Pediatric RESEARCH

BASIC SCIENCE ARTICLE

Does metabolomic profile differ with regard to birth weight?

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BACKGROUND: Macrosomia and child obesity are growing health-care issues worldwide. The purpose of the study was to evaluate how extremely high or low birth weight affects metabolic markers evaluated in newborn screening.
METHODS: The study was register-based and included full-term singletons born in Iceland from 2009 to 2012 with newborn screening samples taken 72–96 h after birth. Three groups based on birth weight were compared: low birth weight (<2500 g), appropriate-for-gestational age, and extreme macrosomia (≥5000 g). The comparison was adjusted for possible confounding factors.
RESULTS: Compared to appropriate-for-gestational age neonates, both low birth weight and extreme macrosomia were associated with higher levels of glutamic acid. The amino acids alanine and threonine were increased in low birth weight neonates. Free carnitine and some medium- and long-chain acylcarnitines were higher in low birth weight infants. Hydroxybutyrylcarnitine was lower in low birth weight infants, but higher in extremely macrosomic neonates. Acetylcarnitine was higher in low birth weight and extremely macrosomic neonates. Succinylcarnitine was lower and hexadecenoylcarnitine higher in macrosomic newborns.
CONCLUSION: Low birth weight and extremely macrosomic neonates show distinctive differences in their metabolomic profile compared to appropriate-for-gestational age newborns. The differences are not explained by gestational age.

Pediatric Research (2021) 89:1144–1151; https://doi.org/10.1038/s41390-020-1033-0

IMPACT:

- The key message of this article is that both low birth weight and extremely macrosomic newborns show dissimilar metabolomic profiles compared to appropriate-for-gestational age neonates.
- The article contributes to knowledge on what affects evaluation of results in newborn screening.
- The impact of this article is to provide information on metabolism at both ends of the birth weight range after accounting for confounding factors including gestational age.

INTRODUCTION

Newborn screening is well-established in most high-income countries and is considered a cost-effective way to detect inborn errors of metabolism at an early stage.[1] The screening usually includes measurements of amino acids and acylcarnitines by tandem mass spectrometry. Several factors may affect the metabolic profiles seen in newborn screening, such as gestational age, age of the neonate when the sample is taken, and mode of nutrition prior to screening.[2–5] Neonates of extremely low birth weight, internationally defined as <1000 g, tend to have higher false-positive rates in screening for inborn errors of metabolism compared to newborns born with birth weight between 2500 and 3999 g.[6] The correlation between amino acids and acylcarnitines and obesity, prediabetes and type 2 diabetes has also been studied in various populations. Increases in branched-chain amino acids (BCAA) and aromatic amino acids (AAA), glycine, glutamic acid and some acylcarnitines (mostly acetylcarnitine (C2), propionylcarnitine (C3) and isovalerylcarnitine (C5)) have been shown in obese compared to non-obese children.[7,8] Positive correlations between the same amino acids and type 2 diabetes/prediabetes compared to those with normal glucose tolerance have been noticed in adults.[9,10] With regard to acylcarnitines there were positive correlations between C2 and hydroxybutyrylcarnitine (C4OH), but negative correlations between tiglylcarnitine (C5:1), linoleoylcarnitine (C18:2), and eicosanoylcarnitine (C20) in the same individuals.[9,10] C2, C4OH, octenoylcarnitine (C8:1), and oleylcarnitine (C18:1) have also been reported to be higher in obese compared to non-obese pregnant women.[11] Kadakia et al.[12] reported a positive association to newborn adiposity using principal components analysis where levels of factor 1 (C2, C3, C5, butanoylcarnitine (C4/Ci4), C4OH, succinylcarnitine (C4DC/Ci4DC), glutamate/glutamine and glycine) explained 28% of the variance in newborn adiposity.

Low birth weight (LBW) is defined as birth weight < 2500 g, regardless of gestational age, since mortality and morbidity tend to rise gradually with lowering weights under 2500 g.[13,14] There

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Received: 15 January 2020 Revised: 3 June 2020 Accepted: 5 June 2020
Published online: 29 June 2020

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are two main causal groups, i.e. preterm birth and intrauterine growth restriction.[14] At the other end of the birth weight range is macrosomia, which is generally defined as birth weight ≥4500 g or ≥4000 g regardless of gestational age, depending on the normal variation in the population.[15] When birth weight is ≥5000 g, it is usually referred to as extreme macrosomia (EM).[16,17] Macrosomic newborns have a higher body fat percentage and mass than those with birth weight within a normal range.[18,19] Macrosomic infants and child obesity are a growing health-care issue with incidences increasing worldwide.[20–22] Risks of maternal and neonatal birth complications and neonatal hypoglycemia rise significantly with increasing birth weight.[15,23] High birth weight is moreover an independent risk factor for childhood obesity, while mothers born large-for-gestational age (LGA, birth weight > 90th percentile for the given nation in relation to gestational age) are more likely to have an LGA-baby of their own.[24–26] The highest risk of LGA births has, however, been noted among mothers with a high body mass index and who were themselves born small-for-gestational age (SGA, birth weight < 10th percentile for the given nation in relation to gestational age).[25]

Considering the risk factors for becoming macrosomic and the subsequent risks, both at birth and later in life, the aim of this study was to examine differences in amino acids and acylcarnitine concentrations across extremes of birth weight and to clarify better the importance of such differences.

MATERIALS AND METHODS

The study was based on a database from the nationwide Icelandic newborn screening program run by the Department of Genetics and Molecular Medicine at Landspitali—The National University Hospital in Reykjavik, where screening supervision is centralized for a country with a homogenous Caucasian population. In the program blood samples are taken on filter paper from newborns and analyzed by tandem mass spectrometry (MS/MS) to screen for inborn errors of metabolism. Background information on the newborns was documented, i.e. gender, gestational weeks attained, time of sampling, birth weight, and maternal age at birth. From the database results of acylcarnitine and amino acid measurements were registered for the study purpose, as was additional background information. This study was approved by Landspitali—The National University Hospital Ethical Committee (33/2013) and the Icelandic Data Protection Authority (2013060794HGK, 31 July 2013).

Study population

The study period was from 1st of January 2009 to 31st of December 2012. Included were all singletons born full term (≥37 weeks gestation) in Iceland. Only full-term neonates were included for minimizing the risk of confounding factors, for example the use of antibiotics or the need for parenteral nutrition. Most newborns in Iceland are exclusively breastfed (86% during the first week of life in the years 2004–2008[27]). At the time of the study, the national guideline was to give all LBW newborns complimentary feeding using infant milk formula in addition to breast milk, so the total amount of feeding was increased over the first week of life. Extremely macrosomic newborns on the other hand were mostly observed clinically and with intermittent glucose measurements. Complementary feeding was only infrequently given, on indication.

Those neonates with incomplete datasets were excluded. Only samples taken 72–96 h after birth were used as this was the time of initial sampling for neonatal screening in the study period. Furthermore, to reduce the risk of repeated sampling, the sample period was limited to this 24-h window. The material was divided into three groups based on birth weight: LBW (<2500 g), birth weight appropriate-for-gestational age (AGA, internationally defined as birth weight between the 10th and 90th percentiles for the given nation in relation to gestational age) according to gender, and EM (≥5000 g). Neonates with birth weight < 2500 g and meeting the AGA criteria were excluded for the reason that they were within the 10th–90th percentile regarding weight but most likely received treatment as babies <2500 g, including supplementary formula feeding, and therefore did not belong in either group. Those neonates not meeting the criteria for birth weight groups were excluded.

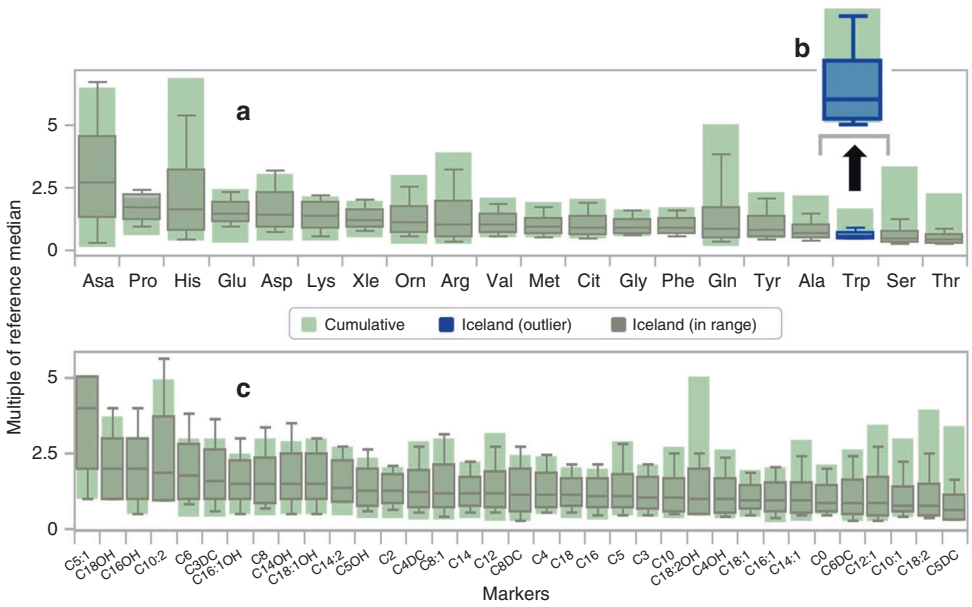

Fig. 1 Comparison of the study results to the CLIR cumulative reference intervals. CLIR productivity tool Reference Plot, an overlay of one site (Iceland) percentiles and cumulative data. Markers are plotted from left to right based on the decreasing distance between the cumulative median, shown as zero, and the location median. **a** Amino acids. **b** Magnification of the only outlier detected by the comparison. Outlier is defined here as the lowest median value among all participants (see also text). **c** Acylcarnitines. Data are shown as box and whisker plots representing the 99, 90, 50, 10, and 1% percentile values after conversion to multiple of the reference median.

Table 1. Descriptive background information on the newborns included, total and by neonatal weight categories.

	Total (*N*: 6131)	LBW (*N*: 36)	AGA (*N*: 6058)	EM (*N*: 37)
Birth weight (g, mean ± SD for total, median, IQR (min, max) for groups)[a]	3619 ± 386	**2335, 174 (1724, 2490)**	3630, 510 (2505, 4495)	**5102, 210 (5000, 6775)**
Gestational age (weeks, mean ± SD)[b]	40.0 ± 1.1	**38.2 ± 0.9**	40.0 ± 1.1	**40.4 ± 1.2**
Maternal age (years, mean ± SD)[c]	30.0 ± 5.3	**28.3 ± 6.1**	30.0 ± 5.3	**32.4 ± 5.6**
Gender[d]				
Male	3137 (51%)	19 (53%)	3091 (51%)	**27 (73%)**
Female	2994 (49%)	17 (47%)	2967 (49%)	**10 (27%)**

Values with significant differences ($\alpha < 0.05$), with AGA as a reference, are shown in bold letters.
AGA appropriate-for-gestational age (birth weight between 10th and 90th percentile in Icelandic population according to gender and gestational age), *LBW* low birth weight (birth weight < 2500 g), *EM* extreme macrosomia (birth weight ≥ 5000 g).
[a]Corrected for gestational weeks, ANOVA F test: $\alpha < 0.0001$.
[b]ANOVA F test: $\alpha < 0.0001$.
[c]ANOVA F test: $\alpha = 0.003$.
[d]Chi-square: $\alpha = 0.005$.

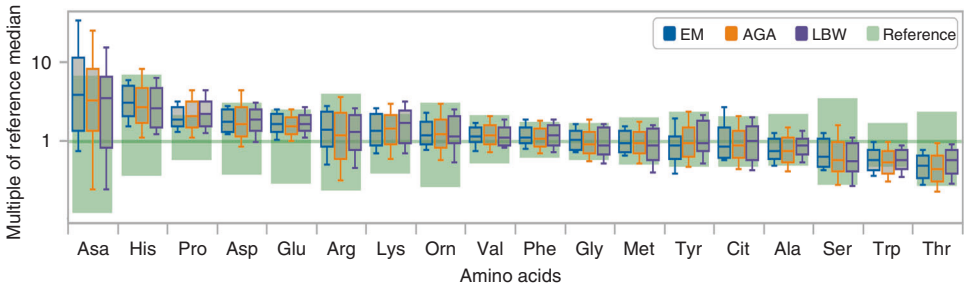

Fig. 2 Amino acids compared between EM (blue), AGA (orange) and LBW (purple). CLIR productivity tool Plot by Multiple Conditions, where birth weight groups are defined as condition. Markers are plotted by multiples of the reference median with CLIR database as reference, median shown as one with 99 and 1% percentile (green). Data are shown as box and whisker plots representing the 99, 90, 50, 10, and 1% percentile values after conversion to multiple of the reference median and arranged by the difference of condition median from the reference median.

Statistical analysis

Descriptive statistics were used for characteristics of study participants and concentrations of amino acids and carnitines. Mean values and standard deviations (SD) were calculated for all variables. For amino acid and acylcarnitine biomarkers, medians and 10^{th}–90^{th} percentiles were additionally calculated. As LBW and EM are skewed variables, the median, interquartile range (IQR), minimum and maximum values were used for descriptive statistics by birth weight groups. Percentages were used to describe dichotomous variables.

We compared the distribution of our measurements of amino acids and acylcarnitines to the reference intervals available from the Collaborative Laboratory Integrated Reports (CLIR, https://clir.mayo.edu), a program that has seen the participation of the Iceland Newborn Screening Program for more than a decade.[28] CLIR is a web-based data collection and analysis system to improve the post-analytical interpretation of complex profiles and to decrease unwanted false-positive tests.[28,29] Over the years it has grown to include millions of newborns from across the world. We uploaded our data into CLIR and used multiples of median boxplots of covariate-unadjusted data for comparison purposes between EM, AGA, and LBW.

For examining associations between birth weight groups, we used linear regression analysis and as a measure of association we used the F test comparing differences across the groups. We also used linear regression analysis for comparing the measurements of most amino acids and acylcarnitines between birth weight groups, adjusted for maternal age, newborn gender, and gestational age. For skewed variables, linear regression models adjusted for the same factors were used, but with logarithmic transformation. As a measure of association we used the F test comparing differences across EM, AGA, and LBW newborns under the null hypothesis that all three groups had the same mean response. A *t* test was used for comparing differences between the two groups. Levels of significance were set at $\alpha = 5\%$ and all tests were two-sided. Chi-squared tests were used for dichotomous variables and statistical significance with α set at 5%. To account for false discovery rate (set at $\alpha = 0.05$), the Benjamini–Hochberg procedure was used. Statistical analyses were done in the RStudio® statistical software program.

RESULTS

There were a total of 18,426 live-born singletons during the study period.[30] Of those 9867 were born at term and had a complete dataset, 1853 were SGA or LGA newborns but did not meet the criteria of being EM (≥5000 g) or having LBW (<2500 g) and were therefore excluded. Ten newborns were excluded as they were born AGA and had birth weight <2500 g. Additionally, 1873 newborns did not meet the criterion of sampling within 3–4 days after birth, leaving a total of 6131 newborns available for analyses. In general, our results were within range for all CLIR reference intervals, except for the amino acid tryptophan that had the lowest median value among contributing laboratories (Fig. 1). However, this is a marker not routinely covered by commercial reagent kits and consequently is measured only by four sites.

Table 2. Relative differences in amino acid concentrations between birth weight groups.

Amino acids	Concentration (μmol/L)	Mean difference (Δ) in concentration relative to AGA (μmol/L or in % for skewed variables)			
	Total *N*: 6131	LBW *N*: 36	AGA *N*: 6058	EM *N*: 37	*p* value[a]
Total	1332 ± 306[b] 1281 (820–1730)[c]	69 (−40 to 177)[d]	—	5.6 (−99 to 110)	0.46
Alanine	201 ± 59 191 (104–279)	**29** (10–48)	—	−1.6 (−20 to 17)	**0.01**
Arginine	11 ± 7 10 (3–19)	3% (−14 to 20%)[e]	—	15% (−2 to 31%)	0.22
Argininosuccinic acid	0.74 ± 0.84 0.55 (0.04–1.36)	−17% (−44 to 10%)	—	26% (−2 to 55%)	0.09
Aspartic acid	60 ± 23 55 (28–88)	4.3 (−3.3 to 12)	—	0.1 (−7.4 to 7.6)	0.54
Citrulline	12 ± 4 12 (6–18)	1.2 (−0.3 to 2.6)	—	0.3 (−1.1 to 1.7)	0.27
Phenylalanine	59 ± 12 57 (37–75)	3.8 (−0.3 to 7.8)	—	2.3 (−1.7 to 6.2)	0.10
Glutamic acid	434 ± 89 424 (271–550)	**38** (9–67)	—	**36** (7–65)	**0.002**[f]
Glycine	389 ± 108 369 (221–525)	6 (−32 to 44)	—	35 (−2–72)	0.16
Histidine	76 ± 35 69 (28–117)	−4 (−15 to 8)	—	7 (−4 to 19)	0.37
Lysine	208 ± 70 199 (80–298)	22 (−0.6 to 45)	—	−7.7 (−30 to 15)	0.13
Methionine	20 ± 6 20 (11–27)	−0.5 (−2.3 to 1.4)	—	0.3 (−1.5 to 2.1)	0.84
Methylhistidine	5 ± 3 5(2–8)	−11% (−23 to 2%)	—	6% (−7 to 19%)	0.17
Ornithine	103 ± 38 96 (45–149)	0.2 (−12 to 13)	—	−1.9 (−14 to 10)	0.95
Proline	394 ± 126 373 (194–557)	23 (−19 to 64)	—	−34 (−75 to 7)	0.15
Serine	96 ± 40 87 (41–142)	−3 (−16 to 10)	—	9 (−4 to 22)	0.35
Threonine	25 ± 8 23 (12–35)	**5** (3–8)	—	2 (−1 to 4)	**0.0002**[f]
Tryptophan	19 ± 5 18 (10–25)	1.5 (−0.1 to 3.1)	—	1.0 (−0.6 to 2.5)	0.08
Tyrosine	85 ± 33 79 (39–123)	−3 (−14 to 8)	—	−6 (−16 to 5)	0.50
Valine	132 ± 30 128 (78–171)	1 (−9 to 11)	—	−5 (−15 to 5)	0.62

The AGA group is used as a reference. The differences presented are adjusted for gestational age, gender of newborn and age of mother. Significant differences are shown in bold letters.
AGA appropriate-for-gestational age (birth weight between 10th and 90th percentile in Icelandic population according to gender and gestational age), *LBW* low birth weight (birth weight < 2500 g), *EM* extreme macrosomia (birth weight ≥ 5000 g).
[a]*p* value from comparison across birth weight groups, with AGA as a reference, using ANOVA F test.
[b]Mean ± SD (all such values).
[c]Median (10th–90th percentile) (all such values).
[d]Mean difference in μmol/L with 95%CI (all such values).
[e]Mean difference in % with 95%CI, for skewed variables (all such values).
[f]Significant after accounting for false discovery rate ($a = 0.05$) using the Benjamini–Hochberg procedure.

Descriptive background information on the newborns is shown in Table 1. Of the 6131 births, 36 were classified as LBW, 37 as EM, and 6058 as AGA. Mean gestational weeks and maternal ages were slightly lower in the LBW groups than the AGA and slightly higher in the EM than the AGA (Table 1), with statistical significance. The EM group had a considerably higher percentage of male newborns (73%) with chi-squared test *p* value of 0.005, while the ratios were more evenly gender-distributed in the AGA and LBW groups (51% vs. 49% and 53% vs. 47%, respectively), not statistically significant.

Mean values with standard deviations and medians with 10th–90th percentiles were calculated for all biomarkers and are shown in Supplementary Tables 1 (amino acids) and 2 (acylcarnitines).

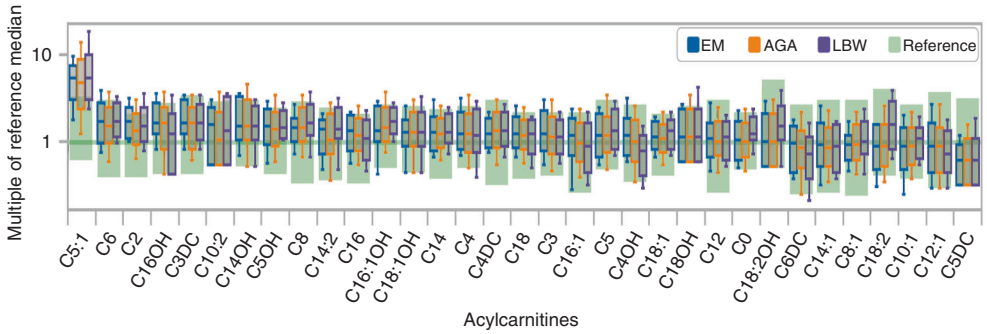

Fig. 3 Acylcarnitines compared between EM (blue), AGA (orange) and LBW (purple). CLIR productivity tool Plot by Multiple Conditions, where birth weight groups are defined as condition. Markers are plotted by multiples of the reference median with CLIR database as reference, median shown as one with 99 and 1% percentile (green). Data are shown as box and whisker plots representing the 99, 90, 50, 10, and 1% percentile values after conversion to multiple of the reference median and arranged by the difference of condition median from the reference median.

Amino acids

Figure 2 displays amino acids comparing EM and LBW to AGA using the CLIR database. There were no obvious differences between AGA and EM neonates. For AGA and LBW there were differences in alanine and threonine, which were higher in LBW compared to AGA newborns. When adjusting for possible confounding factors (gestational age, newborn gender, and maternal age), using linear regression analyses (Table 2), significant differences emerged regarding alanine and threonine between AGA and LBW neonates. Mean differences (Δ) between AGA and LBW were for alanine 29 µmol/L (95%CI 10–48 µmol/L) and threonine 5 µmol/L (95%CI 3–8). The LBW neonates had higher values than those AGA. There were differences regarding glutamic acid when EM and LBW neonates were compared to AGA, i.e. Δ 36 µmol/L (95%CI 7–65 µmol/L) for EM vs. AGA neonates and 38 µmol/L (95%CI 9–67 µmol/L) for LBW vs. AGA.

Acylcarnitines

Figure 3 shows acylcarnitine results comparing EM and LBW to AGA newborns, using the CLIR database. There was a tendency for EM neonates to have higher values of acetylcarnitine (C2), hydroxybutyrylcarnitine (C4OH), and hexadecenoylcarnitine (C16:1) and lower values of succinylcarnitine (C4DC) than seen for those AGA. Differences are notable with higher values for LBW neonates than for those who were AGA as regards free carnitine (C0), octenoylcarnitine (C8:1), tetradecadienoylcarnitine (C14:2), linoleoylcarnitine (C18:2), and 3-hydroxyoctadecadienoylcarnitine (C18:2OH). For LBW there were lower values in hydroxybutyrylcarnitine (C4OH) and methylglutarylcarnitine (C6DC) than among those AGA. After adjusting for possible confounding factors (gestational age, newborn gender, and maternal age), using linear regression analyses (Table 3), the mean differences for EM neonates were higher than for those AGA regarding C2, C4OH, hexadecenoylcarnitine (C16:1), but lower for C4DC. The largest difference between EM and AGA neonates was for C2 with a mean difference of 6 µmol/L (95%CI 2–9 µmol/L) among EM compared to AGA neonates. For LBW the mean differences were higher than for AGA neonates regarding free carnitine (C0), C8:1, tetradecadienoylcarnitine (C14:2), C18:2, while lower for C4OH. The greatest difference between LBW and AGA neonates was regarding C0 (9 µmol/L; 95%CI 6–11 µmol/L). Additionally, we noted higher mean differences for LBW neonates regarding C2, 3-hydroxyhexadecenoylcarnitine (C16:1OH), and C18:1. After adjusting for possible confounding factors, there were no significant differences between LBW and AGA neonates regarding C18:2OH and C6DC. When all acylcarnitines were evaluated together, there were significant differences in the mean values for both EM and LBW neonates compared to those AGA, with a mean difference between EM and AGA of 7 µmol/L (95%CI 0.7–13 µmol/L) and between LBW and AGA of 16 µmol/L (95%CI 10–23 µmol/L).

DISCUSSION

The results of this study, comprising 6131 full-term newborns, suggest that both LBW and EM newborns differ in their metabolomic profile compared to AGA neonates. These differences became clearer when adjusted for gestational age, gender, and maternal age. By collaborating with CLIR, we could verify the differences with more than one method, as well as comparing our data to a large reference set.

In terms of limitations we acknowledge that our findings are to some extent explorative. Given the number of comparisons made, our findings are prone to false-positive findings. When applying the Benjamini–Hochberg procedure, two of the three significant differences observed for the amino acids (threonine and glutamic acid) were still significant. Most of the significant differences for the carnitines were also significant (8 of 11 cases), after applying the Benjamini–Hochberg procedure. Our study population had 6131 newborns, but only 36 were born LBW and 37 EM. In spite of these groups being small, they were distinct from the AGA group and we could estimate the mean differences with considerable accuracy. However, it was not possible to assess what impact these differences may have on false-positive results in newborn screening if the neonate is born LBW or EM. Because of these somewhat explorative findings, confirmation in another independent cohort would be of value.

The unadjusted statistical differences observed on gender across birth weight groups (Table 1) could partly be explained by the difference among the EM newborns, where the male/female ratio was 2.7/1, and by male birth weight being higher in general than among females.[31] The difference in birth weight between AGA and EM was larger than the difference between AGA and LBW neonates, which could explain why similar differences in male/female ratio were not observed in the LBW group.

The amino acid differences observed across birth weight groups suggest an increased breakdown of proteins, exemplified by increased glutamic acid/glutamate levels in both LBW and EM newborns compared to those AGA. Glutamate has a variable role in cell metabolism.[32,33] Higher amounts of the amino acids, alanine and threonine, were also observed in LBW newborns compared to those born AGA. Those differences were, however, not seen for EM newborns, while the increases in glutamic acid/glutamate were similar to those born LBW. This could be due to LBW newborns being more dependent on protein as a source of energy. Muscle proteins are thus broken easily down to synthesize alanine, which then can be used by the liver to synthesize

Table 3. Relative differences in acylcarnitine concentrations between birth weight groups.

Acylcarnitines	Concentration (μmol/L)	Mean difference (Δ) in concentration relative to AGA (μmol/L or in % for skewed variables)			
	Total *N*: 6131	LBW *N*: 36	AGA *N*: 6058	EM *N*: 37	*p* value[a]
Total	57 ± 19[b] 54 (37–82)[c]	**16** (10–23)[d]	—	**7 (0.7–13)**	**<0.0001**[e]
C0	23 ± 9 22 (14–34)	**9** (6–11)	—	0.5 (−2 to 3)	**<0.0001**[e]
C2	31 ± 11 30 (20–45)	**8** (4–11)	—	**6** (2–9)	**<0.0001**[e]
C3	2.1 ± 0.9 1.9 (1.2–3.2)	−0.06 (−0.35 to 0.24)	—	0.34 (0.06–0.63)	0.06
C4	0.31 ± 0.13 0.28 (0.17–0.47)	0.01 (−0.04 to 0.05)	—	0.02 (−0.02 to 0.07)	0.53
C4OH	0.19 ± 0.09 0.18 (0.1–0.31)	**−0.05 (−0.07 to −0.02)**	—	**0.03 (0.001–0.06)**	**0.0009**[e]
C5	0.15 ± 0.09 0.13 (0.08–0.21)	10% (−3 to 23%)[f]	—	0.2% (−13 to 13%)	0.32
C5:1	0.09 ± 0.05 0.08 (0.04−0.15)	15% (−3 to 32%)	—	2% (−15 to 18%)	0.25
C5OH	0.2 ± 0.08 0.19 (0.12−0.31)	0.02 (−0.01 to 0.05)	—	0.01 (−0.01 to 0.04)	0.30
C6	0.09 ± 0.04 0.08 (0.05−0.13)	0.007 (−0.005 to 0.019)	—	0.008 (−0.004 to 0.019)	0.21
C3DC	0.08 ± 0.03 0.08 (0.04−0.12)	0.004 (−0.008 to 0.016)	—	0.012 (−0.0002 to 0.024)	0.12
C4DC	0.32 ± 0.12 0.31 (0.19−0.48)	0.03 (−0.008 to 0.07)	—	**−0.05 (−0.08 to −0.006)**	**0.02**
C5DC	0.04 ± 0.02 0.04 (0.02−0.07)	0.003 (−0.005 to 0.01)	—	−0.004 (−0.01 to 0.004)	0.40
C6DC	0.07 ± 0.06 0.07 (0.04−0.11)	−0.01 (−0.03 to 0.01)	—	0.003 (−0.02 to 0.02)	0.57
C8	0.1 ± 0.04 0.09 (0.06−0.15)	0.01 (−0.004 to 0.025)	—	−0.005 (−0.019 to 0.009)	0.25
C8:1	0.13 ± 0.06 0.12 (0.07−0.2)	**0.05 (0.03−0.07)**	—	−0.006 (−0.02 to 0.01)	**<0.0001**[e]
C10	0.09 ± 0.04 0.09 (0.05−0.14)	0.011 (−0.002 to 0.024)	—	0.005 (−0.008 to 0.017)	0.19
C10:1	0.05 ± 0.02 0.05 (0.03−0.08)	0.007 (−0.0005 to 0.014)	—	−0.0002 (−0.007 to 0.007)	0.19
C10:2	0.03 ± 0.01 0.02 (0.01−0.04)	0.004 (−0.001 to 0.008)	—	0.002 (−0.003 to 0.007)	0.22
C12	0.13 ± 0.05 0.12 (0.08−0.2)	0.002 (−0.01 to 0.02)	—	0.004 (−0.01 to 0.02)	0.85
C12:1	0.06 ± 0.03 0.06 (0.03−0.10)	−0.007 (−0.017 to 0.004)	—	0.006 (−0.005 to 0.016)	0.29
C14	0.29 ± 0.09 0.27 (0.18−0.40)	0.03 (−0.001 to 0.06)	—	0.01 (−0.02 to 0.04)	0.12
C14:1	0.11 ± 0.05 0.1 (0.06−0.17)	0.00005 (−0.02 to 0.02)	—	0.01 (−0.001 to 0.03)	0.20
C14:2	0.04 ± 0.02 0.03 (0.02−0.06)	**26% (10−41%)**	—	3% (−12 to 17%)	**0.004**[e]
C14OH	0.04 ± 0.02 0.03 (0.02−0.06)	−0.006 (−0.01 to 0.00003)	—	0.001 (−0.005 to 0.007)	0.14
C16	3.6 ± 1.2 3.4 (2.2−5.2)	−0.07 (−0.5 to 0.3)	—	0.2 (−0.2 to 0.6)	0.54
C16:1	0.22 ± 0.08 0.2 (0.12−0.33)	−0.004 (−0.03 to 0.02)	—	**0.04 (0.007−0.06)**	**0.04**
C16OH	0.04 ± 0.02 0.04 (0.02−0.06)	−0.003 (−0.009 to 0.003)	—	0.005 (−0.0009 to 0.01)	0.14
C16:1OH	0.07 ± 0.03 0.06 (0.04−0.1)	**0.01 (0.002−0.02)**	—	−0.006 (−0.01 to 0.002)	**0.02**

Table 3. continued

Acylcarnitines	Concentration (μmol/L)	Mean difference (Δ) in concentration relative to AGA (μmol/L or in % for skewed variables)			
	Total *N*: 6131	LBW *N*: 36	AGA *N*: 6058	EM *N*: 37	*p* value[a]
C18	1.1 ± 0.3 1.0 (0.7−1.5)	0.01 (−0.1 to 0.12)	—	0.04 (−0.07 to 0.15)	0.79
C18:1	1.5 ± 0.4 1.4 (1.0−2.1)	**0.29 (0.14−0.43)**	—	0.04 (−0.10 to 0.18)	**0.0005**[e]
C18:2	0.17 ± 0.08 0.16 (0.09−0.28)	**0.15 (0.12−0.18)**	—	−0.02 (−0.04 to 0.01)	**<0.0001**[e]
C18OH	0.02 ± 0.01 0.02 (0.01−0.04)	−0.0007 (−0.005 to 0.003)	—	0.0003 (−0.004 to 0.004)	0.94
C18:1OH	0.03 ± 0.01 0.03 (0.02−0.05)	0.005 (0.00004−0.009)	—	−0.003 (−0.007 to 0.002)	0.07
C18:2OH	0.03 ± 0.01 0.02 (0.01−0.04)	0.005 (0.0002−0.009)	—	−0.002 (−0.006 to 0.002)	0.08

The AGA group is used as a reference. The differences presented are adjusted for gestational age, gender of newborn and age of mother. Significant differences are shown in bold letters.
AGA appropriate-for-gestational age (birth weight between 10th and 90th percentile in Icelandic population according to gender and gestational age), *LBW* low birth weight (birth weight < 2500 g), *EM* extreme macrosomia (birth weight ≥ 5000 g). *C0* free carnitine, *C2* acetylcarnitine, *C3* propionylcarnitine, *C4* butanoylcarnitine, *C4OH* hydroxybutyrylcarnitine, *C5* isovalerylcarnitine, *C5:1* tiglylcarnitine, *C5OH* hydroxyisovalerylcarnitine, *C6* hexanoylcarnitine, *C3DC* malonylcarnitine, *C4DC* succinylcarnitine, *C5DC* glutarylcarnitine, *C6DC* methylglutarylcarnitine, *C8* octanoylcarnitine, *C8:1* octenoylcarnitine, *C10* decanoylcarnitine, *C10:1* decenoylcarnitine, *C10:2* decadienoylcarnitine, *C12* dodecanoylcarnitine, *C12:1* dodecenoylcarnitine, *C14* tetradecanoylcarnitine, *C14:1* tetradecenoylcarnitine, *C14:2* tetradecadienoylcarnitine, *C14OH* 3-hydroxytetradecanoylcarnitine, *C16* hexadecanoylcarnitine, *C16:1* hexadecenoylcarnitine, *C16OH* hydroxyhexadecanoylcarnitine, *C16:1OH* 3-hydroxyhexadecenoycarnitine, *C18* octadecanoylcarnitine, *C18:1* oleylcarnitine, *C18:2* linoleoylcarnitine, *C18OH* hydroxyoctadecanoylcarnitine, *C18:1OH* 3-OH-oleylcarnitine, *C18:2OH* 3-OH-linoleoylcarnitine.
[a]*p* value from comparison across birth weight groups, with AGA as a reference, using ANOVA F test.
[b]Mean ± SD (all such values).
[c]Median (10th–90th percentile) (all such values).
[d]Mean difference in μmol/L with 95%CI (all such values).
[e]Significant after accounting for false discovery rate ($a = 0.05$) using the Benjamini–Hochberg procedure.
[f]Mean difference in % with 95%CI, for skewed variables (all such values).

glucose.[34,35] Why threonine was increased in those born LBW is more difficult to explain, but might be due to a fewer possible metabolic pathways in catabolism than for many of the other amino acids.

EM newborns, with their excess of adipose tissue,[18,19] probably use more triacylglycerol from adipose tissue to form glycerol and fatty acids, which could explain the observed increase in C2, C3, and C4OH. Glycerol can be used in liver gluconeogenesis[34] and fatty acids are released for energy metabolism in the liver, creating ketones at the same time.[35] Glucose and ketone bodies are particularly important in the newborn period as the brain, which is proportionally much larger in newborns than in older children and adults, is dependent on glucose and ketone bodies as a source of energy.

All newborns in our material were mainly in a catabolic state, which is normal for 3–4-day-old neonates adapting to extrauterine life. They were being fed regularly, but not yet receiving sufficient amounts of additional feeding to reach an anabolic state. Over the first week the nutritional intake of a newborn increases gradually and most have reached an anabolic state after their first week of life. This applies also to infants who get supplementary feeding with infant formula milk.

As previously mentioned, both AGA and EM newborns were almost exclusively breastfed from birth, while LBW newborns had routine complimentary feeding with formula. This could in part explain the increase in long-chain acylcarnitines seen in LBW newborns, since most formula milk is rich in long-chain fatty acids. It is also rich in free carnitine, which might explain the increase in C0 among the LBW newborns.

It is somewhat difficult to compare our results to other studies in the newborn period regarding amino acids and acylcarnitines from newborn screening because of the variety of designs among the few studies available. It is important to take into account confounding factors such as the time of blood sampling and that preterm newborns have an immature metabolism. Even though Kadakia et al. took many of the major factors into account, they were looking at cord blood values which may have been affected by both maternal and placental metabolism. This has to be taken into account since amino acids move across the placenta from the mother to the fetus by active transport.[36] Gucciardi et al.[5] reported a correlation between birth weight and acylcarnitine concentrations, both in dried blood spot tests and plasma, but did not adjust their analysis for gestational age when investigating the correlations between birth weight and acylcarnitine concentrations. Since gestational age and birth weight are strongly correlated, it is difficult to compare to our results.

Ryckman et al.[4] and Yang et al.[6] reported changes in BCAA and AAA, as seen in older obese children and adults, as well as adults with type 2 diabetes and insulin resistance.[7–10] Our analyses did not show higher levels of BCAA or AAA in EM or LBW compared to AGA newborns, but our study material excluded preterm newborns, as well as taking gestational age and the time of sampling into account. There is, however, a possibility that differences in BCAA and AAA were not detected due to the small size of the subgroups in our material. There were some similarities regarding changes in acylcarnitine values. These consisted most often of an increase in C2, C3, observed in both LBW and LGA newborns[6,37] with an additional correlation to newborn adiposity regarding C2, C3, C4DC/Ci4DC, and C4OH.[12] Both macrosomic and SGA neonates have been shown to have an increased risk for obesity and metabolic syndrome later in life[24–26,38,39] and studies point in the direction that they differ somewhat in their metabolomic profiling. This may be due to early metabolic adaptive changes and could be dependent on epigenetic factors.

In conclusion, our study showed distinctive differences in the metabolomic profile of LBW and EM neonates born at term compared to term AGA newborns. There is a need for further understanding the dynamics of neonatal metabolic and nutritional adaptation, considering the nutrition given. Our study contributes to the understanding on factors affecting the results of newborn screening. Furthermore, the study provides information on newborn metabolism and shows that being at either end of the birth weight range gives its mark in the first days of life.

ACKNOWLEDGEMENTS

H.V. has received a doctoral grant from the University of Iceland Doctoral Grants Fund. Other authors have no financial support to disclose.

AUTHOR CONTRIBUTIONS

All authors have met the *Pediatric Research* authorship requirements.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-1033-0) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

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