Gestational Diabetes Mellitus Changes the Metabolomes of Human Colostrum, Transition Milk and Mature Milk

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Background:
Gestational diabetes mellitus (GDM) is a pregnancy complication that is diagnosed by the novel onset of abnormal glucose intolerance. Our study aimed to investigate the changes in human breast milk metabolome over the first month of lactation and how GDM affects milk metabolome.

Material/Methods:
Colostrum, transition milk, and mature milk samples from women with normal uncomplicated pregnancies (n=94) and women with GDM-complicated pregnancies (n=90) were subjected to metabolomic profiling by the use of gas chromatography-mass spectrometry (GC-MS).

Results:
For the uncomplicated pregnancies, there were 59 metabolites that significantly differed among colostrum, transition milk, and mature milk samples, while 58 metabolites differed in colostrum, transition milk, and mature milk samples from the GDM pregnancies. There were 28 metabolites that were found to be significantly different between women with normal pregnancies and women with GDM pregnancies among colostrum, transition milk, and mature milk samples.

Conclusions:
The metabolic profile of human milk is dynamic throughout the first months of lactation. High levels of amino acids in colostrum and high levels of saturated fatty acids and unsaturated fatty acids in mature milk, which may be critical for neonatal development in the first month of life, were features of both normal and GDM pregnancies.

MeSH Keywords:
Diabetes, Gestational • Metabolome • Milk, Human

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Background

Gestational diabetes mellitus (GDM) is defined as hyperglycemia that is first diagnosed during mid-pregnancy and its prevalence ranges from 9.3% to 25.5% worldwide [1]. Pregnant women with uncontrolled hyperglycemia have higher incidences of complications for both the mother and the fetus [2,3], such as fetal growth restriction (FGR), embryonic death, fetal malformation, and postpartum type-2 diabetes [4,5]. Although breastfeeding has been assumed to be an important low-cost intervention for women with GDM, and women with GDM are encouraged to breastfeed their babies at least for 3 months [6], it remains to be determined whether GDM affects the composition of breast milk during lactation.

Breast milk is produced by the mammary glands [7], and it is composed of fatty acids, proteins, sugars, immune cells, and bioactive molecules, which help immune system and organ development during a child’s first 6 months of life [8]. The composition of breast milk varies throughout the lactation period; therefore, breast milk is also called colostrum, transition milk, and mature milk in terms of the different stages of lactation. Colostrum is produced until the 5th day of lactation, transition milk is produced until the 14th day; and mature milk is synthesized beginning at the end of the 2nd week of lactation [9]. Breast milk composition can be affected by maternal age, weight, diet, and health condition [10,11], but how GDM influences breast milk content is still unclear.

Metabolomic profiling involves the systematic study of low-molecular-weight (<1 kDa) endogenous and exogenous metabolites (e.g., lipids, amino acids, and organic acids) that represent cellular functions at the intersection of genetic and environmental effects [12,13]. Metabolomic analyses have recently been considered promising tools to study milk nutritional quality [14]. In the present study, we performed gas chromatography-mass spectrometry (GC-MS) to determine the metabolites in breast milk; this may aid in an in-depth understanding of the components and variations in breast milk between women with GDM and women with uncomplicated pregnancies.

Material and Methods

Study population

A total of 100 pregnant women with GDM and 100 women with uncomplicated pregnancies who were seen at the Department of Obstetrics of The First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from January 2016 to December 2016 were randomly recruited (registration number: chiCTR-ROC-17011508). The diagnosis of GDM was defined according to the International Association of Diabetes and Pregnancy (IADPSG) guidelines with a 75 g oral glucose tolerance test (OGTT) that resulted in fasting glucose ≥5.1 mmol/L, 1-hour glucose ≥10 mmol/L, or 2-hour glucose ≥8.5 mmol/L. Individuals with pregestational diabetes mellitus or other major pregnancy complications, such as preeclampsia, intrahepatic cholestasis, etc., were excluded. Due to loss of follow-up, complete sampling of colostrum, transition milk, and mature milk was achieved for 90 women with GDM and 94 women with normal pregnancy (control group). All study participants gave written informed consent, and the study procedures were approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (No. 2016-65).

Breast milk sampling

The breast milk samples were collected on postpartum days 1–3 (colostrum), on days 7–10 (transitional milk), and at 4 weeks (mature milk). The whole milk from each mother was obtained completely by pump expression in the morning of the sampling day before feeding the newborn. After each expression, the whole milk was homogenized via vortexing the tube for 30 seconds, and 6 mL of homogenized milk was transferred into sterile Eppendorf tubes in aliquots and immediately frozen at −80°C. The remainder of the milk was returned to the mothers for infant feeding.

Sample preparation for the GC-MS analysis

The samples were thawed on ice, and 300 μL of breast milk was transferred into a new sterile polypropylene tube. Four internal standards, which included 20 μL of DL-alanine-2, 3, 3, 3-d4 (Sigma, USA, 10 mM), DL-phenylalanine (CIL, USA, 10 mM), DL-tyrosine (CIL, USA, 10 mM), and octanoic acid (CIL, USA, 10 mM), were added into each tube and vortexed for 30 seconds, and then the samples were dried in a SpeedVac with a refrigerated vapor trap (Thermo Scientific, Auckland, New Zealand). The dried breast milk underwent cold methanol extraction using 50% and 80% v/v methanol/water. The samples were centrifuged (17 000 g/15 minute) at 4°C, and then the pooled supernatants were dried. The dried samples were stored at −20°C until derivatization. For the derivation, the samples were derivatized by methyl chloroformate alkylation. A pooled quality control (QC) sample was used to analyze the reproducibility and quantitative robustness and analyzed for each batch by taking 20 μL from each prepared sample within the batch. The QC sample was then run multiple times at the beginning of each batch, in between runs, and at the end of each batch. In addition, blanks were included to facilitate correction for background noise.

GC-MS analysis and data acquisition

A total of 552 human milk samples from 90 GDM patients and 94 controls were subjected to metabolomic analysis by using
Results

Demographic and clinical characteristics

The clinical characteristics of the enrolled study participants are summarized in Supplementary Table 1. The maternal BMI was $21.55\pm2.66$ and $20.55\pm2.06$ kg/m$^2$ in the GDM group and the control group, respectively ($P<0.05$). Maternal weight gain during pregnancy in the GDM group ($14.05\pm5.71$ kg) was significantly lower than that in the control group ($15.98\pm4.79$ kg, $P<0.05$). The average maternal age was $28.67\pm4.23$ years in the control group and $30.78\pm4.19$ years in the GDM group ($P<0.001$). Meanwhile, the average gestational age was $39.50\pm1.17$ weeks in the control group and $39.14\pm1.00$ weeks in the GDM group ($P<0.05$). However, there were no significant differences in neonatal birth weight, height, or head circumference between the GDM group and the control group.

Identified metabolites in human breast milk

A total of 187 metabolites were identified in the breast milk samples (Supplementary Table 2), including 4 alkanes, 17 amino acid derivatives, 21 amino acids, 22 saturated fatty acids, 29 unsaturated fatty acids, 8 TCA cycle intermediates, 3 cofactors or vitamins, 3 keto acids and derivatives, 1 glycolytic intermediate, 43 organic acids, and 36 organic compounds. The coefficient of variation (CV) for the QC samples was listed to analyze the reproducibility and the average values of CV score were 29.5, which indicated that the model is valid for the analysis (Supplementary Table 3).

Metabolites that were altered in the colostrum, transition milk, and mature milk of women with uncomplicated pregnancies

The OPLS-DA results demonstrated that $|R^2|>0.5$ and $|Q^2|>0.4$, which indicated that different metabolite profiles were found in colostrum, transition milk, and mature milk (Figure 1). The empirical $P$-value was less than 0.05, which reflected the reliability of the OPLS-DA model. The analysis of the OPLS-DA loading coefficient plots showed that 59 metabolites were identified as contributing to the differences among the metabolomic profiles of colostrum, transition, and mature milk in normal pregnancies (Figure 2).

According to the Human Metabolome Database (HMDB) and NIST database, the majority of amino acids, including all essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, valine, threonine, and derivatives, were increased in colostrum compared to transition milk and mature milk. Moreover, 6 out of the 7 different saturated fatty acids, including palmitic acid, margaric acid, myristic acid, dodecanoic acid, decanoic acid, and octanoic acid, were found...
to be significantly lower in colostrum compared to transition milk; the levels of hexanoic acid were comparable between colostrum and transition milk but increased in mature milk. Of the 13 differing unsaturated fatty acids, palmitelaidic acid, oleic acid, cis-vaccenic acid, conjugated linoleic acid, 9,12-octadecadienoic (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester, myristoleic acid, 3-hydroxydecanoic acid, and gamma linolenic acid were lower in colostrum, while 3-methyl-2-oxopentanoic acid, butanedioic acid, ethyl methyl ester, 4-methyl-2-oxopentanoic acid, 5-cyano-4-methoxymino-7-phenyl-hept-6-enoic acid, methyl ester, (E,S)-2-hexenoic acid, 4-amino-5-methylmethyl ester were higher in colostrum than in the other 2 milk types. Myristoleic acid, oleic acid, cis-vaccenic acid, and 5-cyano-4-methoxymino-7-phenyl-hept-6-enoic acid were lower in mature milk compared with those in transition milk, while 3-hydroxydecanoic acid, (E,S)-2-hexenoic acid, and 4-amino-5-methylmethyl ester were higher in mature milk compared with those in transition milk. The remaining 6 unsaturated fatty acids were less significantly different between mature milk and transition milk. Moreover, compared to the levels in the transition milk and mature milk, the levels of 2 TCA cycle intermediates, 2-oxoglutaric acid and isocitric acid were higher in colostrum, while the levels of citric acid and malic acid were lower in colostrum. For the class of cofactors and vitamins, the level of nicotinic acid was slightly lower in mature milk and NADP/NADPH was moderately higher in transition and mature milk than in colostrum.

Although the metabolome of transition milk was very similar to that of mature milk, notable discrepancies were also identified between these 2 groups. For instance, the TCA cycle intermediates, such as citric acid, isocitric acid, and 2-phosphoenolpyruvic acid, were increased in mature milk.

Metabolites that were altered in the colostrum, transition milk, and mature milk of women with GDM pregnancies

Similarly, in the GDM group, the OPLS-DA results demonstrated that $|R^2| > 0.5$ and $|Q^2| > 0.4$, indicating that different metabolite profiles were found among colostrum, transition milk, and mature milk (Figure 1). Fifty-eight metabolites were identified as contributing to the differences in the metabolomic profiles among the colostrum, transition, and mature milk of GDM pregnancies (Figure 3). The metabolome composition and differences among the colostrum, transition milk, and mature milk from GDM mothers shared many similarities with those from normal pregnancies. However, alterations in breast milk metabolome due to GDM were identified. For example, the levels of stearic acid, pentadecanoic acid, 9-heptadecanoic, and arachidic acid were significantly lower in GDM transition milk than in GDM colostrum (these fatty acids did not differ significantly between the colostrum and transitional milk from normal pregnancies). Moreover, there were significant differences in 9,12-octadecadienoic (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester and glutamine between colostrum and transition milk from normal pregnancies that were not identified in the GDM group.

Metabolites in the breast milk that showed the differences between GDM and uncomplicated pregnancies

To determine the detailed variations in metabolite enrichment between the GDM group and the control group, linear logistic regression analyses were performed (Figure 4). We found that most of the statistically differential metabolites were lower in the GDM group. In colostrum, 6 metabolites were significantly lower in the GDM group compared to the control group.
Figure 2. Heat Map of key metabolites that differed in colostrum, transitional milk, and mature milk in the uncomplicated pregnancy control group. Comparisons of colostrum versus transitional milk and transitional milk versus mature milk are depicted. Increased and decreased metabolite levels are depicted by red and green colors, respectively.
Figure 3. Heat Map of key metabolites that differed in colostrum, transitional milk, and mature milk in the GDM pregnancy group. Comparisons of colostrum versus transitional milk and transitional milk versus mature milk are depicted. Increased and decreased metabolite levels are depicted by red and green colors, respectively.

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including 1 alkane [heneicosane (NIST: 81.4%)], 1 amino acid and derivatives [G\glycine, N-(methoxyoxoacetyl)-, methyl ester (NIST: 65.7%)] and 4 organic acids (3-aminoisobutyric acid, glutamine, oxaloacetic acid, and 4-aminobutyric acid). In transitional milk, 5 metabolites were reduced in the GDM group, including 2 amino acid and derivatives [D-proline, glycine, N-(methoxyoxoacetyl)-, methyl ester (NIST: 65.7%)], 2 organic acids (hydroxybenzoic acid, malonic acid) and 1 unsaturated fatty acid (9-heptadecenoic acid), while 1 amino acid (asparagine) and 1 TCA cycle intermediate (malic acid) were

Figure 4. Linear logistic regression analysis of the metabolites in breast milk between the GDM group and the healthy control group. Red blocks indicate higher metabolite in the GDM group compare to the control group, whereas green blocks represent lower predicted metabolite levels in the GDM group compared to the control group. Only metabolites with P-value less than 0.05 and q-value were less than 0.15 (colostrum), 0.05 (transitional milk), or 0.05 (mature milk) are shown.
elevated in the GDM group. Importantly, there were 21 metabolites that were significantly different between these 2 groups. There were 17 metabolites that were significantly decreased in the GDM group compared with the control group, including 1 alkane (nonacosane), 1 amino acid (glycine), 3 amino acid and derivatives [glycine, N-ethyl-N-(2-methoxyethoxycarbonyl)-], and 2-methoxyethyl ester (NIST: 79.5%), pyroglutamic acid, beta-alanine], 6 organic acids (2-oxoadipic acid, 3-methyl-2-oxovaleric acid, 4-aminobutyric acid, glutamine, oxalic acid, and oxaloacetic acid), 1 saturated fatty acid (pimelic acid), 4 unsaturated fatty acids (9-heptadecenoic acid, 10-pentadecenoic acid, 2-hydroxyglutaric acid, and nervonic acid), while 4 metabolites were significantly increased in the GDM group including 1 alkane [heneicosane (NIST: 81.4%)], 1 amino acid (cysteine), 1 saturated fatty acid (lignoceric), and 1 TCA cycle intermediate (malic acid).

Discussion

Although nuclear magnetic resonance (NMR) has been applied to investigate changes in maternal milk metabolome [19,20], GC-MS can provide more information [21]. According to Wishart [21], GC-MS can be used to identify and quantify a wide range of metabolites with relatively high sensitivity and reproducibility. However, with chemical derivatization participating, this process may possibly lead to an unsatisfying result by producing some by-products. Recently, GC-MS has been widely used in metabolomics such as cerebrospinal fluid metabolome, blood metabolome, and human breast milk metabolome [22–25].

In our study, we found that maternal age and BMI were higher in the GDM group than in the control group; which was consistent with results from previous studies [26,27]. Breast milk provides various essential nutrients and helps the immune system and organ development in neonates [8,28,29]. The components of human milk vary during lactation, particularly in the first month postpartum. Colostrum is rich in immunologic compounds and growth factors [30,31], while mature milk becomes stable to avoid further fluctuations [32]. In the present study, we analyzed the composition of the metabolites in breast milk at the different stages of lactation from the present study, we analyzed the composition of the metabolome [22–25].

Our data demonstrated that after normal pregnancies, 16 amino acids were increased in colostrum compared to transition and mature milk, including all essential amino acids and branched-chain amino acids (leucine, isoleucine, and valine). Free amino acids are an important source of nitrogen for the growing infant and are more readily absorbed than protein-derived amino acids [33]. Branched-chain amino acids are key nitrogen donors involved in interorgan and intercellular nitrogen shuttling, and leucine is an important nutrient signal [34,35]. Lysine is necessary for protein synthesis, and its deficiency has been found to cause weight loss in neonates [36]. Infants grow rapidly in the first months of life; therefore, high levels of amino acids in colostrum are likely to be a critical resource for neonatal development.

Fatty acids are known to be key to infant growth, brain development, and health [37]. Colostrum and mature milk contain 1.9–2.3% and 3.5–4.5% lipids, respectively [38]. We found increased levels of fatty acids in mature milk compared to those in colostrum, although hexanoic acid, which is a short-chain fatty acid widely used for parenteral nutrition in individuals requiring supplemental nutrition [39], was found to be increased in colostrum in our study. This enrichment of fatty acids in mature milk is likely to be important for infant development. Gamma-linolenic acid in breast milk might reduce the risk of mother-to-child transmission of HIV by breastfeeding [40]. High concentrations of long-chain fatty acids in breast milk, such as oleic acid, might have functional effects on the establishment of gut microbiota in early life [41].

It is known that the offspring of GDM mothers are at a higher risk type-2 diabetes [5]. Several studies have documented alterations in the plasma metabolome of women with GDM, either during pregnancy or postpartum, compared to that in women with uncomplicated pregnancies [42–44]. We found that the components of breast milk in healthy mothers were quite similar to those in GDM mothers as shown by OPLS-DA; most of the difference in milk composition ranging from colostrum to mature milk are subtle. Klein and colleagues reported that there was no significantly difference in free amino acids in breast milk between GDM patients and normal controls [45], while our study demonstrated that quite a lot of free fatty acids in breast milk significantly declined in the GDM group compare to the control group. Considering that both amino acids and free fatty acids are the major nutrients in human breast milk and important for neonatal development, the similarity in amino acids profiles implies that the discrepancies in free fatty acids profile might play a more profound role in breastfeeding related offspring developmental disorders or health-risks in later life. Since free fatty acids are the building blocks for adipogenesis and neurons, disturbed fatty acids composition in breast milk might compromise neonatal adiposity and brain development if breast fed. Therefore, fatty acids composition of formula should be carefully adjusted for infants conceived by GDM mothers.

Conclusions

The metabolomic profile of human milk is dynamic throughout the first month of lactation. High levels of amino acids in
colostrum and high levels of saturated and unsaturated fatty acids in mature milk were features of breast milk from women in had both the normal pregnancy group and GDM pregnancy group. Although the composition of colostrum, transition milk, and mature milk was highly similar between these groups, the GDM group was associated with alterations in the metabolome of breast milk, especially colostrum, which might adversely influence the long-term health of offspring. Fatty acids-optimized formula might be a better choice than maternal breast milk for feeding GDM complicated newborns.

Conflicts of interest
None.

Supplementary Tables

Supplementary Table 1. Patient characteristics.

| Characteristics                              | Controls (n=94) | GDM (n=90) | P value |
|---------------------------------------------|----------------|------------|---------|
| Maternal age (years)                        | 28.67±4.23     | 30.78±4.19 | 0.001*  |
| Pregnancy BMI (kg/m²)                       | 20.55±2.06     | 21.55±2.66 | 0.005*  |
| Gestational Age (week)                      | 39.50±1.17     | 39.14±1.00 | 0.035   |
| OGGT fasting (mmol/L)                       | 4.56±0.29      | 4.9±0.46   | 0.000*  |
| OGGT 1-hour (mmol/L)                        | 7.63±1.25      | 10.21±1.54 | 0.000*  |
| OGGT 2-hour (mmol/L)                        | 6.82±0.91      | 9.48±5.27  | 0.000*  |
| Maternal weight gain at parturition (kg)    | 15.9±4.79      | 14.0±5.71  | 0.014*  |
| Neonate birth weight (g)                    | 3317.13±360.97 | 3373±384.966 | 0.311  |
| Neonate birth head circumference (cm)       | 34.29±1.17     | 34.36±1.06 | 0.703   |
| Neonate birth Height (cm)                   | 49.72±1.68     | 49.71±1.60 | 0.977   |

Student t-test was used for statistical analysis. Adjusted significance value P<0.05(*).

Supplementary Table 2. The Semi-quantitative log values of identified metabolites were provided in the GDM group and the control group including colostrum, transitional milk, and mature milk (median, 1st–3rd quartile) per 100 mL.

| Name                              | Classification | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|-----------------------------------|----------------|----------------------------------------|-------------------------------------|------------------------------------------------|---------------------------------------------|------------------------------------------|----------------------------------------|
| Decane, 2-methyl                  | Alkane         | 4.82 (4.65–4.99)                       | 4.78 (4.62–4.97)                    | 4.88 (4.83–5.08)                               | 4.89 (4.72–5.05)                               | 5.08 (4.93–5.22)                               | 5.06 (4.92–5.25)                               |
| Dodecane                          | Alkane         | 5.19 (4.79–5.61)                       | 5.27 (4.64–5.55)                    | 5.55 (5.49–5.94)                               | 5.77 (5.53–5.92)                               | 5.87 (5.72–6)                                | 5.88 (5.69–6.03)                               |
| Octadecane                        | Alkane         | 5.75 (5.6–5.9)                         | 5.76 (5.59–5.92)                    | 5.8 (5.73–5.92)                                | 5.85 (5.75–5.92)                               | 5.87 (5.79–5.97)                               | 5.83 (5.76–5.96)                               |
| Tricosane                         | Alkane         | 4.96 (4.83–5.09)                       | 4.92 (4.81–5.02)                    | 4.98 (4.92–5.16)                               | 5.06 (4.95–5.18)                               | 5.1 (4.95–5.21)                               | 5.07 (4.96–5.18)                               |
| beta-Alanine                      | Amino acid derivatives | 6.56 (6.36–6.67)                       | 6.57 (6.39–6.71)                    | 6.36 (6.08–6.34)                               | 6.24 (6.11–6.36)                               | 6.28 (6.11–6.39)                               | 6.26 (6.14–6.44)                               |
| beta-Citrull/L-glutamic acid      | Amino acid derivatives | 4.63 (4.34–5.08)                       | 4.6 (4.33–4.89)                     | 4.29 (4.09–4.47)                               | 4.24 (4.09–4.41)                               | 4.22 (4.06–4.39)                               | 4.24 (4.06–4.36)                               |
| Name                     | Classification               | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|-------------------------|------------------------------|----------------------------------------|-------------------------------------|-----------------------------------------------|--------------------------------------------|------------------------------------------|----------------------------------------|
| cis-4-Hydroxyproline    | Amino acid derivatives       | 5.87 (5.74–6.08)                       | 5.92 (5.73–6.01)                    | 6.23 (6.42–6.8)                               | 6.63 (6.47–6.86)                           | 6.51 (6.27–6.65)                         | 6.5 (6.26–6.65)                         |
| D-Norvaline             | Amino acid derivatives       | 5.27                                   | 5.06                                | 5.22                                          | 5.18                                       | 5.13                                     | 5.07                                   |
| D-Proline               | Amino acid derivatives       | 5.9 (5.39–6.35)                        | 5.6 (5.23–6.05)                     | 5.8 (5.38–6.4)                               | 5.67                                       | 6.03                                     | 5.78                                   |
| D-Proline, N-methoxy carbonyl-, pentalyl ester (NIST: 88.5%) | Amino acid derivatives | 8.24 (8.06–8.35)                       | 8.25 (8.14–8.37)                    | 7.77 (7.41–7.58)                             | 7.5 (7.38–7.64)                            | 7.35                                     | 7.36                                   |
| D-Prolyl-d-proline, N-methoxy carbonyl-, methyl ester (NIST: 92.1%) | Amino acid derivatives | 6.39 (5.7–6.73)                        | 6.55 (5.85–6.85)                    | 5.22 (4.83–5.06)                             | 4.93                                       | 4.96                                     | 4.94                                   |
| Glycine, N-ethyl-N-(2-methoxethoxycarbonyl)-, 2-methoxethyl ester (NIST: 97.5%) | Amino acid derivatives | 5.48 (5.36–5.62)                       | 5.42 (5.32–5.54)                    | 5.42 (5.36–5.5)                              | 5.41                                       | 5.41                                     | 5.4 （5.33–5.47）                         |
| L-Isoleucine, N-methoxy carbonyl-, methyl ester (NIST: 91.1%) | Amino acid derivatives | 7.93 (7.65–8.06)                       | 7.99 (7.77–8.09)                    | 7.31 (7.02–7.22)                             | 7.13 (7.01–7.24)                           | 7.19                                     | 7.21                                   |
| L-Leucine, N-methoxy carbonyl-, pentalyl ester (NIST: 66.6%) | Amino acid derivatives | 6.49 (5.83–6.88)                       | 6.57 (5.93–7.07)                    | 5.63 (5.06–5.54)                             | 5.26                                       | 5.22                                     | 5.16                                   |
| L-Proline, N-methoxy carbonyl-, octyl ester (NIST: 77.3%) | Amino acid derivatives | 6.38 (6.17–6.62)                       | 6.41 (6.15–6.66)                    | 6.46 (6.28–6.74)                             | 6.45                                       | 6.58                                     | 6.46                                   |
| L-Prolylglycine, N-methoxy carbonyl-, methyl ester (NIST: 85.7%) | Amino acid derivatives | 6.73 (6.21–7.19)                       | 6.86 (6.38–7.23)                    | 5.97 (5.4–5.8)                               | 5.62                                       | 5.52                                     | 5.49                                   |
| L-Valine, N-methoxy carbonyl-, pentalyl ester (NIST: 62.8%) | Amino acid derivatives | 6.37 (5.61–6.84)                       | 6.48 (5.69–7.08)                    | 4.91 (4.49–4.71)                             | 4.6 (4.43–4.76)                            | 4.66                                     | 4.64                                   |
| Norleucine               | Amino acid derivatives       | 5.04 (4.83–5.22)                       | 4.98 (4.72–5.15)                    | 5.17 (5.18–5.31)                             | 5.21                                       | 5.24                                     | 5.22                                   |
| Norvaline                | Amino acid derivatives       | 5.06 (4.9–5.26)                        | 5.09 (4.86–5.3)                     | 5.0 (4.77–5.09)                              | 4.89                                       | 4.96                                     | 4.91                                   |
| Pyroglutamic acid        | Amino acid derivatives       | 7.67 (7.4–7.81)                        | 7.7 (7.48–7.84)                     | 7.71 (7.56–7.84)                             | 7.71                                       | 7.88                                     | 7.88                                   |
| S-Adenosylmethionine     | Amino acid derivatives       | 6.23 (6.08–6.46)                       | 6.32 (6.16–6.44)                    | 6.32 (6.08–6.5)                              | 6.3                                        | 6.38                                     | 6.31                                   |
| Alanine                  | Amino acids                 | 7.83 (7.65–7.96)                       | 7.86 (7.72–7.98)                    | 7.86 (7.75–7.95)                             | 7.87                                       | 7.89                                     | 7.91                                   |
| Name         | Classification       | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|--------------|----------------------|----------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|---------------------------------------|
| Asparagine   | Amino acids          | 6.73 (6.28–7.06)                       | 6.84 (6.38–7.15)                   | 6.45 (6.12–6.49)                              | 6.27 (6.16–6.45)                             | 6.21 (6.06–6.41)                          | 6.16 (6–6.35)                          |
| Aspartic acid| Amino acids          | 7.4                                    | 7.54                                | 7.06                                          | 7.05                                        | 7.06                                     | 7.05                                  |
| Cysteine     | Amino acids          | 6.22 (5.97–6.66)                       | 6.31 (5.96–6.63)                   | 6.05 (5.81–6.13)                              | 6.01 (5.84–6.14)                             | 5.92 (5.79–6.06)                          | 5.95                                  |
| Glutamic acid| Amino acids          | 7.73 (7.59–7.84)                       | 7.77 (7.62–7.87)                   | 7.84 (7.81–7.99)                              | 7.88 (7.82–7.98)                             | 7.97 (7.87–8.04)                          | 7.97                                  |
| Glutamine    | Amino acids          | 5.87 (5.7–5.92)                        | 5.81 (5.67–5.92)                   | 5.91 (5.61–5.95)                              | 5.93 (5.54–5.89)                             | 5.91 (5.54–5.89)                          | 5.91                                  |
| Glutathione  | Amino acids          | 7.01 (6.77–7.41)                       | 7.2 (6.92–7.42)                     | 7.12 (6.9–7.2)                                | 7.04 (6.93–7.19)                             | 7.24 (7.11–7.39)                          | 7.22                                  |
| Glycine      | Amino acids          | 7.43 (7–7.65)                          | 7.39 (7.24–7.61)                   | 7.27 (6.98–7.27)                              | 7.27 (7.02–7.27)                             | 7.28 (7.17–7.36)                          | 7.27                                  |
| Histidine    | Amino acids          | 6.44 (6.17–6.74)                       | 6.53 (6.26–6.81)                   | 6.22 (5.95–6.21)                              | 6.08 (5.95–6.16)                             | 6.06 (5.92–6.15)                          | 6.06                                  |
| Isoleucine   | Amino acids          | 7.28 (6.99–7.46)                       | 7.38 (7.15–7.54)                   | 6.63 (6.32–6.54)                              | 6.42 (6.21–6.52)                             | 6.39 (6.26–6.55)                          | 6.4                                  |
| Leucine      | Amino acids          | 7.93 (7.65–8.06)                       | 7.99 (7.77–8.09)                   | 7.31 (7.02–7.22)                              | 7.19 (7.01–7.24)                             | 7.21 (7.07–7.21)                          | 7.21                                  |
| Lysine       | Amino acids          | 8.02 (7.64–7.97)                       | 7.89 (7.72–7.98)                   | 7.35 (7.67–7.07)                              | 6.89 (6.52–6.77)                             | 6.64 (6.52–6.77)                          | 6.6                                  |
| Methionine   | Amino acids          | 6.72 (6.46–6.87)                       | 6.72 (6.52–6.88)                   | 6.11 (5.71–6.02)                              | 5.88 (5.74–6.02)                             | 5.86 (5.75–6.02)                          | 5.87                                  |
| Ornithine    | Amino acids          | 7.05 (6.63–7.48)                       | 7.18 (6.68–7.42)                   | 6.42 (6.16–6.48)                              | 6.42 (6.16–6.48)                             | 6.39 (6.04–6.33)                          | 6.06                                  |
| Phenylalanine| Amino acids          | 7.55 (7.22–7.66)                       | 7.53 (7.28–7.69)                   | 7.04 (6.78–6.95)                              | 6.87 (6.8–6.94)                              | 6.9 (6.78–7.03)                           | 6.95                                  |
| Proline      | Amino acids          | 8.24 (8.06–8.35)                       | 8.25 (8.14–8.37)                   | 7.77 (7.41–7.58)                              | 7.5 (7.38–7.64)                              | 7.35 (7.26–7.48)                          | 7.36                                  |
| Serine       | Amino acids          | 6.39 (6.22–6.66)                       | 6.44 (6.25–6.67)                   | 6.33 (6.13–6.39)                              | 6.24 (6.08–6.33)                             | 6.35 (6.18–6.47)                          | 6.23                                  |
| Threonine    | Amino acids          | 7.36 (7.15–7.58)                       | 7.45 (7.18–7.59)                   | 7.2 (6.94–7.2)                                | 7.05 (6.95–7.2)                              | 7.05 (6.92–7.17)                          | 7.02                                  |
| Tryptophan   | Amino acids          | 7.2 (6.87–7.4)                        | 7.16 (6.79–7.44)                   | 6.31 (5.85–6.15)                              | 6.2 (5.86–6.22)                              | 5.87 (5.68–6.04)                          | 5.91                                  |
| Tyrosine     | Amino acids          | 7.39 (7.12–7.61)                       | 7.5 (7.23–7.71)                    | 6.86 (6.59–6.75)                              | 6.69 (6.59–6.75)                             | 6.69 (6.59–6.75)                          | 6.69                                  |
| Valine       | Amino acids          | 7.92 (7.71–8.01)                       | 7.93 (7.72–8.05)                   | 7.53 (7.25–7.44)                              | 7.38 (7.27–7.45)                             | 7.44 (7.34–7.55)                          | 7.45                                  |
| NADP_NADPH   | Cofactors and Vitamins| 4.52 (4.39–4.73)                       | 4.51 (4.39–4.61)                   | 4.68 (4.74–5.03)                              | 4.88 (4.75–5)                                | 4.97 (4.83–5.07)                          | 4.98                                  |
| Nicotinamide | Cofactors and Vitamins| 5.54 (5.39–5.72)                       | 5.56 (5.39–5.68)                   | 5.62 (5.57–5.78)                              | 5.75 (5.65–5.84)                             | 5.64 (5.55–5.76)                          | 5.73                                  |
| Name                                           | Classification                  | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|------------------------------------------------|---------------------------------|-----------------------------------------|--------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|----------------------------------------|
| Nicotinic acid                                 | Cofactors and Vitamins          | 5.08 (4.94–5.23)                        | 5.06 (4.88–5.24)                    | 5.13 (4.98–5.38)                              | 5.17 (5.03–5.38)                             | 5.38 (5.28–5.51)                            | 5.41 (5.3–5.55)                         |
| 2-Phosphoenolpyruvic acid                      | Glycolytic intermediates        | 4.77 (4.38–5.12)                        | 4.6 (4.33–4.96)                     | 5.56 (5.87–6.24)                              | 6 (5.78–6.28)                                | 5.89 (5.63–6.08)                            | 5.89 (5.63–6.06)                         |
| 3-Methyl-2-oxovaleric acid                     | Keto acids and derivatives      | 5.37 (5.21–5.5)                         | 5.29 (5.2–5.48)                     | 5.39 (5.33–5.5)                               | 5.43 (5.32–5.52)                             | 5.45 (5.35–5.55)                            | 5.45 (5.34–5.54)                         |
| Oxaloacetic acid                               | Keto acids and derivatives      | 5.62 (5.07–6.06)                        | 5.38 (5.03–5.82)                    | 5.96 (6.01–6.25)                              | 6.1 (5.94–6.21)                              | 5.97 (5.8–6.12)                             | 5.91 (5.74–6.06)                         |
| Pyruvic acid                                   | Keto acids and derivatives      | 5.53 (5.39–5.74)                        | 5.49 (5.31–5.66)                    | 5.55 (5.44–5.75)                              | 5.56 (5.37–5.65)                             | 5.6 (5.45–5.75)                             | 5.54 (5.35–5.66)                         |
| (1-Methyl-2,6-dioxocyclohexyl)acetic acid, methyl ester (NIST: 58.7%) | Organic acids                  | 5.23 (4.93–5.58)                        | 5.52 (5.07–5.79)                    | 5.01 (4.53–4.93)                              | 4.78 (4.55–5.02)                             | 4.93 (4.67–5.14)                            | 4.91 (4.7–5.24)                          |
| 1-Aminocyclo-pentane-carboxylic acid, N-methoxy-carboxyl, methyl ester (NIST: 51.1%) | Organic acids                  | 5.36 (5.2–5.55)                         | 5.38 (5.19–5.55)                    | 5.23 (5.02–5.25)                              | 5.12 (5.03–5.21)                             | 5.21 (5.1–5.37)                             | 5.19 (5.07–5.29)                         |
| 1-Aminocyclopropane,1-carboxylic acid          | Organic acids                  | 4.98 (4.79–5.14)                        | 5.02 (4.86–5.22)                    | 4.78 (4.55–4.75)                              | 4.64 (4.56–4.75)                             | 4.63 (4.49–4.72)                            | 4.55 (4.44–4.67)                         |
| 2-(4-(2-Acetoxyethyl)-2,5-dimethoxyphenyl)acetic acid, methyl ester (NIST: 63%) | Organic acids                  | 5.84 (5.63–6.11)                        | 5.79 (5.62–6.1)                     | 5.76 (5.59–5.89)                              | 5.76 (5.62–5.87)                             | 5.57 (5.37–5.82)                            | 5.61 (5.41–5.76)                         |
| 2-Aminobutyric acid                            | Organic acids                  | 6.1 (5.91–6.28)                         | 6.3 (5.78–6.24)                     | 6.49 (6.62–7.04)                              | 6.62 (6.63–7.03)                             | 6.62 (6.62–6.96)                            | 6.62 (6.62–6.97)                         |
| 2-Hydroxybutyric acid                          | Organic acids                  | 6.37 (6.17–6.53)                        | 6.34 (6.16–6.5)                     | 6.12 (5.79–6.06)                              | 5.94 (5.82–6.08)                             | 5.85 (5.74–6)                               | 5.93 (5.77–6.07)                         |
| 2-Hydroxyglutaric acid                         | Organic acids                  | 6.26 (6.05–6.43)                        | 6.25 (6.09–6.44)                    | 6.16 (5.92–6.25)                              | 6.05 (5.96–6.22)                             | 6.23 (6.11–6.36)                            | 6.19 (6.07–6.33)                         |
| 2-Hydroxysobutyric acid                        | Organic acids                  | 5.43 (5.24–5.65)                        | 5.34 (5.14–5.56)                    | 5.48 (5.45–5.64)                              | 5.57 (5.42–5.6)                              | 5.57 (5.49–5.73)                            | 5.58 (5.49–5.65)                         |
| 2-Oxoadipic acid                               | Organic acids                  | 5.28 (5.11–5.46)                        | 5.22 (5.07–5.43)                    | 5.31 (5.14–5.64)                              | 5.27 (5.11–5.43)                             | 5.38 (5.07–5.63)                            | 5.26 (5–5.44)                            |
| 2-Oxomalonic acid, methylhydrazone, dimethyl ester (NIST: 62%) | Organic acids                  | 6.03 (5.85–6.31)                        | 6.07 (5.87–6.38)                    | 5.95 (5.79–6.03)                              | 5.89 (5.81–6.03)                             | 6.03 (5.95–6.16)                            | 6.03 (5.89–6.19)                         |
| 2-Oxovaleric acid                              | Organic acids                  | 5.6 (5.32–6.19)                         | 5.65 (5.29–6.07)                    | 5.53 (5.32–5.69)                              | 5.45 (5.29–5.62)                             | 5.81 (5.6–6)                                | 5.91 (5.65–6.11)                         |
| 2-Piperidinecarboxylic acid, 1-acetyl-, ethyl ester (NIST: 69.6%) | Organic acids                  | 5.1 (4.96–5.3)                          | 5.07 (4.89–5.23)                    | 5.21 (5.52–5.42)                              | 5.28 (5.15–5.4)                              | 5.25 (5.12–5.39)                            | 5.22 (5.11–5.34)                         |
| 3,4-Methylenedioxyphenylacetic acid (NIST: 78.4%) | Organic acids                  | 5.33 (5.16–5.58)                        | 5.37 (5.19–5.62)                    | 5.05 (4.75–5)                                 | 4.85 (4.66–4.98)                             | 4.73 (4.62–4.97)                            | 4.71 (4.59–4.82)                         |
| Name                                           | Classification               | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|------------------------------------------------|-----------------------------|-----------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------|
| 3-Aminoisobutyric acid                         | Organic acids               | 5.48 (5.36–5.61)                        | 5.41 (5.3–5.54)                     | 5.42 (5.33–5.5)                               | 5.4 (5.32–5.49)                             | 5.39 (5.32–5.49)                         | 5.39 (5.29–5.45)                         |
| 3-Furanacrylic acid, methyl ester (NIST: 91.8%)| Organic acids               | 5.01 (4.8–5.19)                         | 4.93 (4.79–5.16)                    | 5.03 (4.96–5.26)                              | 5.06 (4.94–5.19)                            | 5.17 (5.02–5.32)                         | 5.14 (5.03–5.27)                         |
| 3-Hydroxy-azetidine-1-carboxylic acid, methyl ester (NIST: 65.9%) | Organic acids               | 5.85 (5.69–6.04)                        | 5.78 (5.65–5.94)                    | 5.93 (5.93–6.13)                              | 6.01 (5.86–6.09)                            | 5.86 (5.77–5.96)                         | 5.84 (5.72–5.95)                         |
| 4-Hydroxyphenylacetic acid                     | Organic acids               | 5.28 (5.1–5.43)                         | 5.3 (5.14–5.47)                     | 5.25 (5.04–5.37)                              | 5.15 (5–5.3)                                | 5.1 (4.97–5.25)                          | 5.14 (5–5.34)                           |
| Acetic acid, 1-hydroxy-4,6-dimethyl-pyridin-2-ylmethyl ester (NIST: 59.2%) | Organic acids               | 5.46 (5.3–5.62)                         | 5.43 (5.29–5.57)                    | 5.49 (5.39–5.68)                              | 5.4 (5.28–5.63)                             | 5.59 (5.46–5.75)                         | 5.54 (5.32–5.67)                         |
| alpha-Acetylisine                              | Organic acids               | 5.03 (4.91–5.21)                        | 5.02 (4.9–5.24)                     | 5.01 (4.87–5.11)                              | 5.01 (4.86–5.13)                            | 5.02 (4.88–5.2)                          | 5.01 (4.89–5.19)                         |
| Azelaic acid                                   | Organic acids               | 6.26 (5.53–6.63)                        | 6.15 (5.5–6.58)                     | 6.64 (6.65–6.78)                              | 6.67 (6.63–6.79)                            | 6.7 (6.51–6.68)                          | 6.6 (6.45–6.68)                         |
| Benzeneacetic acid, methyl ester (NIST: 88.3%) | Organic acids               | 5.06 (4.87–5.3)                         | 5.03 (4.88–5.27)                    | 4.98 (4.71–5.05)                              | 4.84 (4.68–5.04)                            | 4.94 (4.76–5.07)                         | 4.98 (4.77–5.11)                         |
| Benzoic acid                                   | Organic acids               | 5.64 (5.35–5.91)                        | 5.56 (5.34–5.78)                    | 5.8 (5.82–6.25)                               | 6.05 (5.85–6.28)                            | 5.96 (5.76–6.2)                          | 5.94 (5.69–6.21)                         |
| Cabarnic acid                                  | Organic acids               | 4.96 (4.89–5.04)                        | 4.89 (4.79–5.04)                    | 4.92 (4.87–5.01)                              | 4.94 (4.85–4.99)                            | 4.96 (4.89–5.05)                         | 4.93 (4.85–5.02)                         |
| Creatinine                                     | Organic acids               | 7.35 (6.97–6.48)                        | 6.41 (6.04–6.56)                    | 5.75 (5.39–5.64)                              | 5.55 (5.39–5.67)                            | 5.58 (5.38–5.64)                         | 5.59 (5.45–5.7)                         |
| Cyclohexanecarboxylic acid, 2-tridecyl ester (NIST: 53.2%) | Organic acids               | 5.18 (5.02–5.39)                        | 5.15 (5.03–5.29)                    | 5.2 (5.1–5.46)                                | 5.27 (5.1–5.38)                             | 5.35 (5.13–5.58)                         | 5.26 (5.1–5.54)                         |
| D-Indole,3-butyric acid                        | Organic acids               | 5.2 (5.11–5.34)                         | 5.24 (5.13–5.35)                    | 5.18 (5.08–5.23)                              | 5.13 (5.02–5.23)                            | 5.19 (5.11–5.27)                         | 5.18 (5.08–5.35)                         |
| Dehydroascorbic acid                           | Organic acids               | 6.75 (6.55–6.94)                        | 6.64 (6.41–6.83)                    | 6.9 (6.96–7.19)                               | 7.01 (6.9–7.14)                             | 6.89 (6.77–7.02)                         | 6.9 (6.73–6.99)                         |
| Dipicolinic acid                               | Organic acids               | 4.94 (4.75–5.12)                        | 4.94 (4.74–5.14)                    | 4.75 (4.32–4.76)                              | 4.55 (4.34–4.71)                            | 4.28 (4.06–4.5)                          | 4.17 (4.01–4.49)                         |
| Glutaric acid                                  | Organic acids               | 4.59 (4.39–4.81)                        | 4.56 (4.34–4.69)                    | 4.65 (4.62–4.82)                              | 4.69 (4.59–4.82)                            | 4.68 (4.54–4.78)                         | 4.71 (4.59–4.8)                         |
| Glyceric acid                                  | Organic acids               | 5.81 (5.63–6)                           | 5.72 (5.55–5.87)                    | 5.84 (5.81–6.08)                              | 5.91 (5.8–6.01)                             | 5.95 (5.78–6.14)                         | 5.94 (5.82–6.07)                         |
| Glyceric acid                                  | Organic acids               | 5.77 (5.65–5.9)                         | 5.7 (5.6–5.9)                       | 5.75 (5.66–5.91)                              | 5.77 (5.66–5.87)                            | 5.79 (5.71–5.97)                         | 5.8 (5.72–5.97)                         |
| Hippuric acid                                  | Organic acids               | 5.57 (5.3–5.95)                         | 5.61 (5.23–5.92)                    | 5.75 (5.72–6.19)                              | 5.77 (5.72–5.97)                            | 5.79 (5.74–5.97)                         | 5.69 (5.72–5.95)                         |
| Hydroxybenzoic acid                            | Organic acids               | 4.63 (4.48–4.75)                        | 4.6 (4.42–4.76)                     | 4.58 (4.48–4.68)                              | 4.53 (4.45–4.64)                            | 4.52 (4.44–4.62)                         | 4.49 (4.44–4.59)                         |
| Lactic acid                                    | Organic acids               | 7.8 (7.61–7.97)                         | 7.73 (7.59–7.9)                     | 7.43 (7.02–7.26)                              | 7.13 (6.98–7.26)                            | 7.03 (6.91–7.22)                         | 7.09 (6.94–7.28)                         |
| Leuvulinic acid                                | Organic acids               | 4.4 (4.47–4.99)                         | 4.51 (4.54–5.03)                    | 4.42 (4.27–4.48)                              | 4.34 (4.46–4.43)                            | 4.28 (4.32–4.51)                         | 4.28 (4.3–4.43)                         |
| Name                                      | Classification | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|-------------------------------------------|----------------|----------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------|----------------------------------------|
| Malonic acid                              | Organic acids  | 5.39 (5.2–5.52)                        | 5.28 (5.18–5.46)                    | 5.44 (5.43–5.58)                              | 5.49 (5.41–5.56)                            | 5.48 (5.38–5.57)                        | 5.45 (5.37–5.54)                        |
| N-Ethylpyrrolidine-2,2-dicarboxylic acid, dimethyl ester (NIST: 62.2%) | Organic acids  | 5.76 (5.49–6.01)                        | 5.64 (5.45–5.95)                    | 6.01 (6.04–6.23)                              | 6.13 (5.99–6.25)                            | 5.98 (5.88–6.09)                        | 5.99 (5.84–6.1)                         |
| Oxalic acid                               | Organic acids  | 6.1 (5.78–6.48)                        | 5.85 (5.53–6.18)                    | 6.05 (5.9–6.4)                                | 6.11 (5.51–6.28)                            | 6.15 (5.95–6.4)                         | 6.08 (5.73–6.3)                         |
| Phenethyl acetate                          | Organic acids  | 4.32 (4.1–4.48)                        | 4.27 (4.04–4.47)                    | 4.45 (4.28–4.56)                              | 4.37 (4.23–4.54)                            | 4.43 (4.33–4.66)                        | 4.5 (4.35–4.65)                         |
| Propanedioic acid, methyl,ethyl ester (NIST: 64%) | Organic acids  | 5.1 (5.02–5.27)                        | 5.14 (4.95–5.31)                    | 5.12 (5.01–5.22)                              | 5.06 (4.97–5.21)                            | 5.15 (5.05–5.27)                        | 5.11 (5.01–5.23)                        |
| Pyrrolidine-2-one-trans-4,5-dicarboxylic acid, dimethyl ester (NIST: 49.6%) | Organic acids  | 7.37 (7.13–7.54)                        | 7.46 (7.34–7.55)                    | 6.79 (6.43–6.6)                               | 6.53 (6.4–6.65)                             | 6.39 (6.29–6.5)                         | 6.39 (6.26–6.51)                        |
| Stearic acid                              | Organic acids  | 7.92 (7.62–7.89)                        | 7.77 (7.63–7.88)                    | 7.87 (7.86–8.03)                              | 7.94 (7.87–8)                               | 7.92 (7.82–7.99)                        | 7.91 (7.83–8.02)                        |
| Suberic acid                              | Organic acids  | 5.74 (5.53–6.16)                        | 5.64 (5.5–6.14)                     | 6.65 (6.84–7.09)                              | 6.98 (6.86–7.11)                            | 7 (6.86–7.13)                           | 7.01 (6.83–7.12)                        |
| 1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether (NIST: 63.2%) | Organic compounds | 6.34 (6.2–6.52)                        | 6.31 (6.11–6.48)                    | 6.35 (6.21–6.52)                              | 6.32 (6.13–6.49)                            | 6.47 (6.31–6.63)                        | 6.46 (6.27–6.59)                        |
| 2-ethyl-3,3-diphenyloxadizidine (NIST: 67.9%) | Organic compounds | 4.98 (4.68–5.04)                        | 4.79 (4.61–4.96)                    | 4.58 (4.33–4.52)                              | 4.39 (4.28–4.49)                            | 4.41 (4.29–4.56)                        | 4.39 (4.27–4.5)                         |
| 1,2-Hydrazinedicarboxylic acid, dimethyl ester (NIST: 58.3%) | Organic compounds | 5.79 (5.49–6.02)                        | 5.62 (5.4–5.84)                     | 5.72 (5.56–6.19)                              | 5.74 (5.51–6.01)                            | 5.97 (5.52–6.26)                        | 5.82 (5.49–6.07)                        |
| 2-(1-Pentamethylethyl)ethyl-3,4-diphenyloxadizidine | Organic compounds | 5.74 (5.38–5.91)                        | 5.55 (5.34–5.81)                    | 5.76 (5.72–6.05)                              | 5.83 (5.65–6.04)                            | 5.93 (5.66–6.07)                        | 5.86 (5.64–5.97)                        |
| 2,2-Dimethyltetrahydro-1,1-dioxol[4,5-c]pyran-6,7-diol (NIST: 60.3%) | Organic compounds | 6.12 (5.68–6.5)                        | 6.21 (5.64–6.56)                    | 5.36 (4.93–5.21)                              | 5.02 (4.89–5.19)                            | 5.01 (4.88–5.18)                        | 5.01 (4.82–5.15)                        |
| 2,3-Furandione, dihydro-4,4-dimethyl (NIST: 91.3%) | Organic compounds | 4.92 (4.64–5.11)                        | 4.79 (4.57–5.01)                    | 4.96 (4.91–5.3)                               | 4.95 (4.76–5.21)                            | 5.1 (4.96–5.28)                         | 5.02 (4.9–5.21)                         |
| 2-Acetonyl-9-[3-deoxy-β-d-ribouranosyl]hypoxanthine (NIST: 67.8%) | Organic compounds | 5.43 (4.91–5.72)                        | 5.23 (4.79–5.7)                     | 5.79 (5.35–6.13)                              | 5.65 (5.13–6.1)                             | 5.83 (5.33–6.18)                        | 5.61 (5.11–6.09)                        |
| 2-Cyclohexylethanol, dimethyl(ethyl)silyl ether (NIST: 48%) | Organic compounds | 4.97 (4.71–5.21)                        | 5.06 (4.79–5.35)                    | 4.91 (4.67–4.98)                              | 4.86 (4.68–5.01)                            | 4.95 (4.78–5.12)                        | 4.97 (4.72–5.12)                        |
| 2-Methoxy-N-(2-methoxypropyl)-N-methylpropanamide (NIST: 49.9%) | Organic compounds | 5.75 (5.49–5.86)                        | 5.65 (5.46–5.84)                    | 5.75 (5.7–5.6)                                | 5.79 (5.58–5.95)                            | 5.89 (5.74–6.09)                        | 5.8 (5.66–5.99)                         |
| Name                                                                 | Classification   | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|----------------------------------------------------------------------|------------------|----------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|----------------------------------------|
| 3H-Pyrazol-3-one, 2,4-dihydro-2,5-dimethyl-(NIST: 67.2%)             | Organic compounds | 5.19 (5.03–5.31)                      | 5.18 (4.98–5.39)                    | 5.02 (4.77–5.03)                               | 4.9 (4.68–5.02)                              | 5.05 (4.9–5.2)                           | 4.95 (4.85–5.15)                        |
| 3-Pyridinecarboxaldehyde (NIST: 90.7%)                               | Organic compounds | 4.72 (4.58–4.9)                       | 4.68 (4.48–4.83)                    | 4.8 (4.74–5)                                   | 4.89 (4.76–5)                                | 4.84 (4.72–4.94)                         | 4.86 (4.73–4.97)                        |
| 4,4-Dimethyl-5-methylene[1,3]dioxolan-2-one (NIST: 60.5%)            | Organic compounds | 5.02 (4.83–5.31)                      | 5.11 (4.85–5.31)                    | 4.82 (4.58–4.8)                                | 4.65 (4.56–4.79)                             | 4.76 (4.62–4.85)                         | 4.71 (4.59–4.82)                        |
| 4-Pyridinecarboxaldehyde (NIST: 83.6%)                               | Organic compounds | 4.75 (4.56–4.96)                      | 4.58 (4.45–4.79)                    | 4.71 (4.61–5.1)                                | 4.68 (4.47–4.87)                             | 4.9 (4.56–5.06)                          | 4.76 (4.49–4.91)                        |
| 6,8-Dimethyl-7-phenyl-1,3,8-triazaspiro[4,5] decan-2,4-dithione (NIST: 41%) | Organic compounds | 4.55 (4.43–4.73)                      | 4.56 (4.44–4.7)                     | 4.57 (4.47–4.71)                               | 4.56 (4.41–4.67)                             | 4.63 (4.46–4.74)                         | 4.57 (4.39–4.66)                        |
| Acetophenone (NIST: 96.2%)                                           | Organic compounds | 4.57 (4.41–4.86)                      | 4.56 (4.43–4.77)                    | 4.65 (4.55–4.94)                               | 4.67 (4.53–4.84)                             | 4.7 (4.52–4.96)                          | 4.71 (4.52–4.86)                        |
| BHT                                                                  | Organic compounds | 4.6 (4.16–5.06)                       | 4.69 (4.41–4.98)                    | 4.73 (4.46–5.27)                               | 4.5 (4.19–4.56)                              | 4.33 (4.22–4.74)                         | 4.38 (4.22–4.74)                        |
| Bis(2-ethylhexyl) phthalate (NIST: 96.3%)                            | Organic compounds | 5.85 (5.67–6.03)                      | 5.7 (5.54–5.92)                     | 5.85 (5.75–6.09)                               | 5.85 (5.68–6.05)                             | 5.85 (5.73–6.08)                         | 5.89 (5.74–6.02)                        |
| Butanedioyl dihydrazide (NIST: 70.6%)                                | Organic compounds | 6.96 (6.81–7.12)                      | 6.96 (6.78–7.1)                     | 7.04 (7–7.16)                                  | 7.08 (6.99–7.17)                             | 7.06 (6.96–7.14)                         | 7.08 (7–7.17)                           |
| Caffeine                                                             | Organic compounds | 4.57 (4.38–4.85)                      | 4.5 (4.3–4.68)                      | 4.46 (4.33–4.59)                               | 4.39 (4.27–4.53)                             | 4.51 (4.36–4.71)                         | 4.47 (4.35–4.68)                        |
| Cycloheptasioxane, tetradecamethyl-(NIST: 94.2%)                     | Organic compounds | 5.69 (5.6–5.86)                       | 5.68 (5.46–5.9)                     | 5.68 (5.48–5.81)                               | 5.66 (5.42–5.81)                             | 5.7 (5.53–5.89)                          | 5.65 (5.48–5.86)                        |
| Cyclononasioxane, octadecamethyl-(NIST: 91.7%)                       | Organic compounds | 5.52 (5.3–5.8)                        | 5.47 (5.2–5.73)                     | 5.6 (5.47–5.87)                                | 5.71 (5.48–5.84)                             | 5.67 (5.46–5.89)                         | 5.66 (5.49–5.84)                        |
| Cyclopentane, hexyl-(NIST: 65.9%)                                    | Organic compounds | 5.5 (5.26–5.75)                       | 5.41 (5.17–5.6)                     | 5.56 (5.55–5.93)                               | 5.65 (5.52–5.81)                             | 5.77 (5.58–6.12)                         | 5.68 (5.47–5.92)                        |
| Cyclopentylamine, N-tert-butylidemethylsil (NIST: 52.6%)              | Organic compounds | 5.86 (5.6–6.08)                       | 5.97 (5.57–6.2)                     | 5.23 (4.7–5.1)                                 | 4.93 (4.37–5.08)                             | 4.86 (4.69–5.03)                         | 4.82 (4.66–5.05)                        |
| DBP                                                                  | Organic compounds | 5.21 (5.03–5.32)                      | 5.21 (5.08–5.36)                    | 5.24 (5.13–5.38)                               | 5.31 (5.15–5.44)                             | 5.3 (5.14–5.38)                          | 5.3 (5.15–5.47)                         |
| Dehydroabietic acid                                                  | Organic compounds | 4.44 (4.31–4.61)                      | 4.38 (4.21–4.51)                    | 4.44 (4.39–4.57)                               | 4.47 (4.37–4.56)                             | 4.51 (4.43–4.59)                         | 4.5 (4.41–4.58)                         |
| Dimethyl ethyldenedimalonate (NIST: 80.4%)                           | Organic compounds | 5.79 (5.23–6.15)                      | 5.64 (5.2–5.99)                     | 6.14 (6.18–6.35)                               | 6.25 (6.16–6.33)                             | 6.11 (6.04–6.2)                          | 6.12 (6–6.2)                            |
| Furfyl hydroxymethyl ketone (NIST: 88.1%)                            | Organic compounds | 5.01 (4.8–5.19)                       | 4.93 (4.79–5.16)                    | 5.03 (4.96–5.26)                               | 5.06 (4.94–5.19)                             | 5.17 (5.02–5.32)                         | 5.14 (5.03–5.27)                        |
| Heptacosane (NIST: 78.9%)                                            | Organic compounds | 5.37 (5.19–5.59)                      | 5.27 (5.13–5.55)                    | 5.4 (5.36–5.61)                                | 5.39 (5.24–5.59)                             | 5.56 (5.37–5.68)                         | 5.45 (5.31–5.63)                        |
| Methyl 2-ethoxycacetate (NIST: 64.4%)                                | Organic compounds | 7.49 (7.25–7.75)                      | 7.41 (7.22–7.66)                    | 7.7 (7.7–7.91)                                  | 7.77 (7.65–7.86)                             | 7.84 (7.71–7.93)                         | 7.78 (7.67–7.88)                        |
| Name | Classification          | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|------|-------------------------|-----------------------------------------|-------------------------------------|-----------------------------------------------|----------------------------------------------|------------------------------------------|---------------------------------------|
| Methyl 2-methylhexanoate (NIST: 70.4%) | Organic compounds | 5.57 (5.25–5.73) | 5.51 (5.29–5.75) | 5.59 (5.39–5.8) | 5.57 (5.33–5.79) | 5.67 (5.46–5.88) | 5.65 (5.4–5.79) |
| N(1-Methoxycarbonyl-1-methyl)ethyl-2-aza-1,3-dioxane (NIST: 87.4%) | Organic compounds | 5.24 (5.11–5.34) | 5.22 (5.14–5.36) | 5.21 (5.12–5.29) | 5.21 (5.12–5.29) | 5.25 (5.17–5.33) | 5.26 (5.19–5.37) |
| O-Acetylmalic anhydride | Organic compounds | 5.7 (5.02–6.14) | 5.28 (4.68–5.82) | 5.48 (5.48–6.67) | 5.09 (5.09–6.25) | 5.62 (5.62–6.66) | 5.13 (5.13–6.26) |
| Oxetane, 2,3,4-trimethyl- (2e,3e,4e)- (NIST: 64.2%) | Organic compounds | 4.81 (4.71–4.93) | 4.81 (4.69–4.9) | 4.71 (4.56–4.72) | 4.63 (4.52–4.71) | 4.68 (4.61–4.78) | 4.71 (4.58–4.8) |
| Paraldehyde (NIST: 69.9%) | Organic compounds | 6.1 (5.77–6.53) | 6.08 (5.7–6.47) | 6.1 (5.89–6.38) | 6.06 (5.88–6.33) | 6.44 (6.17–6.72) | 6.55 (6.16–6.77) |
| Putrescine | Organic compounds | 6.42 (6.22–6.73) | 6.35 (6.2–6.53) | 6.8 (6.86–6.98) | 6.94 (6.88–7) | 6.9 (6.82–6.96) | 6.9 (6.81–6.97) |
| Trimethyl 2-methoxypropane-1,2,3-tricarboxylate (NIST: 60.4%) | Organic compounds | 6.68 (6.02–7.05) | 6.45 (6.14–6.93) | 7.04 (7.07–7.28) | 7.17 (7.05–7.28) | 7.02 (6.91–7.12) | 7.05 (6.9–7.14) |
| 2-Methyloctadecanoic acid | Saturated fatty acids | 5.14 (4.87–5.4) | 5.21 (4.89–5.46) | 5.21 (5.03–5.43) | 5.16 (4.98–5.3) | 5.18 (5.02–5.4) | 5.17 (4.94–5.3) |
| 3-Hydroxydecanoic acid | Saturated fatty acids | 4.99 (4.89–5.46) | 4.98 (4.81–5.46) | 5.28 (5.32–5.63) | 5.41 (5.31–5.57) | 5.79 (5.6–5.94) | 5.85 (5.61–6.05) |
| 4-Aminobutyric acid (GABA) | Saturated fatty acids | 6.1 (5.99–6.21) | 6.05 (5.95–6.18) | 6.13 (6.1–6.23) | 6.17 (6.06–6.22) | 6.21 (6.15–6.28) | 6.19 (6.12–6.25) |
| Adipic acid | Saturated fatty acids | 5.15 (5.02–5.27) | 5.12 (4.9–5.24) | 5.08 (4.97–5.13) | 5.05 (4.92–5.14) | 5.17 (5.02–5.26) | 5.14 (5.03–5.22) |
| Arachidic acid | Saturated fatty acids | 5.48 (4.75–5.73) | 5.39 (5.15–5.7) | 5.74 (5.81–6.24) | 6.02 (5.81–6.21) | 5.97 (5.75–6.09) | 5.88 (5.75–6.12) |
| Behenic acid | Saturated fatty acids | 4.68 (4.43–4.96) | 4.59 (4.33–4.87) | 5.16 (4.85–5.43) | 5.11 (4.78–5.43) | 5.05 (4.77–5.25) | 5.01 (4.75–5.31) |
| Butanolic acid, 3-(ethylthio)- (NIST: 58.7%) | Saturated fatty acids | 5.26 (5.11–5.46) | 5.19 (5.03–5.38) | 5.26 (5.13–5.48) | 5.28 (5.12–5.38) | 5.36 (5.17–5.51) | 5.3 (5.13–5.44) |
| Decanoic acid | Saturated fatty acids | 6.2 (5.96–6.52) | 6.23 (5.95–6.47) | 7.1 (7.34–7.72) | 7.54 (7.38–7.66) | 7.71 (7.61–7.83) | 7.74 (7.55–7.9) |
| Dodecanoic acid | Saturated fatty acids | 6.78 (6.56–7.13) | 6.71 (6.57–7.1) | 7.69 (7.9–8.13) | 8.03 (7.93–8.14) | 8.05 (7.92–8.15) | 8.05 (7.9–8.15) |
| Hexanoic acid | Saturated fatty acids | 6.23 (5.88–6.65) | 6.18 (5.76–6.49) | 6.39 (6.32–6.9) | 6.51 (6.28–6.87) | 6.93 (6.63–7.18) | 7.03 (6.57–7.31) |
| Lignoceric acid | Saturated fatty acids | 4.18 (4.04–4.38) | 4.15 (4.05–4.35) | 4.28 (4.1–4.52) | 4.27 (4.1–4.53) | 4.18 (4.05–4.35) | 4.24 (4.12–4.34) |
| Margaric acid | Saturated fatty acids | 5.87 (5.67–6.14) | 5.81 (5.6–6.01) | 6.23 (6.34–6.62) | 6.49 (6.34–6.61) | 6.42 (6.25–6.58) | 6.39 (6.28–6.53) |
| Myristic acid | Saturated fatty acids | 6.95 (6.75–7.35) | 6.87 (6.65–7.18) | 7.66 (7.85–8.08) | 7.98 (7.81–8.12) | 7.89 (7.75–8) | 7.85 (7.67–7.99) |
| Nonadecanoic acid | Saturated fatty acids | 4.94 (4.76–5.15) | 4.88 (4.68–5.04) | 5.1 (5.13–5.4) | 5.24 (5.11–5.37) | 5.22 (5.09–5.32) | 5.17 (5.03–5.32) |
| Octanoic acid | Saturated fatty acids | 5.84 (5.58–6.08) | 5.74 (5.47–6.14) | 6.54 (6.88–7.36) | 7.2 (6.82–7.29) | 7.27 (7.08–7.55) | 7.35 (7–7.59) |
| Name                          | Classification       | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|-------------------------------|----------------------|----------------------------------------|------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------------------|--------------------------------------|
| Palmitic acid                 | Saturated fatty acids| 7.63 (7.44–7.92)                      | 7.58 (7.46–7.76)                   | 8 (8.06–8.17)                                 | 8.14 (8.07–8.2)                           | 8.09 (8.02–8.15)                          | 8.09 (8.01–8.16)                      |
| Pentadecanoic acid            | Saturated fatty acids| 5.9 (5.64–6.13)                       | 5.75 (5.59–5.96)                   | 6.14 (6.18–6.47)                              | 6.35 (6.21–6.49)                          | 6.27 (6.13–6.41)                          | 6.27 (6.13–6.46)                      |
| Pimelic acid                  | Saturated fatty acids| 4.65 (4.48–4.85)                      | 4.58 (4.47–4.82)                   | 4.49 (4.48–4.72)                              | 4.49 (4.55–4.75)                          | 4.59 (4.49–4.76)                          | 4.59 (4.49–4.76)                      |
| Tetradecanoic acid, 12-methyl-, methyl ester (NIST: 80.2%) | Saturated fatty acids | 5.34 (5.19–5.55)                      | 5.27 (5.05–5.53)                   | 5.38 (5.29–5.58)                              | 5.44 (5.29–5.57)                          | 5.57 (5.46–5.74)                          | 5.58 (5.37–5.75)                      |
| Tridecanoic acid              | Saturated fatty acids| 5.67 (5.44–5.87)                      | 5.59 (5.23–5.78)                   | 5.76 (5.72–6.09)                              | 5.87 (5.73–6.11)                          | 5.76 (5.57–5.89)                          | 5.78 (5.58–5.9)                       |
| Undecanoic acid               | Saturated fatty acids| 5.12 (4.93–5.29)                      | 5.06 (4.84–5.21)                   | 5.08 (5.01–5.21)                              | 5.14 (5.01–5.25)                          | 5.16 (5.02–5.32)                          | 5.14 (5.02–5.3)                       |
| 2-Oxoglutaric acid            | TCA cycle intermediates | 6.68 (6.48–6.81)                      | 6.66 (6.46–6.82)                   | 6.57 (6.34–6.64)                              | 6.47 (6.3–6.66)                           | 6.71 (6.55–6.81)                          | 6.63 (6.49–6.74)                      |
| cis-Aconitic acid             | TCA cycle intermediates | 6.92 (6.29–7.29)                      | 6.7 (6.27–7.17)                    | 7.27 (7.33–7.55)                              | 7.41 (7.27–7.5)                           | 7.25 (7.16–7.37)                          | 7.26 (7.11–7.37)                      |
| Citramalic acid               | TCA cycle intermediates | 5.7 (5.41–6.01)                      | 5.61 (5.35–5.86)                   | 5.88 (5.91–6.2)                               | 5.97 (5.81–6.12)                          | 6.08 (5.92–6.25)                          | 6.02 (5.84–6.18)                      |
| Citric acid                   | TCA cycle intermediates | 8.17 (7.6–8.45)                       | 8.06 (7.56–8.37)                   | 8.42 (8.44–8.55)                              | 8.49 (8.43–8.55)                          | 8.44 (8.38–8.51)                          | 8.43 (8.36–8.5)                       |
| Fumaric acid                  | TCA cycle intermediates | 5.9 (5.72–6.12)                      | 5.8 (5.61–5.99)                    | 6 (6.01–6.22)                                 | 6.08 (5.97–6.22)                          | 5.98 (5.86–6.07)                          | 5.96 (5.85–6.08)                      |
| Isocitric acid                | TCA cycle intermediates | 5.98 (5.85–6.2)                      | 6.01 (5.78–6.15)                   | 6.04 (5.95–6.16)                              | 6.08 (5.93–6.19)                          | 5.84 (5.7–5.94)                           | 5.84 (5.71–5.94)                      |
| Malic acid                    | TCA cycle intermediates | 6.38 (6.08–6.72)                      | 6.26 (6.07–6.51)                   | 6.78 (7.02–7.25)                              | 7.15 (7.02–7.23)                          | 7.14 (7.02–7.23)                          | 7.12 (6.95–7.21)                      |
| Succinic acid                 | TCA cycle intermediates | 6.98 (6.82–7.13)                      | 6.98 (6.8–7.1)                     | 7.06 (7.02–7.18)                              | 7.09 (7.01–7.18)                          | 7.08 (6.99–7.15)                          | 7.08 (7.01–7.18)                      |
| (E)-2-Hexenoic acid, 4-amino-5-methyl-, methyl ester (NIST: 75.9%) | Unsaturated fatty acids | 5.34 (5.13–5.58)                      | 5.34 (5.17–5.57)                   | 5.16 (4.95–5.16)                              | 4.96 (4.83–5.14)                          | 5.13 (4.95–5.3)                           | 5.06 (4.91–5.2)                       |
| 10-Pentadecenoic acid         | Unsaturated fatty acids | 5.4 (5.21–5.59)                      | 5.3 (5.12–5.5)                    | 5.39 (5.33–5.73)                              | 5.39 (5.23–5.57)                          | 5.52 (5.31–5.81)                          | 5.42 (5.21–5.64)                      |
| 11,14-Eicosadienoic           | Unsaturated fatty acids | 5.94 (5.69–6.25)                      | 5.82 (5.56–6.12)                   | 6.18 (6.24–6.64)                              | 6.42 (6.25–6.59)                          | 6.19 (6.09–6.36)                          | 6.16 (6.03–6.32)                      |
| 13,16-Docosadienoic           | Unsaturated fatty acids | 5.23 (4.84–5.49)                      | 5.06 (4.79–5.35)                   | 5.37 (5.4–5.85)                               | 5.56 (5.3–5.88)                           | 5.65 (5.31–5.88)                          | 5.57 (5.34–5.77)                      |
| Name                                                                 | Classification               | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|----------------------------------------------------------------------|------------------------------|----------------------------------------|--------------------------------------|-----------------------------------------------|---------------------------------------------|-------------------------------------------|-----------------------------------------|
| 14-Methylpentadec-9-enioic acid methyl ester (NIST: 70.8%)            | Unsaturated fatty acids      | 6.23 (6.05–6.42)                       | 6.22 (6.05–6.43)                     | 6.35 (6.32–6.52)                              | 6.44 (6.2–6.53)                             | 6.42 (6.18–6.51)                           | 6.42 (6.09–6.49)                         |
| 3-Hydroxyoctanoic acid                                               | Unsaturated fatty acids      | 5.97 (5.66–6.26)                       | 5.75 (5.49–6.17)                     | 6.06 (6.02–6.41)                              | 6.09 (5.87–6.24)                            | 6.27 (5.98–6.44)                           | 6.12 (5.84–6.29)                         |
| 3-Methyl-2-oxopentanoic acid                                         | Unsaturated fatty acids      | 5.65 (5.43–5.84)                       | 5.68 (5.51–5.89)                     | 5.27 (4.99–5.18)                              | 5.12 (5.5–5.26)                             | 5.06 (4.95–5.17)                           | 5.09 (4.98–5.23)                         |
| 4-Methyl-2-oxopentanoic acid                                         | Unsaturated fatty acids      | 5.78 (5.64–5.96)                       | 5.81 (5.65–5.98)                     | 5.58 (5.31–5.55)                              | 5.42 (5.31–5.55)                            | 5.46 (5.33–5.58)                           | 5.45 (5.3–5.59)                          |
| 5-Cyano-4-methoxyamino-7-phenyl-hept-6-enioic acid, methyl ester (NIST: 54.9%) | Unsaturated fatty acids      | 5.94 (5.74–6.12)                       | 5.85 (5.75–6.24)                     | 5.83 (5.7–5.92)                               | 5.74 (5.56–5.86)                            | 5.68 (5.54–5.82)                           |                                         |
| 9,12-Octadecadienoic acid (2,2)-2-hydroxy-1-(hydroxymethyl)ethyl ester (NIST: 83.7%) | Unsaturated fatty acids      | 4.9 (4.74–5.13)                        | 4.91 (4.69–5.04)                     | 5.09 (5.15–5.48)                              | 5.32 (5.18–5.48)                            | 5.37 (5.18–5.58)                           | 5.32 (5.17–5.53)                         |
| 9-Heptadecenoic acid                                                 | Unsaturated fatty acids      | 5.53 (5.32–5.72)                       | 5.45 (5.25–5.62)                     | 5.56 (5.52–5.91)                              | 5.69 (5.5–5.79)                             | 5.73 (5.52–5.94)                           | 5.71 (5.57–5.86)                         |
| Adrenic acid                                                         | Unsaturated fatty acids      | 5.73 (5.34–6)                          | 5.64 (5.29–5.84)                     | 6.05 (6.19–6.6)                               | 6.44 (6.14–6.66)                            | 5.99 (5.79–6.19)                           | 6.1 (5.85–6.23)                          |
| alpha-Linolenic acid                                                 | Unsaturated fatty acids      | 5.12 (4.95–5.41)                       | 5.03 (4.79–5.22)                     | 5.33 (5.36–6.07)                              | 5.59 (5.33–5.89)                            | 5.84 (5.58–6.14)                           | 5.81 (5.52–6.2)                          |
| Arachidonic acid                                                    | Unsaturated fatty acids      | 6.43 (6.22–6.65)                       | 6.32 (6.16–6.51)                     | 6.56 (6.63–6.92)                              | 6.76 (6.65–6.88)                            | 6.5 (6.35–6.64)                            | 6.51 (6.37–6.67)                         |
| But-2-enedioic acid, dimethyl ester (NIST: 86.5%)                    | Unsaturated fatty acids      | 5.88 (5.75–6)                          | 5.81 (5.68–5.93)                     | 5.9 (5.87–6.08)                               | 5.96 (5.81–6.05)                            | 5.84 (5.73–5.93)                           | 5.82 (5.73–5.95)                         |
| Butanedioic acid, dimethyl ester (NIST: 76.5%)                       | Unsaturated fatty acids      | 5.88 (5.72–6.13)                       | 5.92 (5.66–6.12)                     | 5.92 (5.85–6.06)                              | 5.94 (5.8–6.05)                             | 5.96 (5.87–6.05)                           | 5.96 (5.86–6.03)                         |
| Butanedioic acid, ethyl methyl ester (NIST: 76.3%)                   | Unsaturated fatty acids      | 5.72 (5.49–5.92)                       | 5.84 (5.57–6.05)                     | 5.4 (5.21–5.35)                               | 5.29 (5.2–5.36)                             | 5.32 (5.22–5.4)                            | 5.33 (5.26–5.39)                         |
| cis-Vaccenic acid                                                    | Unsaturated fatty acids      | 7.25 (7.1–7.55)                        | 7.21 (7.07–7.4)                      | 7.63 (7.66–7.77)                              | 7.73 (7.67–7.78)                            | 7.77 (7.65–7.75)                           | 7.77 (7.63–7.76)                         |
| Conjugated-linoleic acid                                             | Unsaturated fatty acids      | 7.25 (7.07–7.59)                       | 7.23 (7.06–7.4)                      | 7.71 (7.76–7.87)                              | 7.83 (7.76–7.87)                            | 7.82 (7.78–7.87)                           | 7.82 (7.75–7.89)                         |
| DHA                                                                | Unsaturated fatty acids      | 5.73 (5.34–6)                          | 5.64 (5.29–5.84)                     | 6.05 (6.19–6.6)                               | 6.44 (6.14–6.66)                            | 5.99 (5.79–6.19)                           | 6.1 (5.85–6.23)                          |
| Erucic acid                                                          | Unsaturated fatty acids      | 5.35 (5.01–5.6)                        | 5.54 (4.94–5.51)                     | 5.78 (5.58–6.02)                              | 5.78 (5.48–6.1)                             | 5.67 (5.44–5.93)                           | 5.58 (5.38–5.91)                         |
| gamma-Linolenic acid                                                 | Unsaturated fatty acids      | 6.08 (5.77–6.51)                       | 5.91 (5.66–6.31)                     | 6.58 (6.76–7.19)                              | 7.04 (6.82–7.22)                            | 7.16 (7.02–7.25)                           | 7.14 (6.94–7.26)                         |
| Gondoic acid                                                         | Unsaturated fatty acids      | 5.82 (5.6–6.12)                        | 5.76 (5.48–6)                        | 6.1 (6.17–6.57)                               | 6.31 (6.2–6.51)                             | 6.08 (5.9–6.26)                            | 6.06 (5.93–6.27)                         |
| Itaconic acid                                                        | Unsaturated fatty acids      | 6.64 (6.04–6.95)                       | 6.46 (5.98–6.79)                     | 6.91 (6.94–7.12)                              | 7.02 (6.93–7.09)                            | 6.88 (6.68–6.95)                           | 6.87 (6.75–6.97)                         |
### Supplementary Table 3. Reproducibility of quality control samples in metabolites.

| Name                        | Classification               | Control-colostrum-median (range)/100 mL | GDM-colostrum-median (range)/100 mL | Control-transitional milk-median (range)/100 mL | GDM-transitional milk-median (range)/100 mL | Control-mature milk-median (range)/100 mL | GDM-mature milk-median (range)/100 mL |
|-----------------------------|------------------------------|----------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------|---------------------------------------|
| Linolelaidic acid           | Unsaturated fatty acids      | 6.06 (5.85–6.24)                       | 5.94 (5.66–6.28)                    | 6.23 (6.22–6.42)                              | 6.3 (6.16–6.39)                             | 6.36 (6.27–6.47)                        | 6.34 (6.26–6.44)                      |
| Myristoleic acid            | Unsaturated fatty acids      | 6.23 (6.04–6.6)                        | 6.16 (5.95–6.47)                    | 6.93 (7.1–7.35)                               | 7.25 (7.08–7.39)                            | 7.15 (7.01–7.27)                        | 7.12 (6.94–7.26)                      |
| Nervonic acid               | Unsaturated fatty acids      | 4.75 (4.58–4.97)                       | 4.69 (4.51–4.93)                    | 4.75 (4.49–4.94)                              | 4.69 (4.47–4.96)                            | 4.75 (4.51–4.94)                        | 4.73 (4.49–4.91)                      |
| Oleic acid                  | Unsaturated fatty acids      | 7.25 (7.1–7.55)                        | 7.21 (7.07–7.4)                     | 7.63 (7.66–7.77)                              | 7.73 (7.67–7.78)                            | 7.7 (7.65–7.75)                         | 7.7 (7.63–7.76)                       |
| Palmitelaidic acid          | Unsaturated fatty acids      | 6.58 (6.27–6.82)                       | 6.43 (6.04–6.82)                    | 6.78 (6.76–7.03)                              | 6.88 (6.76–6.98)                            | 6.91 (6.81–7.01)                        | 6.9 (6.79–7.01)                       |
| Name                                                                 | Classification                      | CV  |
|----------------------------------------------------------------------|-------------------------------------|-----|
| Glycine, N-ethyl-N-(2-methoxyethoxycarbonyl)-2-methoxyethyl ester (NIST: 97.5%) | Amino acid derivatives              | 22.5|
| Alanine                                                              | Amino acids                         | 13.1|
| Asparagine                                                           | Amino acids                         | 17.8|
| Aspartic acid                                                        | Amino acids                         | 9.3 |
| Creatinine                                                           | Amino acids                         | 23.8|
| Cysteine                                                             | Amino acids                         | 21.1|
| Glutamic acid                                                        | Amino acids                         | 12.5|
| Glutamine                                                            | Amino acids                         | 17.3|
| Glutathione                                                          | Amino acids                         | 15.4|
| Glycine                                                              | Amino acids                         | 14.0|
| Histidine                                                            | Amino acids                         | 10.5|
| Isoleucine                                                           | Amino acids                         | 12.1|
| Leucine                                                              | Amino acids                         | 12.5|
| Lysine                                                               | Amino acids                         | 9.3 |
| Methionine                                                           | Amino acids                         | 21.5|
| Ornithine                                                            | Amino acids                         | 13.2|
| Phenylalanine                                                        | Amino acids                         | 12.0|
| Proline                                                              | Amino acids                         | 10.6|
| Serine                                                               | Amino acids                         | 15.2|
| Threonine                                                            | Amino acids                         | 14.7|
| Tryptophan                                                           | Amino acids                         | 27.3|
| Tyrosine                                                             | Amino acids                         | 10.0|
| Valine                                                               | Amino acids                         | 11.9|
| NADP_NADPH                                                           | Cofactors and Vitamins              | 21.0|
| Nicotinamide                                                         | Cofactors and Vitamins              | 50.2|
| Nicotinic acid                                                       | Cofactors and Vitamins              | 26.5|
| 2-Phosphoenolpyruvic acid                                           | Glycolytic intermediates            | 25.3|
| 3-Methyl-2-oxovaleric acid                                          | Keto acids and derivatives           | 61.2|
| Oxaloacetic acid                                                     | Keto acids and derivatives           | 35.7|
| Pyruvic acid                                                         | Keto acids and derivatives           | 30.7|
| Dimethyl aminomalonic acid                                           | Organic acids                       | 38.6|
| O-Acetymalic anhydride                                               | Organic acids                       | 81.5|
| 1-Aminocyclopropane-1-carboxylic acid                               | Organic acids                       | 18.6|
| 2-Aminobutyric acid                                                 | Organic acids                       | 11.3|
| 2-Hydroxybutyric acid                                               | Organic acids                       | 15.1|
| 2-Hydroxyglutaramic acid                                            | Organic acids                       | 44.5|

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| Name                                                                 | Classification      | CV  |
|----------------------------------------------------------------------|---------------------|-----|
| 2-Hydroxyisobutyric acid                                             | Organic acids       | 29.9|
| 2-Oxoadipic acid                                                     | Organic acids       | 31.3|
| 2-Oxovaleric acid                                                    | Organic acids       | 33.7|
| 3-Acetoxy-3-Hydroxy-2-methylpropionic acid (NIST: 88.6%)             | Organic acids       | 44.7|
| 4 Hydroxybenzene sulphonic acid                                      | Organic acids       | 45.1|
| 4-Aminobutyric acid (GABA)                                           | Organic acids       | 21.5|
| 4-Hydroxyphenylacetic acid                                           | Organic acids       | 19.1|
| Azelaic acid                                                         | Organic acids       | 11.0|
| Benzoic acid                                                         | Organic acids       | 42.4|
| Cabamic acid                                                         | Organic acids       | 28.4|
| Caffeine                                                             | Organic acids       | 14.7|
| D_Indole-3-butyric acid                                              | Organic acids       | 15.7|
| DBP                                                                  | Organic acids       | 24.0|
| Dehydroabietic acid                                                  | Organic acids       | 63.5|
| Dehydroascorbic acid                                                 | Organic acids       | 19.4|
| Dipicolinic acid                                                     | Organic acids       | 17.5|
| Glutaric acid                                                        | Organic acids       | 32.5|
| Glyceric acid                                                        | Organic acids       | 21.8|
| Glyoxylic acid                                                       | Organic acids       | 33.1|
| Hippuric acid                                                        | Organic acids       | 19.0|
| Hydroxybenzoic acid                                                  | Organic acids       | 17.4|
| Lactic acid                                                          | Organic acids       | 14.8|
| Levulinic acid                                                       | Organic acids       | 25.6|
| Malonic acid                                                         | Organic acids       | 22.8|
| Oxalic acid                                                          | Organic acids       | 52.7|
| Phenethyl acetate                                                    | Organic acids       | 30.3|
| Suberic acid                                                         | Organic acids       | 18.0|
| Methyl 2-ethoxyacetate (NIST: 64.4%)                                 | Organic acids       | 24.1|
| Paraldehyde (NIST: 69.9%)                                            | Organic acids       | 41.5|
| Cyclopentane, hexyl- (NIST: 65.9%)                                   | Organic acids       | 61.6|
| Oxetane, 2,3,4-trimethyl-, (2α,3α,4 β)- (NIST: 64.2%)                 | Organic acids       | 20.7|
| 2-Acetonyl-9-[3-deoxy-β-d-ribofuranosyl]hypoxanthine (NIST: 67.8%)   | Organic acids       | 65.0|
| Heptacosane (NIST: 78.9%)                                            | Organic acids       | 30.3|
| Cycloheptasiloxane, tetradecamethyl- (NIST: 94.2%)                   | Organic acids       | 42.5|
| Cyclononasiloxane, octadecamethyl- (NIST: 91.7%)                     | Organic acids       | 58.1|
| 2-Piperidinecarboxylic acid, 1-acetyl-, ethyl ester (NIST: 69.6%)    | Organic acids       | 22.0|
| Name                                                                 | Classification      | CV   |
|----------------------------------------------------------------------|---------------------|------|
| 4,4-Dimethyl-5-methylene[1,3]dioxolan-2-one (NIST: 60.5%)             | Organic acids       | 20.2 |
| 3-Hydroxy-azetidine-1-carboxylic acid, methyl ester (NIST: 65.9%)      | Organic acids       | 25.8 |
| Methyl 2-methylhexanoate (NIST: 70.4%)                                | Organic acids       | 82.9 |
| Benzenecacetic acid, methyl ester (NIST: 88.3%)                       | Organic acids       | 60.2 |
| 3-Furancarboxylic acid, methyl ester (NIST: 91.8%)                    | Organic acids       | 24.6 |
| Furfuryl hydroxymethyl ketone (NIST: 88.1%)                           | Organic acids       | 46.0 |
| Propanedioic acid, methyl,ethyl ester (NIST: 64%)                     | Organic acids       | 21.4 |
| Acetophenone (NIST: 96.2%)                                            | Organic acids       | 42.1 |
| 4-Pyridinecarboxaldehyde (NIST: 83.6%)                                | Organic acids       | 43.3 |
| 3-Pyridinecarboxaldehyde (NIST: 90.7%)                                | Organic acids       | 27.0 |
| 3H-Pyrazol-3-one, 2,4-dihydro-2,5-dimethyl- (NIST: 67.2%)              | Organic acids       | 41.3 |
| Butanedioyl dihydrazide (NIST: 70.6%)                                 | Organic acids       | 15.7 |
| 2-Methoxy-N-(2-methoxy-propyl)-N-methyl-propionamide (NIST: 49.9%)   | Organic acids       | 38.1 |
| Dimethyl ethylidenemalonate (NIST: 80.4%)                             | Organic acids       | 27.6 |
| 2-Oxomalonic acid, methylhydrazone, dimethyl ester (NIST: 62%)        | Organic acids       | 21.5 |
| 2,3-Furandione, dihydro-4,4-dimethyl (NIST: 91.3%)                    | Organic acids       | 46.4 |
| 2-(1-Pentamethylphenyl)ethyl-3,3-diphenyloxaziridine (NIST: 67.9%)   | Organic acids       | 15.4 |
| 3,4-Methylenedioxyphenylacetic acid (NIST: 78.4%)                    | Organic acids       | 21.1 |
| Bis(2-ethylhexyl) phthalate (NIST: 96.3%)                             | Organic acids       | 62.2 |
| N-Ethylpyrrrolidine-2,2-dicarboxylic acid, dimethyl ester (NIST: 62.2%)| Organic acids       | 15.8 |
| 2-[(4-(2-Acetoxyethyl)-2,5-dimethoxyphenyl)acetic acid, methyl ester (NIST: 63%)] | Organic acids       | 21.3 |
| BHT (Antioxidant)                                                     | Organic acids       | 39.1 |
| Putrescine                                                           | Organic acids       | 10.6 |
| 1-(2-Methoxyethoxy)-2-methyl-2-propanol, methyl ether (NIST: 63.2%)   | Organic acids       | 17.0 |
| N(1-Methoxycarbonyl-1-methyl)ethyl-2-aza-1,3-dioxane (NIST: 87.4%)   | Organic acids       | 23.3 |
| Stearic acid (C18_0)                                                  | Saturated fatty acids| 13.6 |
| 2-Methyloctadecanoic acid                                            | Saturated fatty acids| 46.3 |
| 3-Hydroxydecanoic acid                                               | Saturated fatty acids| 14.3 |
| Myricic acid                                                         | Saturated fatty acids| 27.9 |
| Adrenic acid (C22_4n-6,9,12,15c)                                     | Saturated fatty acids| 32.4 |
| Arachidic acid (C20_0)                                               | Saturated fatty acids| 31.1 |
| Behenic acid (C22_0)                                                 | Saturated fatty acids| 42.3 |
| Decanoic acid (C10_0)                                                | Saturated fatty acids| 25.6 |
| Dodecanoic acid (C12_0)                                              | Saturated fatty acids| 17.0 |
| Hexanoic acid (C6_0)                                                 | Saturated fatty acids| 45.8 |
| Lignoceric acid (C24_0)                                              | Saturated fatty acids| 65.2 |
| Name                                           | Classification          | CV  |
|------------------------------------------------|-------------------------|-----|
| Margaric acid (C17_0)                          | Saturated fatty acids   | 23.3|
| Myristic acid (C14_0)                          | Saturated fatty acids   | 18.8|
| Nonadecanoic acid (C19_0)                      | Saturated fatty acids   | 29.1|
| Octanoic acid (C8_0)                           | Saturated fatty acids   | 43.3|
| Palmitic acid (C16_0)                          | Saturated fatty acids   | 10.0|
| Pentadecanoic acid (C15_0)                     | Saturated fatty acids   | 22.2|
| Pimelic acid                                   | Saturated fatty acids   | 17.4|
| Tridecanoic acid (C13_0)                       | Saturated fatty acids   | 95.0|
| Undecanoic acid (C11_0)                        | Saturated fatty acids   | 22.9|
| Tetradecanoic acid, 12-methyl-, methyl ester (NIST: 80.2%) | Saturated fatty acids   | 18.8|
| Tridecanoic acid, 12-methyl-, methyl ester (NIST: 89.6%) | Saturated fatty acids   | 23.4|
| 2-Oxoglutaric acid                             | TCA cycle intermediates| 29.6|
| cis-Aconitic acid                              | TCA cycle intermediates| 20.8|
| Citramalic acid                                | TCA cycle intermediates| 28.5|
| Citric acid                                    | TCA cycle intermediates| 28.0|
| Fumaric acid                                   | TCA cycle intermediates| 16.8|
| Isocitric acid                                 | TCA cycle intermediates| 18.7|
| Malic acid                                     | TCA cycle intermediates| 43.7|
| Succinic acid                                  | TCA cycle intermediates| 15.7|
| 10-Pentadecenoic a+A2: A134cid (C15_1n-5c)    | Unsaturated fatty acids | 38.3|
| 11,14-Eicosadienoic (C20_2n-6,9c)              | Unsaturated fatty acids | 25.9|
| 13,16-Docosadienoic acid (C22_2n-6,9c)         | Unsaturated fatty acids | 39.1|
| 3-Hydroxyoctanoic acid                         | Unsaturated fatty acids | 38.2|
| 3-Methyl-2-oxopentanoic acid                   | Unsaturated fatty acids | 20.4|
| 4-Methyl-2-oxopentanoic acid                   | Unsaturated fatty acids | 16.8|
| 9-Heptadecenoic acid (C17_1n-8t)               | Unsaturated fatty acids | 34.7|
| alpha-Linolenic acid (C18_3n-3,6,9c)           | Unsaturated fatty acids | 87.6|
| Arachidonic acid (C20_4n-6,9,12,15c)           | Unsaturated fatty acids | 22.0|
| cis-Vaccenic acid (C18_1n-7c)                  | Unsaturated fatty acids | 10.4|
| Conjugated linoleic acid (C18_2n-9,11c)        | Unsaturated fatty acids | 11.6|
| DHA (C22_6n-3,6,9,12,15,18c)                   | Unsaturated fatty acids | 32.4|
| Erucic acid (C22_1n-9c)                        | Unsaturated fatty acids | 35.5|
| gamma-Linolenic acid (C18_3n-6,9,12c)          | Unsaturated fatty acids | 52.9|
| Gondoic acid (C20_1n-9c)                       | Unsaturated fatty acids | 26.6|
| Itaconic acid                                  | Unsaturated fatty acids | 24.5|
| Linolelaidic acid (C18_2n-9,12c)               | Unsaturated fatty acids | 62.1|
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