDM who breastfed for over three months showed increased plasma levels of phosphatidylcholine compared to those who did not, and their metabolomic profile was closely comparable to women with type 2 diabetes.<sup>13</sup>

On the contrary, chlorine was increased in the urine of women who went on to develop GDM than those who had healthy-term pregnancies. This metabolite has been associated with the early stages of GDM.<sup>14</sup> Moreover, phosphocholine was found increased in the serum of mothers with GDM.<sup>15</sup>

While lipids and phospholipids levels were altered in seven studies, Walejko showed increased levels of fatty acids in the serum of mothers with GDM. They also observed that women in the first trimester who went on to develop GDM displayed a metabolic profile that was closely comparable to non-pregnant women with type 1 or type 2 diabetes.<sup>16</sup> Another interesting fact is that different classes of lipids may behave differently in the same biofluid, in this case

TABLE 1 Details of metabolomics studies about GDM, IUGR and breast milk

Condition	Studies	Patients	Methods	Samples (n)
GDM	32 (2010–2021)	Mothers with GDM: 3694 Controls: 3120 Neonates born to mothers with GDM: 312 Controls: 862	LC-MS: 15 <sup>1</sup> H-NMR: 10 GC-MS: 7 MS: 1	Urine: 12 Plasma: 10 Serum: 7 Umbilical cord blood: 4 Amniotic fluid: 3 Meconium: 3 Breast milk: 3 Mothers' faeces: 1
IUGR	18 (2011–2020)	Mothers of neonates with IUGR: 365 Controls: 998 Neonates with IUGR: 338 Controls: 337	LC-MS: 8 <sup>1</sup> H-NMR: 7 GC-MS: 3 HR-MAS-MRS: 1	Umbilical cord blood: 7 Urine: 6 Serum: 2 Placenta: 1 Plasma: 1 Hair: 1 Milk: 1
Breast milk	21 (2014–2020)	Mothers: 1497 Neonates: 781	<sup>1</sup> H-NMR: 15 LC-MS: 4 GC-MS: 3 CE-MS: 1 MRS: 1	Milk: 16 Urine: 3 Faeces: 2 Blood: 1 Formula: 1

Abbreviations: <sup>1</sup>H-NMR, nuclear magnetic resonance; GC-MS, gas chromatography mass spectrometry; GDM, gestational diabetes mellitus; HR-MAS-MRS, high resolution magic angle spinning magnetic resonance spectroscopy; IUGR, intrauterine growth restriction; LC-MS, Liquid chromatography-mass spectrometry.

plasma. Some classes of lipids increased, and some decreased. Lai et al analysed the blood lipids of women with a history of GDM who progressed to type 2 diabetes. Diacyl-glycerol-phospholipids increased, and sphingolipids and phospholipids decreased. These results proved to be predictive of the progression to type 2 diabetes.<sup>17</sup> Law and Zhang linked the decreased phospholipids in GDM plasma to low-grade persistent inflammation.<sup>18</sup>

While phospholipids were decreased in the plasma of mothers with GDM, and in their infant's umbilical cord blood, their foetuses had a strong tendency to sequester these metabolites with very high lipid accumulation and adiposity.<sup>9</sup> This pattern may lead the foetus to develop metabolic disorders later in life. Nevertheless, in another study, the umbilical cord blood showed decreased levels of these metabolites, probably due to the prolonged exposure to hyperglycaemia.<sup>19</sup> One study found that the amniotic fluid of female and male foetuses with GDM showed some metabolic differences that were just based on their gender. The authors reported that female foetuses showed increased lipid content compared to males. This could be one explanation for the different complications experienced by newborn male and female infants when their mothers have GDM.<sup>20</sup>

Glycine was decreased in GDM patients in all five studies that analysed different biological fluids and it is considered a biomarker of type 2 diabetes.<sup>16,21</sup> The decrease of this amino acid in the serum of women who go on to develop GDM and in infants' umbilical cord blood could be due to both the alterations in its metabolism and placental production and transfer, respectively.<sup>16</sup> In one study, glycine in plasma was associated with five clinical parameters: body mass index, fasting plasma glucose, two-hour plasma glucose, fasting insulin and the homeostatic model assessment of insulin resistance.<sup>21</sup>

In breast milk, glycine contributed to the definition of the molecular fingerprint of breast milk in GDM.<sup>22</sup> Other research indicated that decreased levels of glycine in the amniotic fluids of women who went on to develop GDM may also have been due to foetal protein demand. Moreover, glycine conjugates were related to metabolic disorders.<sup>11</sup>

Sphingomyelins were found to be lower in the plasma of mothers with GDM, in common with other classes of lipids, due to their role in brain development. While they were elevated in the amniotic fluid of female foetuses for the same reason, they protected the foetus from GDM exposure.<sup>20</sup> They are considered biomarkers of type 2 diabetes progression and have been found to have decreased up to seven years before the onset of type 2 diabetes.<sup>17,21</sup> Indeed, their breakdown into ceramides induced beta-cell apoptosis.<sup>21</sup>

Carnitine and acylcarnitine were present in four studies with different results. High levels of acyl-carnitine in the urine and plasma of GDM patients were reported to be a common metabolic event of glucose homeostasis and were related to insulin resistance.<sup>10,23</sup> In umbilical cord blood, decreased levels of carnitine and acylcarnitine may have been due to the inability of the foetus to synthesise carnitine and the reduced initiation of fatty acid oxidation as a consequence of foetal hyperglycaemia.<sup>9</sup> Interestingly, another research group found lower levels of carnitine in children with type 1 diabetes.<sup>24</sup>

Increased alanine was found in three studies, and it was different in the faecal samples of women with GDM and non-GDM controls. In the same study, the authors investigated the neonatal blood metabolome infants born to mothers with GDM and found a connection between the maternal faecal metabolome

TABLE 2 Main metabolites seen in GDM and their biological significance

Metabolite	Number of studies	Results	Biological significance
Choline, lyso-phosphocholine, Phosphocholine	7 <sup>9-15</sup>	↑cord blood, urine, serum, umbilical cord blood, plasma, ↓serum, umbilical cord blood, amniotic fluid, plasma	High demand from the foetus, associated with type 2 diabetes. Putative role of GDM associated with preterm birth. Distinction between mothers with GDM breastfeeding or not. Associated with early stages of GDM. Biomarker of alteration of lipid metabolism.
Lipids and phospholipids	7 <sup>9,16-20,23</sup>	↑serum, plasma, amniotic fluid of female ↓plasma, umbilical cord blood, urine	Energy source for the mother. Increased lipolysis. Biomarkers of type 2 diabetes progression. Protective for brain and immune function. Alteration of lipid enzymatic activity. Low-grade inflammation. High foetal exposure to hyperglycaemia.
Glycine	5 <sup>9,11,15,16,21,22</sup>	↓cord blood, plasma, serum, breast milk, amniotic fluid	Biomarker for type 2 diabetes progression. Alteration of placental production and transfer. Metabolic alterations of GDM. Breast milk fingerprint of GDM. Glycine conjugate associated with metabolic disorder.
Sphingomyelins	4 <sup>9,17,20,21</sup>	↑amniotic fluid ↓plasma	High foetal demand. Biomarker of type 2 diabetes progression. Protective for brain development.
Carnitine and acylcarnitine	4 <sup>9,10,21,23</sup>	↑urine, plasma ↓umbilical cord blood, plasma	Common metabolic event of glucose homeostasis. Foetus unable to synthesise carnitine. Impaired fatty acid oxidation. Correlated with insulin resistance.
Alanine	3 <sup>19,25,26</sup>	↑maternal faecal sample, serum, cord serum	Discriminant metabolite. Substrate of gluconeogenesis. Derangement of the metabolism of women with GDM.
Betaine	3 <sup>16,27,28</sup>	↑cord blood ↓plasma, urine	Choline metabolism. Altered foetal uptake. Metabolite of microbial origin. Decreased in women who went on to develop GDM.
Creatinine	3 <sup>11,16,26</sup>	↓serum, amniotic fluid	Increased glomerular filtration rate. Development of hyperglycaemia. Changes in renal function
Histidine	3 <sup>16,19,23</sup>	↑plasma, umbilical cord blood ↓serum	Elevated blood glucose. Hepatic gluconeogenesis. Prolonged foetal exposure to hyperglycaemia.

Abbreviation: GDM, gestational diabetes mellitus.

and the neonatal blood metabolome. This relationship depicted, at least partly, the molecular basis of the negative effects of GDM on newborn infants.<sup>25</sup> Indeed, alanine has been shown to be a biomarker of perturbations in glucose and amino acid metabolism.<sup>19,26</sup>

Betaine levels, which are part of choline metabolism, were altered in three studies. It was decreased in the urine and plasma of women who went on to develop GDM<sup>27,28</sup> and increased in the umbilical cord blood of foetuses born to such women.<sup>16</sup> Increases in umbilical cord blood betaine may be related to altered foetal uptake, while plasma betaine decreases may have a microbial origin, because increasing evidence has demonstrated an association between GDM and gut dysbiosis.<sup>27</sup>

Creatinine was found decreased in three studies. In women who went on to develop GDM and already had GDM in their serum, this could have been due to the progressive development of hyperglycaemia during pregnancy and the increased glomerular filtration rate at the early stage of gestation.<sup>16,26</sup> Nevertheless, further studies are needed to validate its predictive power. Decreased levels of creatinine in the amniotic fluid of foetuses carried by women with GDM suggested that changes in renal function were associated with this condition.<sup>11</sup>

Histidine was found increased in urine, plasma and umbilical cord blood, confirming altered amino acid metabolism.<sup>19,23</sup> Moreover, it increased in the serum of women who went on to develop GDM, reflecting hyperglycaemia and alterations in hepatic gluconeogenesis.<sup>16</sup>

### 1.3 | Intrauterine growth restriction

IUGR alters foetal growth, and the foetus is much smaller than it should be at a given gestational age.

The seven most relevant metabolites in these 18 studies, and the number of studies they were mentioned in, were as follows: tyrosine (n=5), valine (n=5), alanine (n=5), myoinositol (n=4), glutamine and acetyl-glutamine (n=4), choline and phosphocholine (n=3) and methionine (n=3). These metabolites and their biological significance are summarized in Table 3.

Tyrosine was detected in six studies. It was increased in mono-chorionic twins and singletons who had IUGR.<sup>29,30</sup> This demonstrated that this intrauterine growth discordance was caused by extrauterine and intrauterine factors rather than genetics. Another study found decreased levels of tyrosine in cord blood plasma and suggested that it could be associated with early-onset severe

IUGR.<sup>31</sup> Tyrosine was also decreased in the urine of mothers who went on to develop GDM at the end of the first trimester and in the plasma of mothers who delivered babies with IUGR.<sup>32,33</sup> Thus, tyrosine can have predictive power for IUGR. In addition, tyrosine has been associated with the gut microbiota and women who deliver newborn infants with IUGR could have a peculiar microbiota.<sup>33</sup> Sulek et al<sup>34</sup> analysed the hair of mothers who delivered IUGR infants using metabolomics, which was not a common choice. The authors justified this investigation by stating that hair growth was slow and its structure was relatively stable. Furthermore, hair growth incorporates endogenous compounds and environmental exposure from blood; hair sampling is much less invasive than blood sampling, and storing hair is relatively simple. The mechanisms of amino acid transportation into the hair follicles in IUGR is still not clear. Thus, the reason for the tyrosine decreased levels in IUGR maternal hair is still unknown.<sup>34</sup>

Alanine and phenylalanine were found altered in five studies of umbilical cord blood and the urine of mothers who later delivered infants with IUGR. They were decreased in urine, which reflected the early stage of amino acid metabolism perturbation in IUGR. Three studies showed increased levels of phenylalanine and alanine in the umbilical cord blood of twins and singletons.<sup>29,30,35</sup> These amino acids are precursors of tyrosine and thus of monoamine neurotransmitters. Hence, they could be associated with worse neurological clinical outcomes,<sup>31</sup> but this hypothesis needs further investigation.

Conversely, these amino acids were decreased in umbilical cord blood, suggesting that phenylalanine and alanine were the best discriminants between foetuses with IUGR and controls who were adequate for gestational age during healthy pregnancies. This could be due to the altered placental tissue subsequent to the hypercatabolic state of IUGR.<sup>31</sup>

Valine was discussed in five studies. It was decreased in the umbilical cord blood of twins and singletons born with IUGR.<sup>31,35</sup> In twins, valine was related to umbilical artery doppler abnormalities.<sup>35</sup> That study supported the hypothesis that the amino acid deficit in twins with IUGR was due to impaired placental transport rather than intertwin transfusion.<sup>35</sup> In contrast with previous studies, Abd El Wahed et al<sup>30</sup> showed increased levels of valine in umbilical cord blood. The authors hypothesised that elevated levels of cortisol in stressful situations, such as IUGR, could reduce its specific catabolic enzymes, leading to increased levels.<sup>30</sup> Valine was also decreased in the milk of mothers who delivered infants with IUGR. This amino acid plays a role in the development of the brain and immune system and glucose metabolism. The decreased levels may be due to the different needs of newborn infants with IUGR.<sup>36</sup>

Myoinositol is a derivative of inositol, is a cyclic sugar alcohol, and is the isomer most frequently present in nature.<sup>37</sup> In immature lung tissue, myoinositol is a substrate for the production of the surfactant.<sup>38</sup> It is also a second messenger of insulin metabolism. Studies have reported that it was altered in the amniotic fluid of foetuses carried by mothers

**TABLE 3** The most relevant IUGR metabolites and their biological significance

Metabolites	Number of studies	Results	Biological significance
Tyrosine	6 <sup>29-34</sup>	↑umbilical cord blood ↓urine, hair of the mothers, umbilical cord blood, plasma	Derangement of phenylalanine pathway. Associated with poor foetal growth. Associated with microbiota. Amino acid alteration. Associated with early-onset severe IUGR. Predictive for IUGR.
Alanine and Phenylalanine	5 <sup>29-31,33,35</sup>	↑umbilical cord blood ↓ umbilical cord blood, urine	Precursor of tyrosine and neurotransmitters. Altered placental transport. Associated with worse clinical outcomes. Alterations of amino acid transfer and fetoplacental perfusion.
Valine	5 <sup>30,31,34-36</sup>	↑umbilical cord blood ↓milk, umbilical cord blood, hair	Role in brain function. Immune system and glucose metabolism. Reduced catabolic enzyme for this amino acid. Linked to starvation and lack of energy supply. Related to umbilical artery doppler abnormalities.
Myoinositol	4 <sup>39-42</sup>	↑urine	Biomarker of derangement of glucose metabolism. Cellular damage
Choline	3 <sup>31,35,36</sup>	↑milk, ↓umbilical cord blood	Needed for the proper development of foetal brain and liver. Related to severity of brain damage. Energy demand of the foetus
Glutamine and Acetyl-glutamine	3 <sup>30,31,36</sup>	↑milk, umbilical cord blood	Contributes to neurodevelopment and to gut colonisation of the infant gut by microbiota. Main sources of energy for foetal cells.
Methionine	3 <sup>29,30,34</sup>	↑umbilical cord blood ↓hair of mothers	Substrate for protein synthesis. Source of the methyl group.

TABLE 4 Breast milk main metabolites and biological significance

Metabolite	Number of studies	Results	Biological significance
Human Milk Oligosaccharides	7 <sup>43-49</sup>	↑ milk of preterm, normal weight mothers Different ethnic groups had different Human Milk Oligosaccharides Distinction among phenotypes ↓ milk at term	Preterm milk played protective role and met neonates' developmental needs. Obese metabolic signature of breast milk. Term versus preterm milk met different nutritional and developmental needs that were different over time.
Lipids	6 <sup>49-53,60</sup>	↑non-esterified fatty acid in human milk, short-chain fatty acids in faeces of breastfed neonates with human milk and enriched formula, SCFAs in milk during breastfeeding 98 previously unidentified lipids ↓ Docosahexaenoic acid derivatives in milk of fasting women, SCFAs in faeces of polluted breastfed newborn infants, aminobutyrate in women with irritable bowel disease	Usefulness of metabolomics in breastmilk analysis. Short Chain Fatty Acids related to gut microbiota. Docosahexaenoic acid related to brain development. Lipids were main source of energy for the infant
Lactose	6 <sup>36,43,54-56,58</sup>	↑Milk of Large for Gestational Age, Intrauterine Growth Restriction, Milk of preterm, extremely preterm, Urine of breastfeeding mother ↓ Milk of mothers with Inflammatory Bowel Disease, milk in late lactation stage	Prevent hypoglycaemia. Altered gut microbiota. Changes in carbohydrates biosynthesis. Distinctive of breastfeeding mothers
Choline and phosphocholine	5 <sup>36,38,57-59</sup>	↑milk fortified with bovine fortifier, Intrauterine Growth Restriction, Urine Variation in blood depending on formula or breast milk	Brain development. Variation according to the type of nutrition

with GDM and decreased in the mothers' serum.<sup>11,16</sup> Myoinositol was found to be elevated in the urine of infants with IUGR in all of those four studies. Marincola et al<sup>39</sup> stated that myoinositol could be considered a biomarker for the derangement of glucose metabolism in newborn infants with IUGR.<sup>39</sup> Barberini et al<sup>40</sup> highlighted how this metabolite was the main discriminant between foetuses who had IUGR or were large for gestational age versus controls at birth.<sup>40</sup> Indeed, infants with IUGR or who were large for gestational age showed perturbations in glucose metabolism, confirming previous results.<sup>41</sup> Moreover, since myoinositol is a second messenger, it is located within cells. Thus, urine myoinositol originates from cellular damage. Indeed, oxidative stress is one of the characteristics of IUGR.<sup>42</sup>

Other metabolites of interest were choline and its derivatives, which were altered in three studies. The alteration of these metabolites has been shown to be present in GDM as well.<sup>12</sup> Choline and phosphocholine were found elevated in the milk of women who delivered newborn infants with IUGR. Choline is an important precursor of acetyl-choline, a methyl donor of various metabolic processes that is also involved in lipid metabolism. Moreover, it is directly related to neonatal growth. Thus, these increased levels are needed by foetuses with IUGR to meet their energy and developmental needs.<sup>36</sup>

Glutamine and acetyl-glutamine were found to be elevated in IUGR in three studies. In breast milk, glycine is one of the most abundant amino acids, and it is related to the infant's neurodevelopment and gut colonisation by the microbiota.<sup>36</sup> Studies also reported that

glutamine was also decreased in the umbilical cord blood of foetuses with IUGR.<sup>30,31</sup> It is reasonable to assume that glutamine is one of the main energy sources for the foetus and that further energy supplies, such as glucose, are lacking due to the catabolic state of IUGR.<sup>31</sup>

In addition, methionine was detected in three studies. It was increased in the umbilical cord blood of twins and singletons with IUGR.<sup>29,30</sup> It is the substrate of protein synthesis and a methyl donor involved in many pathways all over the human body. Thus, the alteration of this amino acid could lead to altered foetal and neonatal development. Methionine was decreased in the hair sample of mothers who delivered an infant with IUGR.<sup>34</sup> The reason is still unclear, but it could be a biomarker of the derangement of amino acid metabolism in IUGR.

## 1.4 | Breast milk

As already mentioned, breast milk is the gold standard for neonatal nutrition. It is unique for each neonate and is tailored to their needs. Our search highlighted that the four most relevant metabolites in breast milk metabolomic studies were human milk oligosaccharides (HMOs) (mentioned in seven studies), lipids (n=7), lactose (n=6) and choline and its derivatives (n=5).

The information concerning these metabolites and their biological significance are summarised in Table 4.



HMOs were detected in seven studies, and these provided very interesting information including the fact they are the third most prevalent class of nutrients in breast milk. The levels of HMOs were higher preterm than term milk.<sup>43,44</sup> This could be due to the unlimited biological properties of this class of molecules and to the different nutritional needs of preterm infants. There are also different metabolic signatures in the breast milk of obese and normal weight mothers.<sup>45</sup> In addition, it was possible to detect difference in HMOs production in the different regions of the world.<sup>46</sup> Furthermore, metabolomics made it possible to distinguish different phenotypes.<sup>47</sup> It is also possible to study each HMO individually. For example, sialyllactose is decreased in term milk,<sup>48</sup> and fucose is increased in term milk.<sup>49</sup> This could be due to the different phenotypes of the mothers or the different lactation stages, since their quantity changes over time.

Different classes of lipids changed in six studies. One study found 98 lipids that had never been detected in breast milk before, showing how metabolomics is very useful in investigating breast milk.<sup>50</sup> The faeces of breastfed newborn infants in a polluted environment displayed lower levels of short-chain fatty acids (SCFAs). Since SCFAs are produced by the gut microbiota, these breastfed children also displayed lower diversity in their gut bacteria.<sup>51</sup> In fasting women four and six months after delivery, docosahexaenoic acid and its derivatives were decreased, implying a temporal modification of the trend in metabolites in breast milk.<sup>52</sup> When human milk, bovine milk and formula were compared, human milk showed a higher content of non-esterified fatty acids. This is because lipids are the main source of energy for infants and play several important physiological roles in their development. The comparison among enriched formula, human milk and normal formula showed higher levels of SCFAs in human milk and enriched formula and in the faeces of these newborn infants. Since they are biomarkers of gut microbial activity, this demonstrated that they vary according to the type of milk.<sup>53</sup> These results were comparable with those found in a previous study of term milk.<sup>49</sup>

Lactose was detected in six studies and was elevated in the breast milk of women who delivered infants that were large for gestational age and those that had IUGR. This metabolite has been correlated with growth, and it could be protective against hyperglycaemia, a common condition for such neonates.<sup>36</sup> It is also increased in the breast milk mothers give to their preterm infants, due to their high energy demand.<sup>43</sup> Lactose was also very high in the milk of extremely preterm newborn infants.<sup>54</sup> Another study found that lactose levels were decreased in women with inflammatory bowel disease. This disaccharide is hydrolysed into glucose and galactose in the small intestine, and this process modulates the gut microbiota and the immune response of breastfed neonates. The decreased lactose in the breast milk of mothers with this disease may alter the composition of the gut microbiota and compromise the health of their offspring, even in the long term.<sup>55</sup>

Lactose was also detected in the urine of women who breastfed, demonstrating a completely different metabolic profile to those who did not breastfeed.<sup>56</sup>

Finally, choline was discussed in five studies. Giribaldi et al<sup>57</sup> described elevated levels of choline in the urine of very low birth weight newborn infants, whose milk was fortified with a bovine milk-based fortifier.<sup>57</sup> This showed how metabolomics could be useful for monitoring the metabolism of these very fragile small patients and choose the best type of fortifier for them. Choline, which is related to infant growth and involved in brain development, was increased in the breast milk of women who delivered infants with IUGR. Thus, these high levels may protect newborn infants against neurodevelopmental problems.<sup>36</sup> Indeed, higher levels of choline and reduced levels of phosphocholine were found in term milk, maybe for the same reason.<sup>58</sup> In addition, different levels of choline were detected in the blood of newborn infants according whether they were given breast milk or formula.<sup>59</sup> Moreover, choline and betaine showed the same trend over time in the urine of newborn infants fed human milk, formula milk and enriched formula.<sup>38</sup>

## 2 | CONCLUSION

The growing number of studies using metabolomics to investigate perinatal nutrition reflects the increasing interest of the scientific community in this topic. Questions are now being answered, such as the pathophysiology of IUGR and GDM and new aspects of breast milk. This has only been made possible by metabolomic analyses of individuals. Although metabolomics is relatively young, we are convinced that it will prove increasingly useful in investigating perinatal nutrition. It provides the key to personalised medicine, and it will help clinicians to provide the best care possible for both mothers and their neonates.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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