Extreme macrosomia

– birth outcome for mother and infant, and metabolomic profile

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Thesis for the degree of Philosophiae Doctor

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Miklir þungburar

- fylgikvillar fæðingar fyrir móður og barn, og mynstur efnaskiptamerkja

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"Don't let anyone rob you of your imagination, your creativity, or your curiosity."

Mae Jemison, physician and astronaut

Ágrip

Markmið: Að kanna áhættuþætti fyrir fæðingu mikilla þungbura (fæðingarþyngd ≥5000 g) og hugsanlegar afleiðingar þess fyrir móður og barn (grein 1). Að meta efnaskipti mikilla þungbura með því að skoða mynstur þeirra amínósýra og acylkarnitína sem mæld eru í nýburaskimun og bera saman við börn með eðlilega fæðingarþyngd og börn með lága fæðingarþyngd (<2500 g) (grein II). Jafnframt að meta hvaða áhrif fæðingarmáti hefur á efnaskipti barns við fæðingu með því að mæla magn ofangreindra þátta í naflastrengsblóði og við nýburaskimun þegar barnið er 2-3 daga gamalt (grein III).

Aðferðir: Fyrsti hluti verkefnisins var ferilrannsókn með öllum fæðingum mikilla þungbura sem fæddust á árunum 1996-2005. Fyrir hvern þungbura voru valin til samanburðar tvö fullburða börn sem höfðu eðlilega fæðingarþyngd (10.-90.‰ í íslensku þýði). Annar hlutinn var gagnagrunnsrannsókn byggð á niðurstöðum úr íslensku nýburaskimuninni með fullbura einburum fæddum á Íslandi á árunum 2009-2012. Þrír hópar með mismunandi fæðingarþyngd voru bornir saman: lág fæðingarþyngd (<2500 g), eðlileg fæðingarþyngd miðað við meðgöngulengd (10.-90.‰) og miklir þungburar (≥5000 g). Þriðji hluti verkefnisins var framskyggn rannsókn á börnum fæddum með eðlilegri fæðingu eða valkeisaraskurði og mæðrum þeirra frá nóvember 2013 til apríl 2014. Blóð frá móður fyrir fæðingu, naflastrengsblóð nýbura við fæðingu og niðurstöður nýburaskimunar voru notuð til að bera saman amínósýrur og acýlkarnitín milli fæðingarmáta.

Niðurstöður: 343 miklir þungburar fæddust á árunum 1996-2005 eða 0,9% allra einbura (grein I). Mæður þeirra voru líklegri til að vera með hærri líkamsþyngdarstuðul og þyndaraukning þeirra á meðgöngu var marktækt hærri en mæðra barna með eðlilega fæðingarþyngd. Einnig var framköllun fæðingar ólíklegri til að takast hjá þeim og bráðakeisaraskurðir voru líklegri, en ekki valkeisaraskurðir. Miklir þungburar voru líklegri til að lenda í axlarklemmu í fæðingu og fá fæðingaráverka, en ekki að verða fyrir fósturköfnun. Minni háttar fæðingargallar og efnaskiptatruflanir voru algengari hjá miklum þungburum.

Í öðrum hluta verkefnisins (grein II), höfðu bæði nýburar með lága fæðingarþyngd og miklir þungburar hærri gildi af glútamínsýru en börn með eðlilega fæðingarþyngd miðað við meðgöngulengd. Þá var amínósýran þreónín hærri hjá börnum með lága fæðingarþyngd. Frítt karnitín og sum af meðal- og langra-keðju acýlkarnitínum voru hærri hjá börnum með lága

fæðingarþyngd. Hýdroxýbútýrýlkarnitín var lægra hjá börnum með lága fæðingarþyngd en hærra hjá miklum þungburum. Acetýlkarnitín var hærra hjá börnum með lága fæðingarþyngd og miklum þungburum.

Í samanburði við nýbura sem fæddir voru með valkeisaraskurði (grein III) voru nýburar fæddir á eðlilegan máta með hærri meðaltalsmun fyrir alanín, lysín, fenýlalanín, prólín, valín, samanlögð acýlkarnitín, própíónýlkarnitín, súkkinýlkarnitín, tetradekanóýlkarnitín, hexadekanóýlkarnitín, oktadekanóýlkarnitín og óleýlkarnitín við fæðingu. Enginn marktækur munur var á amínósýrum í blóðsýni móður og barns við nýburaskimun eftir leiðréttingu fyrir fjölda tölfræðilegra prófanna. Amínósýrur og minni stuttkeðju acýlkarnitín voru, óháð fæðingarmáta, hærri í naflastrengsblóði nýbura en blóði móður, að frátöldum arginínósúkkiniksýru, asparssýru og cítrúllini. Amínósýrur og acýlkarnitín voru hærri við nýburaskimun en í naflastrengssýnum í báðum fæðingarmátahópum, að frátöldum alaníni, arginíni, fenýlalaníni, þreóníni, valíni, fríu karnitíni og línóleóýlkarnitíni.

Ályktanir: Hár líkamsþyngdarstuðull og of mikil þyngdaraukning móður á meðgöngu auka áhættu á fæðingu mikilla þungbura. Í slíkum tilfellum er aukin hætta á fylgikvillum í fæðingu, bæði hjá móður og barni. Það virðist munur á efnaskiptum mikilla þungbura og barna með eðlilega fæðingarþyngd, sem kemur fram sem munur á mynstri þeirra amínósýra og acýlkarnitína sem mæld eru við nýburaskimun. Sá munur er ólíkur því mynstri sem kemur fram við samanburð fullburða barna með lága fæðingarþyngd miðað við eðlilega. Fæðingarmáti virðist tengjast mynstri þessara efna í blóði barna við fæðingu, þar sem börn sem fæðast með eðlilegri fæðingu hafa hærra gildi margra amínósýra og acýlkarnitína en börn fædd með valkeisaraskurði. Í nýburaskimuninni er þessi munur lítill sem enginn, sem bendir til þess að fæðingarmáti hafi ekki langvarandi áhrif.

Lykilorð:

Acylkarnitín, amínósýra, fæðingaráverki, fæðingarmáti, miklir þungburar.

Abstract

Aims: To investigate risk factors for extreme macrosomia (birth weight ≥5000 g) and to assess associated morbidities of extreme macrosomia for both mother and neonate during gestation and birth (paper I). To compare the metabolomic profile of extremely macrosomic babies in newborn screening to term babies born with a normal birth weight for gestational age and those born with low birth weight (paper II). Furthermore, to estimate the association between mode of delivery and amino acids and acylcarnitines in cord blood at birth and at the time of newborn screening (paper III).

Methods: The first part was a cohort study including all births of extremely macrosomic babies in 1996-2005 (exposed) and a comparison cohort with babies born at term with birth weight between the 10.-90. percentile for the Icelandic population (non-exposed), identified in the national birth register. The second part was a register study based on the Icelandic Newborn Screening program and included full term singletons born in Iceland from 2009 to 2012. We compared the metabolomic profile of babies born with low birth weight (<2500 g), appropriate-for-gestational age and extreme macrosomia (≥5000 g). The third part was a prospective study including normal vaginal births and elective cesarean sections from November 2013 to April 2014 in Iceland. Maternal blood samples before birth, neonatal cord blood samples at birth and newborn screening results were used to compare amino acids and acylcarnitines by delivery mode.

Results: There were 343 macrosomic babies born in 1996-2005 or 0.9% of all singletons (paper I). Compared to the normal birth weight cohort, mothers of extremely macrosomic newborns were more likely to be categorized with a higher body-mass index at the 20th week of gestation and to have experienced higher weight gain during pregnancy. Compared to the normal birth weight cohort, extremely macrosomic babies were at increased odds of shoulder dystocia, delivery by emergency cesarean section and failed labour induction, but there was not increased risk of delivery by elective section. Minor congenital malformations were more frequent among extremely macrosomic babies, as were birth injuries and minor metabolic disturbances, but not birth asphyxia.

In the second part of the project (paper II), both neonates born with low birth weight and extreme macrosomia were associated with higher levels of

glutamic acid compared to those with weight appropriate-for-gestational age. The amino acid threonine was 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.

Compared to neonates born by elective cesarean section, neonates born vaginally (paper III) had higher levels of alanine, lysine, phenylalanine, proline, valine, total acylcarnitines, propionylcarnitine, succinylcarnitine, tetradecanoylcarnitine, hexadecanoylcarnitine, octadecanoylcarnitine and oleylcarnitine at birth. Maternal blood samples and newborn screening results showed no differences after correction for multiple testing. For both delivery modes, amino acids and smaller short-chain acylcarnitines were higher in cord blood compared to maternal blood, with the exception of argininosuccinic acid, aspartic acid and citrulline. Amino acids and acylcarnitines were higher at the time of newborn screening compared to cord blood for both delivery modes, except for alanine, arginine, phenylalanine, threonine, valine, free carnitine and linoleoylcarnitine.

Conclusions: High maternal body-mass index and excess gestational weight gain increase the risk of extreme macrosomia. Extreme macrosomia carries increased risks for birth complications, both for the mother and her offspring. There are distinctive differences in the metabolomic profile of extremely macrosomic neonates, newborns with normal birth weight and those born at term with low birth weight. Furthermore, there are distinct differences in metabolomic profile between newborns across delivery modes, in cord blood, where neonates born by vaginal delivery show transiently higher values of many amino acids and acylcarnitines than neonates born by elective cesarean section. At the time of newborn screening there were small, if any, differences. This suggests that any influence of delivery mode on the neonate‘s metabolomic profile is not a lasting phenomenon.

Keywords:

Acylcarnitine, amino acid, birth mode, birth trauma, extreme macrosomia.

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Furthermore, I would like to thank Atli Dagbjartsson, who along with Reynir started the first part which this thesis is built on and Hildur Harðardóttir for her participation and guidance in the first part. Special thanks to Leifur Franzson for his contribution to parts two and three, based on his expertise in newborn screening program. He also convinced Dr. Piero Rinaldo to let us collaborate with CLIR, and who also shared his expertise in the field of inborn errors of metabolism.

To the midwives working in the Women´s Clinic at Landspitali University Hospital, who helped with recruiting women and collected the samples for the third part of this project, I would like to give a special thanks. Furthermore, I would like to give special thanks to the staff at the Newborn Screening Program in Iceland who analyzed the samples from the mothers and the newborns after birth for the third part of this project.

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Contents

List of abbreviations

AAA	Aromatic amino acids
AGA	Appropriate-for-gestational age
BCAA	Branched chain amino acids
BMI	Body-mass index (kg/m2)
CI	Confidence interval
CLIR	Collaborative Laboratory Integrated Reports (CLIR, https://clir.mayo.edu)
DBS	Dried blood spot paper
ECS	Elective cesarean section
EM	Extreme macrosomia (≥5000 g)
ICD-9	International Statistical Classification of Diseases and Related Health Problems, 9th edition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th edition
IQR	Interquartile range
LBW	Low birth weight (<2500 g)
LGA	Large-for-gestational age
min-max	Minimum-maximum
NBS	Newborn Screening Program
n.s.	not significant
PI	Ponderal Index (kg/m^3)
SD	Standard deviation
SGA	Small-for-gestational age
VD	Vaginal delivery
Δ	Mean difference

Acylcarnitines:

C0: Free carnitine, C2: Acetylcarnitine, C3: Propionylcarnitine, C3DC: Malonylcarnitine, C4: Butanoylcarnitine, C4DC: Succinylcarnitine, C4OH: Hydroxybutyrylcarnitine, C5: Isovalerylcarnitine, C5DC: Glutarylcarnitine, C5OH: Hydroxyisovalerylcarnitine, C5:1: Tiglylcarnitine, C6: Hexanoylcarnitine, C6DC: Methylglutarylcarnitine, C8: Octanoylcarnitine, C8:1: Octenoylcarnitine, C10: Decanoylcarnitine, C10:1: Decenoylcarnitine, C10:2: Decadienoylcarnitine, C12: Dodecanoylcarnitine, C12:1: Dodecenoylcarnitine, C14: Tetradecanoylcarnitine, C14OH: 3-hydroxytetradecanoylcarnitine, C14:1: Tetradecenoylcarnitine, C14:2: Tetradecadienoylcarnitine, C16: Hexadecanoylcarnitine, C16OH: Hydroxyhexadecanoylcarnitine, C16:1: Hexadecenoylcarnitine, C16:1OH: 3-hydroxyhexadecenoycarnitine, C18: Octadecanoylcarnitine, C18OH: Hydroxyoctadecanoylcarnitine, C18:1: Oleylcarnitine, C18:1OH: 3-OH-oleylcarnitine, C18:2: Linoleoylcarnitine, C18:2OH: 3-OH-linoleoylcarnitine.

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. Vidarsdottir H, Geirsson RT, Hardardottir H, Valdimarsdottir U, Dagbjartsson A. Obstetric and neonatal risks among extremely macrosomic babies and their mothers. American Journal of Obstetrics and Gynecology. 2011; 204(5): 423.e1-6.

II. Vidarsdottir H, Thorkelsson Th, Halldorsson Th, Bjarnason R, Geirsson RT, Rinaldo P, Franzson L. Does metabolomic profile differ with regard to birth weight? Pediatric Research. 2020 Jun 29. doi: 10.1038/s41390-020-1033-0. Online ahead of print.

III. Harpa Vidarsdottir, Thorhallur Ingi Halldorsson, Reynir Tomas Geirsson, Ragnar Bjarnason, Leifur Franzson, Unnur Anna Valdimarsdottir, Thordur Thorkelsson. Mode of delivery was associated with transient changes in the metabolomic profile of neonates. Manuscript accepted for publication in Acta Paediatrica.

Declaration of contribution

Paper I: I participated in the planning of the project together with Professor Reynir T. Geirsson in collaboration with Assoc. Professor Atli Dagbjartsson and Professor Unnur A. Valdimarsdóttir. I applied for the ethics approvals and for funding of the project with the co-authors. I collected the data, got clinical advices from Reynir, Hildur Harðardóttir and Atli and did the analysis under the guidance of Unnur. I drafted the paper with the guidance of Reynir and participated in the revision process with Reynir with the approval of the co-authors.

Paper II: I planned the project together with the co-authors. I applied for the ethics approvals and for funding with assistance from the co-authors. I received the raw data from the Newborn Screening program in Iceland (NBS) from Leifur Franzson, at the Department of Genetics and Molecular Medicine at Landspitali. I analysed the data from the newborn screening program under the guidance of Professor Þórhallur Ingi Halldórsson. I wrote the manuscript and handled the revision process with the assistance and approval of the co-authors.

Paper III: I planned the project together with the co-authors and applied for the ethics approvals. I recruited the women with the help of the labor ward midwives at the Women's Clinic at Landspitali and collected background data from the medical records and the hospital´s main biochemical laboratory on NBS. I received the results from the measurements on maternal and neonatal cord blood samples and results from the NBS from Leifur Franzson and the staff at the Department of Genetics and Molecular Medicine at Landspitali. I analyzed the data with the guidance of Þórhallur. I wrote the manuscript and coordinated the revision process with the co-authors.

1 Introduction

1.1 Birth weight

The first publications of birth weight were reported in the 17th century. However, recognition of the importance of birth weight was not apparent until the 18th century (Cone, 1961). Interest in fetal growth and development has since only increased, where the main focus of research has mostly been on low birth weight (LBW), rather than high birth weight. However, in the last four decades with increasing obesity worldwide the birth weight has increased considerably and there is an increasing trend for babies to be born with a high birth weight (Alberman, 1991; Henriksen, 2008; Koyanagi et al., 2013). This has led to an increased interest on the short- and long-term morbidity and mortality associated with high birth weight.

1.1.1 The definition of birth weight categories

The World Health Organization has defined birth weight by different categories ("ICD-11," 2018). Appropriate-for-gestational age (AGA) is defined as birth weight between the 10th to 90th percentiles for a given population, corrected for gestational age. Similarly, small-for-gestational age (SGA) is <10th percentile and large-for-gestational age (LGA) is >90th percentile. However, LGA is also often defined as birth weight between 4000 g and 4499 g at birth regardless of gestational age. Birth weight of more than 4000 g or 4500 g has also been referred to as macrosomia ("Practice Bulletin No. 173: Fetal Macrosomia," 2016) and extreme macrosomia (EM) is generally defined as birth weight ≥5000 g (Alsunnari et al., 2005; Anoon et al., 2003), or as WHO defines it now as the “exceptionally large newborn” ("ICD-11," 2018). On the lower end of the birth weight scale there is LBW, defined as birth weight between 1500 g and 2499 g, with very low birth weight (VLBW) between 1000 g and 1499 g and finally extremely low birth weight (ELBW) with birth weight under 999 g ("ICD-11," 2018). These are all categories defined by weight, regardless of gestational age and gender.

Birth weight categories, both low and high, have been shown to be related to increased short- and long-term morbidity risks and mortality (Class et al., 2014; Cnattingius et al., 2012; Kramer, 1987). Birth weight categories are much used in clinical settings to help to decide on mode of delivery, levels of observation and treatment, both before and during delivery and in the neonatal period.

1.1.2 What determines birth weight?

Birth weight is primarily determined genetically but modified by many regulating factors, including fetal hormones, multiple functions of the placenta, maternal hormones and maternal nutritional status both before and during pregnancy (*Textbook of Perinatal Medicine*, 2006). It is vital that these regulating factors can interact in complex ways to ensure the optimal growth and development of the fetus to give in order to give it the best chance of surviving the birth and continue life outside the protective environment of the womb.

Insulin is the primary growth hormone produced by the fetus and even though growth hormone (GH) is also expressed it does not regulate fetal growth, but it becomes an important growth regulator later in childhood and up to adulthood (Murphy et al., 2006; *Textbook of Perinatal Medicine*, 2006). The somatomedins, insulin-like growth factor I and II (IGF I and II), produced by the fetus are also important regulators of fetal growth. Although the role of somatomedins is not fully understood they seem to promote cell growth, cell differentiation and influence transport of glucose and amino acid over the placenta (Murphy et al., 2006; *Textbook of Perinatal Medicine*, 2006).

The placenta produces hormones and secretes these into the maternal and fetal circulation, as well as hormones to promote placental implantation and growth, all of which are essential for optimal fetal growth (Murphy et al., 2006). The placenta seems to receive and transmit endocrine signals between the mother and the fetus to ensure that the fetus gets what is needed and the fetus is protected from unwanted factors. Thereby optimal fetal growth at the right time is promoted (Costa, 2016; Murphy et al., 2006). By mediating metabolic changes in the mother (human placental GH (HPGH) and human placental lactogen (HPL) being most prominent), and by increasing blood flow to the uterus through the action of multiple hormones (human chorionic gonadotropin (HCG), progesteron and estrogens), the placenta ensures a constant flow of nutrients to the fetus throughout the whole pregnancy (Costa, 2016; Murphy et al., 2006). The placenta also produces proteins that are favorable for fetal growth, such as pregnancy-associated plasma protein A (PAPP-A) and placental protein 13 (PP-13) although their mechanisms of action are not fully understood (Costa, 2016; *Textbook of Perinatal Medicine*, 2006). The placenta secretes HPL into the fetal circulation stimulating the production of insulin and IGF-1 by the fetus, thereby in turn stimulating fetal growth (Costa, 2016).

The placenta is also a metabolically active organ which uses considerable amounts of glucose for its own metabolism and transport of both glucose,

amino acids and essential fatty acids from the mother to the fetus (Battaglia & Regnault, 2001; Cetin et al., 2005; Herrera & Ortega-Senovilla, 2014; Murphy et al., 2006). Glucose is transported by diffusion transport proteins of the GLUT family that are highly correlated to the maternal glucose concentration. On the other hand, amino acids and essential fatty acids are transported via active transport by placental transport proteins. More is known about the placental transport of amino acids than essential fatty acids, though it seems in both cases that the transport mechanism is mostly independent of concentrations in maternal blood, except in extreme cases of severe starvation or malnutrition (Battaglia & Regnault, 2001; Cetin et al., 2005; Herrera & Ortega-Senovilla, 2014; Murphy et al., 2006). Furthermore, it seems that the placenta is capable of producing some non-essential amino acids to meet the demands of the fetus (Battaglia & Regnault, 2001; Holm et al., 2017). There are indications that the placenta can modulate the length of fatty acid chains, since there are proportionally higher amounts of long-chain polyunsaturated fatty acids in the fetal than in the maternal circulation, though fatty acids in general are in lower concentrations in the fetal circulation (Cetin et al., 2009; Herrera & Ortega-Senovilla, 2014).

Changes in maternal hormones influencing fetal growth are partly mediated by placental hormones and seem to have the main purpose of ensuring a favorable flow of nutrition and oxygen to the fetus along with promoting changes needed for lactogenesis (Costa, 2016; Murphy et al., 2006). Glucose is the most important substrate for energy metabolism in the fetus and placenta (Cetin et al., 2005; Herrera & Ortega-Senovilla, 2014; Murphy et al., 2006; Şengül & Dede, 2014), sparing amino acids and fatty acids for fetal growth and development. Gluconeogenesis and ketogenesis are not active in the fetus, - illustrating how dependent the fetus is on maternal glucose supply, but both can become active in extreme cases, such as severe maternal starvation (Herrera & Amusquivar, 2000; S. Kalhan & Parimi, 2000). A mixture of placental hormones, primarily IGF II, HPGH and HPL, mediate hyperinsulinemia and increased insulin sensitivity in the mother resulting in increased synthesis and storage of triacylglycerides (TAG) as fat deposits in the mother (Costa, 2016; Herrera & Ortega-Senovilla, 2014; Murphy et al., 2006; Stanley et al., 1998). During gestation the accumulation of fat deposits slows down. In the last trimester this starts to decrease and lipolysis is promoted resulting in hyperlipidemia (Herrera & Ortega-Senovilla, 2014). This is due to changes in the dynamic mixture of placental hormones (HPL, HPGH and estrogens), which promote increased peripheral insulin resistance and lipolysis during the latter half of pregnancy (Herrera & Ortega-Senovilla, 2014;

Murphy et al., 2006; Stanley et al., 1998). This results in an increased use of fatty acids and glycerol for maternal energy metabolism and keton body utilization, providing the increased maternal glucose production for the placenta and fetus (Butte, 2000; Herrera & Ortega-Senovilla, 2014; Murphy et al., 2006). Furthermore, there is an increase in essential fatty acids and long-chain polyunsaturated fatty acids in the maternal cirulation, allowing increased transport across the placenta to the fetus for growth and development along with amino acids (Cetin et al., 2009; Herrera & Amusquivar, 2000; Şengül & Dede, 2014). In the last trimester the fetus starts to accumulate glycogen and build up fat deposits, preparing for extrauterine life. These changes are believed to be primarily from glycogenesis and lipogenesis in the fetal liver, using glucose from the maternal circulation and possibly non-essential fatty acids (Herrera & Amusquivar, 2000; Hillman et al., 2012; Williams, 1997).

Since the human fetus also needs various nutrients to ensure successful fetal growth and development, there are more factors that are important than fat, amino acids and glucose. It has been reported that not only is adequate nutrition during pregnancy of importance, but also the maternal pre-pregnancy nutritional status where amino acids and iron appear to be of great importance (Cetin et al., 2005; Manta-Vogli et al., 2018; Picciano, 2003). If some nutritional factors are missing from early pregnancy it can leave it´s marks on fetal growth and fetal development.

Even though fetal growth is primarily controlled by genes, it is a complex process with multiple factors involving the mother, the placenta and the fetus itself, all of which must interact. This process is not fully understood as it is dynamic and changes during the course of pregnancy in response to the different needs of the fetus throughout gestation.

1.1.3 The risks of becoming and being extremely macrosomic?

Known risk factors for fetal macrosomia include maternal obesity and pre-existing or gestational diabetes, while both maternal and paternal stature also affect birth weight through genetic mechanisms (Clausen et al., 2005; Dai et al., 2018; Schaefer-Graf et al., 2005). Mild glucose and lipid metabolic disturbances are of importance along with excessive gestational weight gain and excessive interpregnancy weight gain of the mother (Bonomo et al., 2005; Clausen et al., 2005; Crosby et al., 2017; Thorsdottir et al., 2002).

Macrosomic babies have a higher risk of neonatal death, stillbirth and birth injuries, such as cervical plexus injuries and various neonatal complications such as hypoglycemia and respiratory problems (Beta et al., 2019; Bjorstad et

al., 2010; Boulet et al., 2004; Oral et al., 2001; Zhang et al., 2008). Induced labour is more often required, with a higher chance of cesarean section, high likelihoods of serious pelvic floor damage, hemorrhage at or after delivery and prolonged recovery after the birth (Beta et al., 2019; Bjorstad et al., 2010; Boulet et al., 2004; Casey et al., 2005; Stones et al., 1993).

A previous study on 111 births of extremely macrosomic infants showed that only 44% were born by vaginal delivery (VD) and in those instances a fifth were complicated by shoulder dystocia and 6% developed Erb‘s palsy (Alsunnari et al., 2005). A more recent study on 182 births of extremely macrosomic infants showed that 66% were born vaginally whereof 14.2% were complicated with shoulder dystocia and 2.5% developed Erb’s palsy (Hehir et al., 2015). A smaller case-control study with 47 cases showed similar results (Anoon et al., 2003). Furthermore, children born macrosomia have a doubled chance for prolonged neonatal unit care at and above a 5000 g birth weight (Gillean et al., 2005). However, most studies on macrosomic newborns have concerned babies with birth weights >4000 g or >4500 g (Boulet et al., 2004). Much less is known about EM, even though the maternal and fetal risks among these deliveries can be expexted to be highest (Boulet et al., 2004; Gillean et al., 2005; Zhang et al., 2008). When such babies have been suspected before birth it is not straightforward to choose the appropriate time and mode of delivery (Boulet et al., 2004; "Practice Bulletin No. 173: Fetal Macrosomia," 2016).

Macrosomic newborns have a higher body fat percentage and mass than those with birth weight within a normal range (Hammami et al., 2001; Lee et al., 2012). High birth weight is moreover an independent risk factor for childhood obesity, while women born LGA are more likely to have an LGA-baby of their own (Cnattingius et al., 2012; Kain et al., 2009; Sparano et al., 2013). The highest risk of LGA births has, however, been noted among mothers with a high body mass index and who were themselves born SGA (Cnattingius et al., 2012).

In summary there are constantly increased risks for the mother and child associated with the birth of an extremely macrosomic neonate, and this knowledge has in turn led to an increased use of induction of delivery around term when a macrosomic baby is suspected or alternately an elective cesarean section (ECS) is resorted to and vaginal delivery deliberately avoided. Making a birth plan does, however, not change a possible increased risk of congenital abnormalities, nor the risk for hypoglycemia after birth, or the potential risks of increased morbidity and mortality later in life that associate with a high birth

weight. Both diabetes and obesity are treatable risk factors in later life, even if they are the consequences of EM that develops during intrauterine life. This makes it even more important to study extremely macrosomic neonates and their epidemiological conditioning in order to find the best optimal prevention for morbidity in later life.

1.2 Energy metabolism at birth and the first days after

Birth is a remarkable process. Maternal and fetal preparation before and under labour to ensure the survival of the newborn is as fascinating as it is complex. The changes required to adapt during birth and immediately thereafter include multiple factors that affect most organ systems that need to function together to give the newborn the best chance of survival outside the womb (Hillman et al., 2012; Morton & Brodsky, 2016).

As mentioned previously, the metabolism of the fetus is primarily an anabolic state, to build and grow (Bloomfield et al., 2013; Cetin et al., 2005). In the last trimester the preparation begins by building up glycogen and fat storage but the fetal liver at term contains proportionally three times more glycogen than the adult liver (Herrera & Amusquivar, 2000; Hillman et al., 2012; Morton & Brodsky, 2016; Rao et al., 2013; Williams, 1997). At birth the metabolism needs to switch from an anabolic to a catabolic state to get the baby through the birth and the first days after delivery, while the maternal lactogenesis is commencing and the newborn gastrointestinal tract adapting for handling the amounts of new nutrient needed (Herrera & Amusquivar, 2000; Hillman et al., 2012; Morton & Brodsky, 2016; Rao et al., 2013; Williams, 1997).

Initiation of birth is again a complex mechanism with complicated interactions required between maternal, placental and fetal factors, and is far from being fully understood (Vannuccini et al., 2016). However, studies have shown that stress hormones, such as catecholamines and cortisol, favor the metabolic adaptation that is needed for survival during birth and the days of life (Hillman et al., 2012; Lagercrantz & Slotkin, 1986; Morton & Brodsky, 2016; Williams, 1997). Catecholamines stimulate glycogenolysis, the mobilization of glucogenic and ketogenic amino acids into the circulation, lipolysis and fatty acid oxidation and seem to induce the fetal ability for gluconeogenesis and ketogenesis (Herrera & Amusquivar, 2000; Hillman et al., 2012; S. Kalhan & Parimi, 2000; Lagercrantz & Slotkin, 1986; Morton & Brodsky, 2016; Rao et al., 2013; Williams, 1997). It seems that gluconeogenesis is active 4-6 hours after birth (S. C. Kalhan et al., 2001).

After birth and during the first days postpartum, the rapid drop in maternal progesteron and estrogen levels stops the inhibitition of prolactin so the second stage of lactogenesis is initiated (Sriraman, 2017). Untill that is fulfilled the newborn gets colostrum from the mother, a product of the breast glands which is small in amount but rich in nutrients with a different composition from mature breast milk, i.e. it is richer in proteins and poorer in fat and lactose (Gidrewicz & Fenton, 2014; Sriraman, 2017). Both colostrum and mature breast milk promote ketogenesis in the newborn, assisting the newborn by supplying alternative substrates for energy metabolism. This is particulary favorable for the brain as that organ can use ketone bodies as an energy substrate, necessary not least because the brain is proportionally much larger in newborns than older children and adults.

1.3 Newborn screening

1.3.1 Purpose and a brief history of neonatal screening programs

The Newborn Screening Program is a screening program used to detect inborn errors of metabolism, i.e. a genetically determined and large group of diseases in metabolic pathways where irreversible organ damage, or in worst cases death, can be the first symptoms. If diagnosed early, however, many of these conditions are preventable and/or treatable, preferably before symptoms of damage are seen (Almannai et al., 2016; Saudubray & Garcia-Cazorla, 2018). Newborn screening is now well established in most middle- and high-resource countries and this has significantly improved the outcome of children with disease suitable for screening (Almannai et al., 2016).

The history of newborn screening started in the early 1960´s when Dr. Robert Guthrie and Ada Susi published their method on detecting phenylketonuria in dried blood drops on filter paper in large populations of newborns (Guthrie & Susi, 1963; King & Hammarström, 2018). This initial screening was extended to congenital hypothyroidism and other metabolic diseases, including maple syrup urine disease and homocysteinuria, and in the 1980´s the introduction of fluorimetric assays changed the screening method (Almannai et al., 2016; King & Hammarström, 2018). In the 1990´s the introduction of using the methods of tandem mass spectrometry (MS/MS) has further revolutionized the screening by enabling measurements of multiple analytes at the same time (King & Hammarström, 2018; Millington et al., 1990). Since then, there has been both an increase in the number of diseases screened, in 2016 up to 30, and number of countries that have established the method in all inhabitated continents, even though there is considerable

variability of how many diseases/conditions a country or even regions within the same country are screening for (Villoria et al., 2016)

The Newborn Screening Program in Iceland started in 1972 with screening for phenylkentonuria, but has since then developed and today screens for 46 diseases including fatty acid oxidation defects, organic acid disorders, urea cycle defects and endocrine disorders such as hypothyroidism and congenital adrenal hypoplasia.

1.3.2 The method of neonatal screening and what to expect

Blood is taken from a newborn baby, usually by a midwife or an other caregiver, at 48-72 hours after birth and dropped on to filter paper. This is called a dried blood spot test (DBS) and in Iceland it is practiced to take the sample with a heel prick test. When the blood has dried the filter paper is sent to the laboratory, in Iceland to the Landspitali University Hospital where all samples are analysed for the whole population. Background information on the newborns is documented and sent with the test to the laboratory were it is documented into a database for the results from the newborn screening. The variables are gender, gestational weeks attained, date and time of birth, date and time of sampling and birth weight.

The blood samples are analyzed using the method of tandem mass spectrometry, where samples are prepared. For example in the case of amino acids and acylcarnitines they are extracted from the filter paper using methanol and then isotopically-labelled amino acids, or acylcarnitine standards are used for quantification of the individual amino acids and acylcarnitines (Chace, 2001). When a test is positive, the newbornwill be brought in for further testing and confirmation of diagnosis.

During the metabolic and nutritional adaptation of a newborn in the first days after birth, the energy metabolism is using glycogenolysis, proteolysis and lipolysis to ensure substrates for gluconeogenesis, ketogenesis and fatty acid oxidation (Herrera & Amusquivar, 2000; S. Kalhan & Parimi, 2000; Rao et al., 2013; Williams, 1997). These conditions are optimal for diagnosing defects in those pathways, as many of the diseases connected to inborn errors of metabolism (IEM) will then be evident. However, many factors have been shown to affect the results of the screening program with increases in false positive results, such as preterm birth, age of the newborn at the time of sampling or mode of nutrition (Clark et al., 2014; Gucciardi et al., 2015; Ryckman et al., 2013). Many of these factors are difficult to control for, but timing is considered vital and can be controlled for. Therefore, timing the

newborn screening correctly and in line with the reference values used, is essential.

1.3.3 Previous studies on amino acids and acylcarnitines measured with mass spectrometry

Apart from studies reporting what affects the results of newborn screening, there have been studies done to measure amino acids, acylcarnitines or both in conditions were there are known metabolic disturbances present, such as in childhood and adult obesity, in individuals with diabetes and prediabetes and pregnant women.

Correlations between amino acids and acylcarnitines and obesity, prediabetes and type 2 diabetes have been studied in various populations. Increases in branched-chain amino acids (BCAA) and the aromatic amino acids (AAA), glycine, glutamic acid and some acylcarnitines (mostly acetylcarnitine (C2), propionylcarnitine (C3) and isovalerylcarnitine (C5)), have been reported in obese compared to non-obese children (Butte et al., 2015; Zhao et al., 2016). Positive correlations between the same amino acids and type 2 diabetes/prediabetes compared to those with normal glucose tolerance have been reported in adults (Guasch-Ferré et al., 2016; Menni et al., 2013). 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 (Guasch-Ferré et al., 2016; Menni et al., 2013). C2, C4OH, octenoylcarnitine (C8:1) and oleylcarnitine (C18:1) have also been reported to be higher in obese compared to non-obese pregnant women (Donovan et al., 2016). Kadakia et al 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 (Kadakia et al., 2018). Wright and Baker reported in their study on newborn screening results in Colorado that macrosomic newborns (>4000g in their study) had elevations in C2, C3, dicarboxylic, and long-chain acylcarnitines (specifically C16 and C18 species), compared to normal birth weight (2500-3999 g) (Wright & Baker, 2020). Furthermore, their results showed that C3 and C18:1 were two to three times more likely to be above the predetermined state cut-offs in macrosomic compared to non-macrosomic neonates.

It is complex how these substances interact at and after birth. There is a continuing need to cast light on what affects them and what not. The base of

our metabolomic studies is set in what was previously noted as specific when comparing differences between normal babies and those born exceptionally heavy. In this way the studies link to constitute this thesis.

2 Aims

Paper I: To investigate risk factors for extremely macrosomic fetuses/neonates and to estimate the morbidity for mother and neonate.

Hypothesis 1: There are increased risks for both the fetus/neonate and the mother for adverse outcomes which are connected to the birth of extremely macrosomic neonates compared to normal weight neonates.

Paper II: To evaluate metabolomic profiles of multiple amino acids and acylcarnitines measured in the newborn screening program, with a particular emphasis on the extremes of birth weight.

Hypothesis 2: Neonates born at the extremes in birth weight differ in their metabolomic profiles as measured in the newborn screening program.

Paper III: To estimate the effects of mode of delivery on amino acids and acylcarnitines in cord blood and to evaluate if and how those effects are preserved at the time of the newborn screening.

Hypothesis 3: Mode of delivery affects the metabolomic markers in cord blood. At the time of sampling in the newborn screening program these differences are minimal.

3 Materials and methods

3.1 Study design and cohorts

3.1.1 Obstetric and neonatal risks among extremely macrosomic babies and their mothers (paper I)

This first study was a retrospective, register-based cohort study including all singleton babies with birth weight ≥5000 g in Iceland from 1996 through 2005 (exposed cohort). A comparison cohort with birth weight between the 10th - 90th percentiles according to gestational weeks and gender (defined as normal birth weight) by Icelandic neonatal birth weight standards (Biering et al., 1985) was selected at a ratio of 1:2 exposed as the comparison cohort. Neonates in the comparison cohort were individually matched with the exposed births for a) calendar time (date), i.e. the comparison cohort neonates were born immediately before and after the index babies, b) the same gestational age (±14 days), c) gender and d) maternal parity (primi- and multiparity). If these conditions were fulfilled, the comparison births were matched as closely as possible for e) place of birth.

The exposed cohort was identified from the files of the Icelandic Medical Birth Registration (IMBR) and verified by inspection of the individual birth notification forms. Each set of the relevant notes for all mothers, including the neonatal records and discharge summaries, were inspected by one of the authors (HV) and verified as appropriate (RTG and HH for maternal, AD for neonatal data). The ICD-9 system was used up to and through 1996 and ICD-10 thereafter. Additional population information was obtained from Statistics Iceland (national statistical bureau).

3.1.2 Does metabolomic profile differ with regard to birth weight? (paper II)

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 University Hospital in Reykjavik. From the database results of acylcarnitine and amino acid measurements were extracted for the study purpose, as was additional background information.

The study period extended from 1st January 2009, to 31st December 2012. Included were all singletons born at full term (≥37 weeks gestation) in Iceland.

Only full-term neonates were included in order to minimize the influence of gestational age as a potential confounding factor, and thus also avoid possible effects from the use of antibiotics or the need for parenteral nutrition. Neonates with incomplete data were excluded.

Most newborns in Iceland are exclusively breastfed (86% during the first week of life in the years 2004-2008 (Sigurbjornsdottir & Gunnarsdottir, 2012)). 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.

The material was divided into three groups based on birth weight: LBW (<2500 g), AGA (birth weight between the Icelandic 10th – 90th newborn population percentiles by gender and gestational age (Biering et al., 1985)) and EM (≥5000 g). Neonates with birth weight <2500 g and meeting the AGA criteria were excluded. Neonates not meeting the criteria for birth weight groups were also excluded.

3.1.3 Mode of delivery was associated with transient changes in the metabolomic profile of neonates (paper III)

This was a prospective study of 115 births done at the Women‘s Clinic at Landspitali University Hospital. Neonatal blood samples were collected by the delivering midwife on a DBS from the umbilical cord immediately after delivery by letting the blood drop on the filter paper directly from the cord, giving a mixture of arterial and venous blood. Maternal blood was obtained at the time when a peripheral intravenous cannula was placed, also by letting the blood drop directly on the filter paper, both for mothers delivering vaginally and those undergoing elective cesarean section. In most cases of normal vaginal deliveries, no intravenous access was needed. Mothers delivering vaginally were well into the delivery process by the time an intravenous cannula was inserted, with an unknown nutritional status, while mothers having elective cesarean section were in the preparatory phase before the operation with the sample taken after at least six hours of fasting. The neonatal and maternal samples were sent to the laboratory to be handled according to procedures of the Newborn Screening Program system for Iceland. The samples were analysed for a range of amino acid and acylcarnitine substances. When the newborn screening was done on the newborn at 48-72 hours after birth, as the national guidlines at that time recommended, the midwife registered the weight

of the neonate. Additional information was collected from maternity records.

The study period was from first of November 2013 to 30th of April 2014 with the goal to include at least 30 ECS and at least 90 normal vaginal births. Excluded were woman with pre-eclampsia/hypertension, non-singleton births, births were birth defects were suspected, births were the newborn had an Apgar score of ≤6 after five minutes, needed antibiotics, parenteral nutrition, glucose infusion or other care in the neonatal intensive care unit. All emergency cesarean sections were excluded.

The material was devided into two groups by delivery mode, i.e. normal VD and ECS. Furthermore, the three time points of sampling (mother, umbilical cord and newborn screening) were compared between the modes of delivery.

3.2 Variables and measurements

3.2.1 Obstetric and neonatal risks among extremely macrosomic babies and their mothers (paper I)

Variables for the mother and baby were collected from the IMBR, from individual birth notification forms and in a few cases from the medical records of the newborn.

Maternal variables were height and weight at mid-pregnancy (as near to 20 weeks gestation as possible), gestational weight gain, estimated birth weight (clinical and ultrasound), maternal age at birth (≤20, 21-25, 26-30, 31-35 and ≥36 years), smoking, complications during the pregnancy or labor. Body-mass index (BMI) at mid-pregnancy was categorized into four groups (<19, 19-24, 25-30, >30). BMI was also estimated at the end of pregnancy as well as BMI increase. Mean symphysis-fundal height and mean in-patient stay after delivery were recorded. The beginning of labour was also recorded (spontaneous, induction, failed induction). Maternal complications were categorized into 11 groups: infections, gestational hypertension, musculoskeletal complications and exhaustion in pregnancy, gestational and pre-existing diabetes mellitus, other pregnancy-related disease, complications during delivery (not elsewhere specified), instrumental delivery, pelvic floor trauma, elective or emergency cesarean section (defined as section at <8 hours from decision).

Neonatal variables included weight, length and head circumference, gestational age, complications during delivery and ponderal index (PI). Neonatal diagnoses were categorized into nine groups: infections, congenital malformations, birth trauma, shoulder dystocia, asphyxia, breathing difficulties,

metabolic disturbances including hypoglycemia, hematologic complications and other.

3.2.2 Metabolomics studies (paper II and III)

In paper II only neonatal blood samples taken 72–96 hours after birth were used as this was the nationally recommended time of initial sampling for neonatal screening during the study period. For reducing the risk of repeated sampling, the sample period was limited to this 24-hour period. The additional background information from the newborn screening database used for paper II were the maternal date of birth, offspring birth weight, gestational age, gender, time of birth and time of sampling. From the dates of birth of the mother and the newborn maternal age was calculated. From the respective times of birth and of sampling the age of the newborn at sample time was calculated in hours. Variables used in paper II and III from the NBS are listed in Table 1. The combined concentrations of leucin, isoleucin and hydroxyproline were only used in paper III.

Table 1. Amino acids and acylcarnitines in papers II and III.

Amino acids	**Acylcarnitines**	
Alanine	Free carnitine (C0)	Tetradecanoylcarnitine (C14)
Arginine	Acetylcarnitine (C2))	3-hydroxytetradecanoyl-carnitine (C14OH)
Argininosuccinic acid	Propionylcarnitine (C3)	Tetradecenoylcarnitine (C14:1)
Aspartic acid	Malonylcarnitine (C3DC)	Tetradecadienoylcarnitine (C14:2)
Citrulline	Butanoylcarnitine (C4)	Hexadecanoylcarnitine (C16)
Phenylalanine	Succinylcarnitine (C4DC)	Hydroxyhexadecanoylcarnitine (C16OH)
Glutamic acid	Hydroxybutyrylcarnitine (C4OH)	Hexadecenoylcarnitine (C16:1)

Glycine	Isovalerylcarnitine (C5)	3-hydroxyhexade-cenoycarnitine (C16:1OH)
Histidine	Glutarylcarnitine (C5DC)	Octadecanoylcarnitine (C18)
Leucin, isoleucine, hydroxyproline*	Hydroxyisovalerylcarnitine (C5OH)	Hydroxyoctadecanoylcarnitine (C18OH)
Lysine	Tiglylcarnitine (C5:1)	Oleylcarnitine (C18:1)
Methionine	Hexanoylcarnitine (C6)	3-OH-oleylcarnitine (C18:1OH)
Methylhistidine	Methylglutarylcarnitine (C6DC)	Linoleoylcarnitine (C18:2)
Ornithine	Octanoylcarnitine (C8)	3-OH-linoleoylcarnitine (C18:2OH)
Proline	Octenoylcarnitine (C8:1)	
Serine	Decanoylcarnitine (C10)	
Threonine	Decenoylcarnitine (C10:1)	
Tryptophan	Decadienoylcarnitine (C10:2)	
Tyrosine	Dodecanoylcarnitine (C12)	
Valine	Dodecenoylcarnitine (C12:1)	

*Only in paper III.

The additional backround information items collected for paper III were neonatal gender, birth weight and gestational length, parity, mode of delivery, Apgar scores at one and five minutes and weight of the newborn at the time of the NBS. Maternal age, newborn age in hours at the time of NBS and the weight change from birth to NBS was calculated.

3.3 Statistical analysis

3.3.1 Obstetric and neonatal risks among extremely macrosomic babies and their mothers (paper I)

The Statistical Package for the Social Sciences (SPSS for Windows rel. 12.0.1., Chicago, USA) was used for digital recording of the data and statistical analyses. Descriptive analysis was used to describe maternal, birth and offspring characteristics. Multivariate logistic regression analysis was used to examine association between exposed and non-exposed cohort using odds ratios (OR) with 95% confidence intervals (95%CI). Paired samples t-test were performed, with significance set a level of $p<0.05$ if multivariable regression was not appropriate. All data were processed in SPSS except for body mass index and PI which were calculated using Microsoft Excel software.

3.3.2 Does metabolomic profile differ with regard to birth weight? (paper II)

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 continuous variables. Due to skewed distribution of amino acid and acylcarnitine medians and the 10th to 90th percentiles were also reported. The median, interquartile range (IQR), mininum and maximum values were used for descriptive statistics for birth weight, both for total and by birth weight groups. Percentages were used to describe dichotomous variables.

The distribution of the study measurements of amino acids and acylcarnitines were compared 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 (McHugh et al., 2011). 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 (Bahn, 2017; McHugh et al., 2011). Over the years it has grown to include millions of newborns from across the world. The data were uploaded into CLIR and used multiples of median boxplots of covariate-unadjusted data were used for comparison purposes between EM, AGA and LBW.

For examining associations between concentrations of amino acids and acylcarnetines across birth weight groups multivariate linear regression analysis was used. As a measure of association the F-test comparing differences across the groups was used. In our multivariate models we adjusted for maternal age, newborn gender and gestational age, under the null hypothesis that all three groups had the same mean response. For skewed variables linear regression models adjusted for the same factors were used, but with logarithmic transformation. As a measure of association the F-test was

used comparing differences across EM, AGA and LBW newborns under the same null hypothesis. A t-test was used for comparing differences between two-groups. Levels of significance were set at α = 5% and all tests were two-sided. Chi-squared tests were used for dichotomous variables and statistical significance with α set at 5%.

Due to the number of comparisons performed for amino acids and acylcarnitines the Benjamini–Hochberg procedure was used to account for false discovery rates, set at α = 0.05 (Thissen et al., 2002). Statistical analyses were done in the RStudio® (RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com).

3.3.3 Mode of delivery was associated with transient changes in the metabolomic profile of neonates (paper III)

Descriptive statistics were used to describe characteristics of study participants and concentrations of amino acids and acylcarnitines. Mean values and SD were calculated for all variables except for gestational age, weight loss ratio from birth to newborn screening and Apgar scores after one and five minutes, where medians and IQRs were used. Medians and 10th - 90th percentiles were established for all amino acid and acylcarnitine biomarkers. Percentages were used to describe dichotomous variables such as parity and neonatal gender. To assess potential differences between the two groups, we used the t-test for variables which were normally distributed, while the Mann-Whitney test was used for skewed variables. The chi-square test was used to test for differences between groups for dichotomous variables.

Concentrations of amino acids and acylcarnitines were compared between those born by VD and those born by ECS for each of the three sample types collected (i.e. maternal, cord blood and NBS samples). Relative differences in concentrations by mode of delivery, using elective cesarean section as reference, were examined using linear regression analyses adjusting for different sets of covariates used in the regression model for different types of collected samples. The covariates were selected from known factors affecting newborn screening results as well as those known factors connected to a more stressful delivery. Weight change from birth to NBS was used in the regression models for NBS samples reflecting the neonatal nutritional status at the time of NBS. To which extent the statistically significant covariates in the regression model impact on the biological markers was not a topic of the present study. For maternal samples the covariates were gestational age, birth weight, gender, maternal age and parity. For cord blood samples the covariates were

gestational age, birth weight, gender, age of mother, parity, and Apgar scores assessed at one and five minutes. For NBS samples the covariates were gestational age, birth weight, gender, age of mother, parity, Apgar scores assessed at one and five minutes, weight change and age in hours of the neonate from birth to NBS. All continuous covariates were entered as linear terms in the regression model. All outcomes variables were log-transformed due to right-skewed distributions and for each analysis normality of model residuals were inspected visually with Q–Q plots. These inspections did not reveal any obvious outliers and it was concluded that the model residuals did not deviate from normality. Significant results were presented in figures as a mean change in percentages with ECS as a reference (all results presented in the Tables 13,14 and 15).

Differences in concentrations of amino acids and acylcarnitines in the maternal and cord blood were presented using the ratio of concentrations in the two samples, maternal/cord blood. These ratios were compared by mode of delivery using linear regression analysis adjusted for gestational age, birth weight, gender, age of mother, parity, Apgar scores at one and five minutes. The same comparison was made for the ratio between cord blood samples and NBS, NBS/cord blood, after adjusting for gestational age, birth weight, gender, age of mother, parity, Apgar scores at one and five minute, weight change and age of the neonate at the time of newborn screening.

In all analyses the level of significance was set at $\alpha = 5\%$ and all tests were two-sided. Due to the number of comparisons performed for amino acids and acylcarnitines the Benjamini–Hochberg procedure was used to account for false discovery rates, set at $\alpha = 0.05$ (Thissen et al., 2002). All analyses were done in the RStudio® (RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com).

3.4 Informed consent and ethics

The Icelandic National Bioethics Committee (VSNb2006060001/03.11), relevant hospital authorities and the Icelandic Data Protection Authority (2006060365AGG) approved the study on the obstetric and neonatal outcome of extremely macrosomic babies and their mothers (paper I).

The study on results from NBS regard to birth weight (paper II) was approved by Landspitali University Hospital Ethical Committee (33/2013) and the Icelandic Data Protection Authority (2013060794HGK).

In the study on the effect of mode of delivery on the metabolomic profile on newborns after birth and their mothers (paper III) an informed consent was

collected from the mothers shortly after admission to the labor ward by the attenting midwife. All participants received a copy of the informed consent with contact information if they chose to decline participation at a later date. There were no such cases. The study was approved by the Chief Medical Officer and the Ethical Committee of Landspitali – The National University Hospital of Iceland (33/2013) and notification sent as required to the Icelandic Data Protection Authority.

4 Results

4.1 Obstetric and neonatal risks among extremely macrosomic babies and their mothers (paper I)

From 1996 through 2005 there were 41 377 deliveries in Iceland, including 40 319 singleton babies, of whom 343 were extremely macrosomic, all liveborn and constituting 0.9% of singeltons and 0.8% of all newborns. Matched deliveries of children of normal birth weight were 686. Sixteen deliveries were excluded, i.e. 10 where the mother had delivered a second baby during the study period (8 exposed and 2 non-exposed deliveries). Case records on six deliveries were not found (1 exposed, 5 non-exposed). The total mother-infant pairs analysed were 1013, i.e. 334 exposed and 679 non-exposed births.

Maternal characteristics in exposed and non-exposed births are compared in Table 2. Emergency cesarean section occured more often among exposed mothers, while VDs were fewer. There was no difference between exposed and non-exposed in ECS rates.

Table 2. Maternal characteristics in deliveries of extremely macrosomic babies and matched comparison deliveries.

	Macrosomic deliveries (n = 334)		**Normal birth weight deliveries (n = 679)**		**OR (95%CI)[a]**
	n	%	n	%	
VD	231	69.2	607	89.4	0.3 (0.2-0.4)
ECS	21	6.3	32	4.7	1.4 (0.8-2.4)
Emergency cesarean section	82	24.6	40	5.9	5.2 (3.5-7.8)
BMI <19	1	0.3	8	1.2	0.3 (0.0-2.0)
BMI 19≤ to ≥25	76	22.8	296	43.6	0.4 (0.3-0.5)
BMI 25< to ≤30	134	40.1	240	35.3	1.2 (0.9-1.6)
BMI >30	100	29.9	112	16.5	2.2 (1.6-2.9)
Nonsmokers	298	89.2	537	79.1	2.2 (1.5-3.2)
Smokers[b]	35	10.5	142	20.9	0.4 (0.3-0.7)
≤20 years	19	5.7	34	5.0	1.1 (0.6-2.0)

21-25 years	54	16.2	120	17.7	0.9 (0.6-1.3)
26-30 years	118	35.3	223	32.8	1.1 (0.8-1.5)
31-35 years	107	32.0	190	29.0	1.2 (0.9-1.6)
≥36 years	36	10.8	112	16.5	0.6 (0.4-0.9)
Gestational diabetes mellitus	8	2.4	9	1.3	1.8 (0.7-4.8)

[a]Adjusted for gender, gestational age (±14 days), parity (nulli- and multiparity).
[b]Sometime during pregnancy.

The mothers of extremely macrosomic babies were more likely to be categorized with a higher BMI than controls (Table 2). The mean BMI was 28.6 (± 5.14 SD) among exposed and 26.1 (±4.40 SD) among non-exposed mothers ($p = 0.005$) and increased more over the gestation (4.2 ± 1.57 SD vs. 3.5 ± 1.43 SD, $p = 0.03$). Exposed mothers were less likely to be smokers. Age distribution was similar between exposed and non-exposed mothers, with the only difference seen for the oldest age group, where exposed mothers were less likely to be included. There were no significant differences with regard to gestational diabetes.

The mean length of labor of exposed births was 435 minutes (min-max: 62-2030 min), but 449 minutes (min-max: 40-2305 min) for non-exposed births. There were no differences in the occurrence of prolonged first stage (exposed 20.7%, non-exposed 31.2%; OR 0.8, 95% CI 0.6-1.1) or second stage of labor (exposed 1.5%, non-exposed 2.7%; OR 0.7, 95%CI 0.2-1.9) among the mothers, but the length of the second stage was longer (mean 30 minutes; min-max: 3-188 min) for exposed mothers compared to non-exposed (21 minutes; min-max: 0-315 min, $p = 0.03$).

Deliveries among exposed mothers were less often spontaneous than among the non-exposed (Table 3). Induction of labor was more likely to fail among exposed mothers with resulting higher rates of emergency cesarean section.

Table 3. Multivariate analyses of EM and potential delivery hazards/characteristics compared to non-exposed cohort.

	Macrosomic deliveries (n=334)		Normal birth weight deliveries (n=679)		OR (95%CI)[a]
	n	%	n	%	
Spontaneous labor	216	64.7	560	82.5	0.4 (0.3-0.5)
Induction of labor	84	25.1	77	11.3	2.5 (1.8-3.6)
Failed induction of labor, cesarean section	14	4.2	7	1.0	4.3 (1.7-11.0)

[a]Adjusted for gender, gestational age (±14 days), parity (nulli- and multiparity), age at delivery of child, smoking, mothers BMI at 20th week of gestation and gestational diabetes.

Exposed mothers were significantly heavier than non-exposed with a mean weight at 20 weeks gestation of 83.5 kg (± 15.5 kg SD) compared to 73.0 kg (± 13.0 kg SD) for non-exposed ($p < 0.001$), while height was not statistically different (exposed 170.8 cm ± 5.4 cm SD, non-exposed 167.1 cm ± 5.7 cm SD). Weight gain was significantly greater for exposed mothers than non-exposed (12.2 kg ± 4.4 kg SD vs 9.7 kg ± 3.9 kg SD, $p < 0.008$). Fundal height and in-patient stay after delivery did not differ between exposed and non-exposed mothers (Table 4). Exposed mothers were, however, twice as often recorded to have had an infectious complication during pregnancy or following delivery (Table 5). The incidence of gestational hypertension, musculoskeletal disorders and other gestational diseases, pre-gestational disease and instrumental deliveries was not different between exposed and non-exposed mothers. Exposed mothers were more likely than non-exposed to have suffered trauma to pelvic structures or needed episiotomy.

Table 4. Maternal and babies characteristics of extremely macrosomic deliveries compared to non-exposed cohort. Values are presented by mean and SD.

	Macrosomic deliveries (n=334)	**Normal birth weight deliveries (n=679)**
Mean symphysis fundal height, mothers	41.1 cm (±2.3 cm)	37.7 cm (±2.1 cm)
Mean in-patient stay	5.2 days (±2.1 days)	5.0 days (±2.1 days)
PI (median), babies	2.91[a] (2.09-3.62)	2.65[a] (2.00-3.44)
Mean birth length, babies	56.4 cm (±1.7 cm)	52.1 cm (±1.7 cm)
Mean head circumference	38,2 cm (±1.1 cm)	35.9 cm (±1.1 cm)

[a]p<0.001

The birth weight of babies from exposed births ranged from 5000 g to 6340 g with a median value of 5135 g, while the median for non-exposed children was 3770 g (range 2880 - 4415 g). The PI of exposed babies was significantly larger (Table 5), but the mean length at birth was not significantly different between exposed and non-exposed babies. In Table 5 the maternal and neonatal outcomes are shown, including the almost 27-fold higher incidence of shoulder dystocia and 29-fold higher risk of Erb‘s palsy among the exposed babies.

Birth trauma was almost four times as common among exposed babies, particularly clavicular fracture (OR 3.8, 95%CI 1.5-9.1). The odds of being born with any congenital malformation was double among exposed babies (OR 2.1, 95%CI 1.2 – 3.7), where heart malformations (ICD-10 Q10-Q26) were seen in 2.1%, but 0.9% among non-exposed babies, (OR 2.6, 95%CI 0.8 – 8.4, n.s.). Minor metabolic disorders were more common among exposed babies, mostly hypoglycemia (exposed 3.9%; non-exposed 0.6%, OR 2.9, 95%CI 0.9 – 10.1, n.s.). There was no statistical difference between exposed and non-exposed babies regarding rates of asphyxia. The same was true for other birth outcomes, including head circumference, neonatal infection, respiratory and hematologic disorders.

Table 5. Multivariate analyses of maternal outcome and birth complications for mothers and babies of extremely macrosomic deliveries compared to non-exposed cohort.

	Macrosomic (n=334)		Normal birth weight (n=679)		OR (95%CI)[a]
	n	%	n	%	
Mothers					
Infections	22	6.6	16	2.4	2.3 (1.1-4.7)
High gestational blood pressure	17	5.1	27	4.0	1.0 (0.5-2.0)
Musculoskeletal complications and exhaustion in pregnancy	14	4.2	36	5.3	0.7 (0.4-1.4)
Gestational diseases	11	3.3	27	4.0	0.8 (0.4-1.6)
Pre-existing diseases	4	1.2	2	0.3	2.3 (0.3-15.3)
Various birth complications	213	63.8	249	36.7	2.1 (1.6-2.9)
Forceps or vacuum delivery	17	5.1	45	6.6	0.9 (0.5-1.6)
Trauma to pelvic structures	200	59.9	462	68.0	2.0 (1.3-3.0)
Infants					
Infections	13	3.9	22	3.2	1.2 (0.6-2.5)
Congenital malformations	26	7.8	27	4.0	2.1 (1.2-3.7)
Birth trauma	30	9.0	21	3.1	3.7 (2.1-6.8)
Shoulder dystocia	46	13.8	6	0.9	26.9 (11.1-65.1)
Erb‘s palsy	11	3.3	1	0.1	29.1 (3.7-230.6)
Asphyxia	6	1.8	11	1.6	1.0 (0.4-3.0)
Respiratory disorders	17	5.1	18	2.7	1.7 (0.8-3.4)
Metabolic disorders	18	5.4	9	1.3	2.5 (1.1-6.2)
Hematologic disorders	0	0	6	0.9	-
Other	4	1.2	11	1.6	0.9 (0.3-3.0)

[a] Adjusted for gender, gestational age (±14 days), parity (nulli- and multiparity), age at delivery of child, smoking, mode of delivery, mothers BMI at 20th week of gestation and gestational diabetes.

The mean recorded clinically estimated birth weight by a midwife before delivery was 4500 g (min-max: 3600-6000 g) among the exposed children and 3700 g (min-max: 2800-4800 g) among non-exposed children. About a quarter of exposed and non-exposed babies did not have a clinical estimate done

before delivery. Ultrasonic weight estimation was done in a few exposed pregnancies and at varible times, precluding further assessment.

4.2 Does metabolomic profile differ with regard to birth weight? (paper II)

There were a total of 18 426 live-born singletons during the study period ("Live births and late fetal deaths by sex 1951-2018,"). 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 (Figure 1). However, this is a marker not routinely covered by commercial reagent kits and consequently is measured only by four sites.

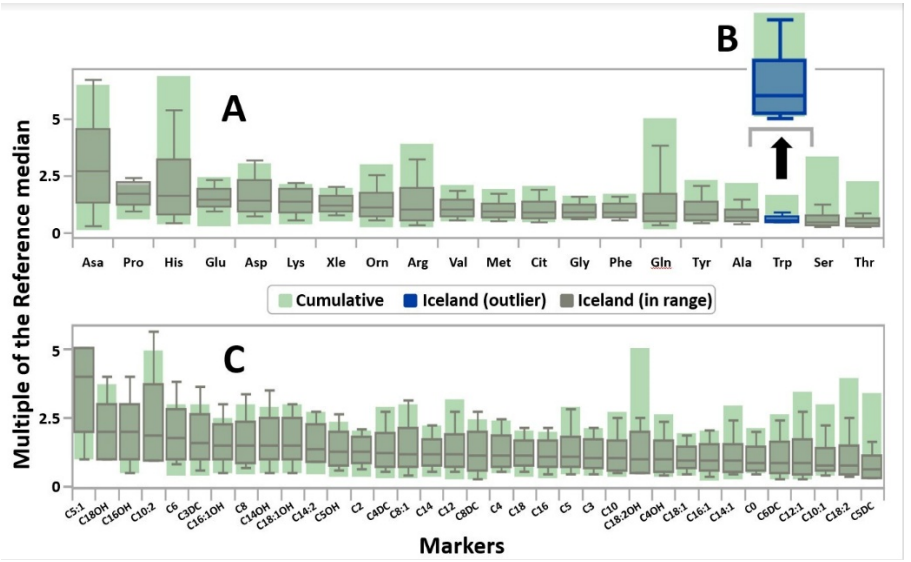

Figure 1. Comparison of the study results to the CLIR cumulative reference intervals.
CLIR productivity tool Reference Plot, an overlay of one site (Iceland) percentiles and cumulative data. Markers are plotted from left to right based on the decreasing distance between the cumulative median, shown as zero, and the location median. Panel A, amino acids; Panel 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); Panel 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.

Descriptive background information on the newborns is shown in Table 7. 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 7), with statistical significance. The EM group had a considerably higher percentage of male newborns (73%) with a 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, n.s.).

Table 6. Descriptive background information on the newborns included, total and by neonatal weight categories. Values with significant differences ($\alpha < 0.05$), with AGA as a reference, are shown in bold letters (p-value and types of test shown in footnotes).

	Total (N 6131)	**LBW (N 36)**	**AGA (N 6058)**	**EM (N 37)**
Birth weight (median, IQR, (min, max))*	3630, 515 (1724, 6775)	2335, 174 (1724, 2490)	3630, 510 (2505, 4495)	5102, 210 (5000, 6775)
Gestational age (weeks, mean ±SD)**	40.0 ±1.1	38.2 ±0.9	40.0 ±1.1	40.4 ±1.2
Maternal age (years, mean ±SD)†	30.0 ±5.3	28.3 ±6.1	30.0 ±5.3	32.4 ±5.6
Gender‡				
- Male	3137 (51%)	19 (53%)	3091 (51%)	27 (73%)
- Female	2994 (49%)	17 (47%)	2967 (49%)	10 (27%)

*Corrected for gestational weeks, ANOVA F-test: $\alpha < 0.0001$.
**ANOVA F-test: $\alpha < 0.0001$.
†ANOVA F-test: $\alpha = 0.003$.
‡Chi-square: $\alpha = 0.005$.

4.2.1 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.

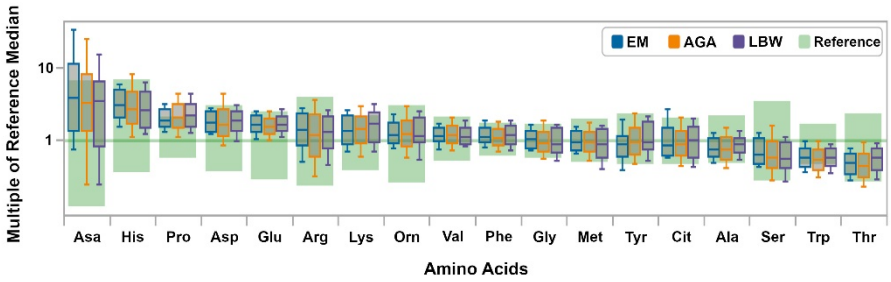

Figure 2. Amino acids compared between EM (blue), AGA (orange) and LBW (purple).
CLIR productivity tool Plot by Multiple Conditions, where birth weight groups are defined as condition. Markers are plotted by multiples of the reference median with CLIR database as reference, median shown as one with 99% 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.

When adjusting for possible confounding factors (gestational age, newborn gender and maternal age), using linear regression analyses (Table 8), 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. Both glutamic acid and threonine remained significant after correction with the Benjamini-Hochberg procedure.

Table 7. Relative differences in amino acid concentrations between birth weight groups. 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.

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

[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 percentil) (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).
[NS] or [S] Correction for multible testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as NS

and those who were significant after correction are marked with S.

4.2.2 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 C2, C4OH and hexadecenoylcarnitine (C16:1) and lower values of C4DC than seen for those AGA. Differences are notable with higher values for LBW neonates than for those who were AGA as regards C0, C8:1, C14:2, C18:2, and C18:2OH. For LBW there were lower values in C4OH and C6DC than among those AGA.

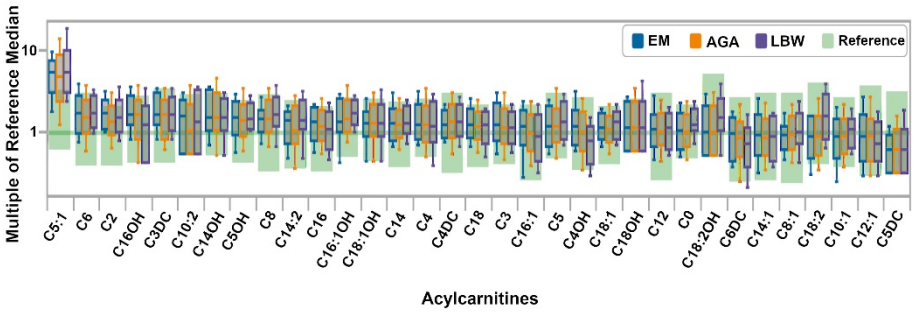

Figure 3. Acylcarnitines compared between EM (blue), AGA (orange) and LBW (purple).
CLIR productivity tool Plot by Multiple Conditions, where birth weight groups are defined as condition. Markers are plotted by multiples of the reference median with CLIR database as reference, median shown as one with 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.

After adjusting for possible confounding factors (gestational age, newborn gender and maternal age), using linear regression analyses (Table 9), the mean differences for EM neonates were higher than for 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 C0, C8:1, 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). C0, C2, C4OH, C8:1, C14:2, C18:1, C18:2 and the total of acylcarnitines remained significant after correction with the Benjamini-Hochberg procedure.

Table 8. Relative differences in acylcarnitine concentrations between birth weight groups. 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.

	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]**
C0	23 ±9 22 (14-34)[c]	**9** **(6-11)[d]**	**-**	0.5 (-2-3)	**<0.0001[S]**
C2	31 ±11 30 (20-45)	**8** **(4-11)**	**-**	**6** **(2-9)**	**<0.0001[S]**
C3	2.1 ±0.9 1.9 (1.2-3.2)	-0.06 (-0.35-0.24)	-	0.34 (0.06-0.63)	0.06
C3DC	0.08 ±0.03 0.08 (0.04-0.12)	0.004 (-0.008-0.016)	-	0.012 (-0.0002-0.024)	0.12
C4	0.31 ±0.13 0.28 (0.17-0.47)	0.01 (-0.04-0.05)	-	0.02 (-0.02-0.07)	0.53
C4DC	0.32 ±0.12 0.31 (0.19-0.48)	0.03 (-0.008-0.07)	-	**-0.05** **(-0.08- -0.006)**	**0.02[NS]**
C4OH	0.19 ±0.09 0.18 (0.1-0.31)	**-0.05** **(-0.07- -0.02)**	**-**	**0.03** **(0.001-0.06)**	**0.0009[S]**
C5	0.15 ±0.09 0.13 (0.08-0.21)	10% (-3-23%)[e]	-	0.2% (-13-13%)	0.32
C5DC	0.04 ±0.02 0.04 (0.02-0.07)	0.003 (-0.005-0.01)	-	-0.004 (-0.01-0.004)	0.40
C5OH	0.2 ±0.08 0.19 (0.12-0.31)	0.02 (-0.01-0.05)	-	0.01 (-0.01-0.04)	0.30
C5:1	0.09 ±0.05 0.08 (0.04-0.15)	15% (-3-32%)	-	2% (-15-18%)	0.25
C6	0.09 ±0.04 0.08 (0.05-0.13)	0.007 (-0.005-0.019)	-	0.008 (-0.004-0.019)	0.21
C6DC	0.07 ±0.06 0.07 (0.04-0.11)	-0.01 (-0.03-0.01)	-	0.003 (-0.02-0.02)	0.57

C8	0.1 ±0.04 0.09 (0.06-0.15)	0.01 (-0.004-0.025)	-	-0.005 (-0.019-0.009)	0.25
C8:1	0.13 ±0.06 0.12 (0.07-0.2)	**0.05** **(0.03-0.07)**	**-**	-0.006 (-0.02-0.01)	**<0.0001[S]**
C10	0.09 ±0.04 0.09 (0.05-0.14)	0.011 (-0.002-0.024)	-	0.005 (-0.008-0.017)	0.19
C10:1	0.05 ±0.02 0.05 (0.03-0.08)	0.007 (-0.0005-0.014)	-	-0.0002 (-0.007-0.007)	0.19
C10:2	0.03 ±0.01 0.02 (0.01-0.04)	0.004 (-0.001-0.008)	-	0.002 (-0.003-0.007)	0.22
C12	0.13 ±0.05 0.12 (0.08-0.2)	0.002 (-0.01-0.02)	-	0.004 (-0.01-0.02)	0.85
C12:1	0.06 ±0.03 0.06 (0.03-0.10)	-0.007 (-0.017-0.004)	-	0.006 (-0.005-0.016)	0.29
C14	0.29 ±0.09 0.27 (0.18-0.40)	0.03 (-0.001-0.06)	-	0.01 (-0.02-0.04)	0.12
C14OH	0.04 ±0.02 0.03 (0.02-0.06)	-0.006 (-0.01-0.00003)	-	0.001 (-0.005-0.007)	0.14
C14:1	0.11 ±0.05 0.1 (0.06-0.17)	0.00005 (-0.02-0.02)	-	0.01 (-0.001-0.03)	0.20
C14:2	0.04 ±0.02 0.03 (0.02-0.06)	**26%** **(10-41%)**	**-**	3% (-12-17%)	**0.004[S]**
C16	3.6 ±1.2 3.4 (2.2-5.2)	-0.07 (-0.5-0.3)	-	0.2 (-0.2-0.6)	0.54
C16OH	0.04 ±0.02 0.04 (0.02-0.06)	-0.003 (-0.009-0.003)	-	0.005 (-0.0009-0.01)	0.14
C16:1	0.22 ±0.08 0.2 (0.12-0.33)	-0.004 (-0.03-0.02)	**-**	**0.04** **(0.007-0.06)**	**0.04[NS]**
C16:1OH	0.07 ±0.03 0.06 (0.04-0.1)	**0.01** **(0.002-0.02)**	**-**	-0.006 (-0.01-0.002)	**0.02[NS]**
C18	1.1 ±0.3 1.0 (0.7-1.5)	0.01 (-0.1-0.12)	-	0.04 (-0.07-0.15)	0.79
C18OH	0.02 ±0.01 0.02 (0.01-0.04)	-0.0007 (-0.005-0.003)	-	0.0003 (-0.004-0.004)	0.94
C18:1	1.5 ±0.4 1.4 (1.0-2.1)	**0.29** **(0.14-0.43)**	**-**	0.04 (-0.10-0.18)	**0.0005[S]**
C18:1OH	0.03 ±0.01 0.03 (0.02-0.05)	0.005 (0.00004-0.009)	-	-0.003 (-0.007-0.002)	0.07
C18:2	0.17 ±0.08 0.16 (0.09-0.28)	**0.15** **(0.12-0.18)**	**-**	-0.02 (-0.04-0.01)	**<0.0001[S]**
C18:2OH	0.03 ±0.01 0.02 (0.01-0.04)	0.005 (0.0002-0.009)	-	-0.002 (-0.006-0.002)	0.08
Total	57 ±19b 54 (37-82)	**16** **(10-23)**	-	**7** **(0.7-13)**	**<0.0001[S]**

[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 percentil) (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).
[NS] or [S] Correction for multible testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as NS and those who were significant after correction are marked with S.

4.3 Mode of delivery was associated with transient changes in the metabolomic profile of neonates (paper III)

Births in Iceland under the study period were 2064 ("Live births and late fetal deaths by sex 1951-2018," 2019) and around 75% of all births at that time were at the Women's Clinic at Landspitali. The estimated number of births during the study period at Landspitali was therefore approximately 1550. A total of 115 births were included, 83 VD and 32 ECS. One cord blood sample obtained from an ECS could not be analyzed. Maternal blood was collected from 11 mothers (of 83, 13%) having VD, and from 26 mothers (81%) of those having ECS. The median and 10th – 90th percentiles for all amino acids and acylcarnitines are shown in Tables 13 (maternal), 14 (cord blood) and 15 (NBS).

Maternal and newborn characterisitcs and birth outcomes are shown in Table 10. Mothers who had an ECS tended to be older, had attained fewer gestational weeks and were multiparous in comparison to mothers delivering vaginally. Birth weight was not different between modes of delivery, but babies born by ECS had a greater weight loss from birth until NBS.

Table 9. Descriptive background information on the births included, total and by mode of delivery. Values with significant differences are shown in bold letters.

	All (N 115)	**VD (N 83)**	**ECS (N 32)**	**p-value***
Age of mother (mean, SD)	30.8 years, 5.2y	**30.1y, 5.0y**	**33.0y, 5.5y**	**0.02**
Birth weight (g, mean, SD)	3676g, 442g	3707g, 440g	3599g, 442g	0.24
Newborn age at time of NBS (mean, SD)	63.5 hours, 14.7h	63.2 hours, 16.5h	64.3 hours, 9.0h	0.74
Newborn weight at time of NBS (g, mean, SD)	3418g, 401g	**3480g, 391g**	**3274g, 395g**	**0.03**
Weight loss ratio at time of NBS (%, mean, (IQR))	6% (3%)	**5%, (4%)**	**7%, (4%)**	**0.02**
Gestational Age (median, (IQR))	39 weeks + 5 days, (1w+4d)	**40w+1d, (1w+4d)**	**39w+1d, (0w+3d)**	**<0.0001**

Apgar 1 min (median, (IQR))	9, (1)	9, (1)	9, (1)	0.27
Apgar 5 min (median, (IQR))	10, (1)	10, (1)	10, (1)	0.43
Parity of mother				
- nullipara	35 (30%)	31 (37%)[a]	4 (12,5%)[b]	
- multipara	80 (70%)	52 (63%)	28 (87,5%)	
Gender of newborn[c]				
- Male	52 (45%)	39 (47%)	13 (41%)	
- Female	63 (55%)	44 (53%)	19 (59%)	

*P-value from comparison across delivery method, using ECS as a reference.
[a]Chi-square: p = 0.02.
[b]Chi-square: p < 0.0001.
[c]Chi-square: p = 0.69.

Figure 4 shows the mean difference in maternal samples among women with VD relative to those who give birth by ECS. Overall, there were no significant differences for most amino acids and acylcarnitines. Those who gave birth through VD had, however, lower concentrations of aspartic acid (mean difference (Δ) -30%, 95%CI -47%; -8%, p = 0.02), histidine (Δ: -25%, 95%CI -42%; -4%, p = 0.03) and lysine (Δ: -18%, 95%CI -31%; -4%, p = 0.03). The corresponding mean difference for C5 was -40% (95%CI -62%; -5%, p = 0.04). These differences were not significant when correcting for multiple testing using the Benjamini-Hochberg procedure.

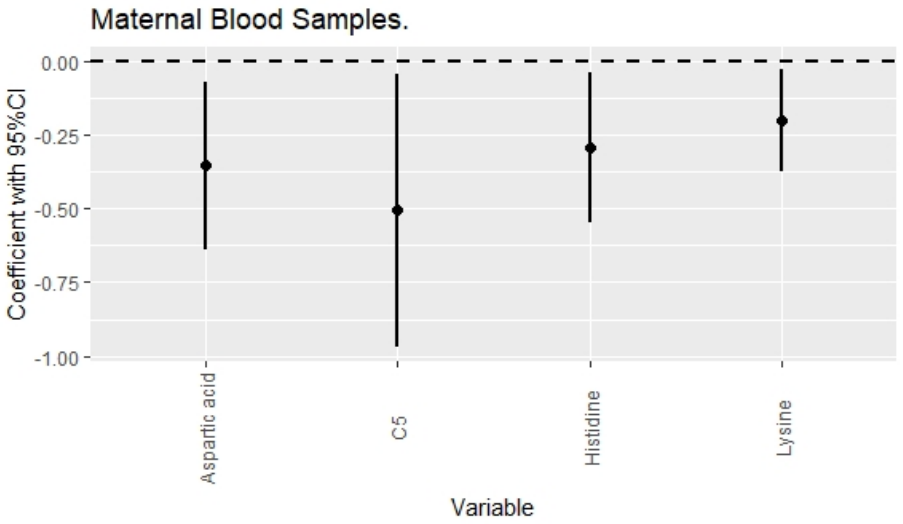

Figure 4. Differences in metabolomic profile in mothers across mode of delivery. Adjusted mean differences (in %) in amino acid and acylcarnitine concentrations with 95% CI among women giving birth with VD compared to ECS (N 37, 11 VD and 26 ECS). The mean difference was adjusted for gestational age, birth weight, neonatal gender, age of mother and parity. Of the 21 amino acids and 35 carnitines examined only those who reached statistical significance are presented (see otherwise Table 13). None remained significant after adjustment for multiple comparisons using the Benjamini-Hochberg procedure.

The mean difference in the cord blood samples across modes of delivery for various amino acids and acylcarnitines is shown in Figure 5. Amino acids and acylcarnities were increased in newborns born by VD compared to those born by ECS, where alanine (Δ: 27%, 95% CI 13%; 42%, p = 0.0001) and proline (Δ: 26%, 95% CI 13%; 41%, p = 0.0001) showed the highest increase in amino acids. The highest mean difference among the acylcarnitines was for C3, with a mean difference of 48% (95% CI 23; 79%, p < 0.0001). The changes for five of eight significant amino acids and seven of 14 acylcarnitines kept their significance after the Benjamini-Hochberg correction.

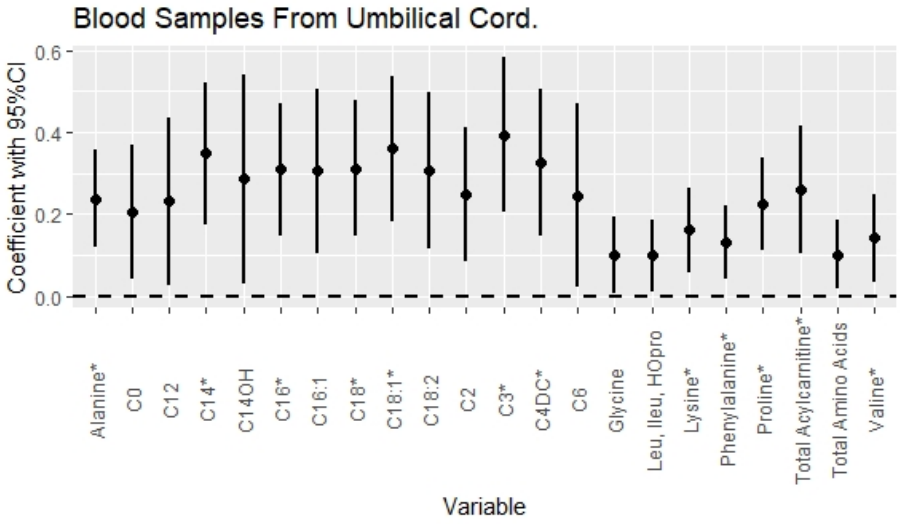

Figure 5. Differences in metabolomic profile in newborn directly after birth across mode of delivery.
Adjusted mean differences (in %) in amino acids and acylcarnitines concentrations with 95% CI among newborn born by VD compared to ECS (N 114, 83 VD and 31 ECS). The mean difference was adjusted for gestational age, birth weight, neonatal gender, age of mother, parity and Apgar score at one and five minutes. Of the 21 amino acids and 35 carnitines examined only those who reached statistical significance are presented (see otherwise Table 14). Those marked with * remained significant after correction for multiple comparisons using the Benjamini-Hochberg procedure.

In Figure 6, the mean differences between NBS samples from VD and ECS are shown. Of the amino acids, only histidine had a significant mean difference of 28% (95% CI 3%; 60%, p = 0.03) after VD compared to neonates born by ECS. Six of the measured acylcarnitines had a lower mean difference in neonates born after VD as compared to ECS, where the greatest decrease was for C10:1 (Δ: -33% with 95% CI -50%; -10%, p = 0.01). None were significant after multiple testing correction (Benjamini-Hochberg procedure).

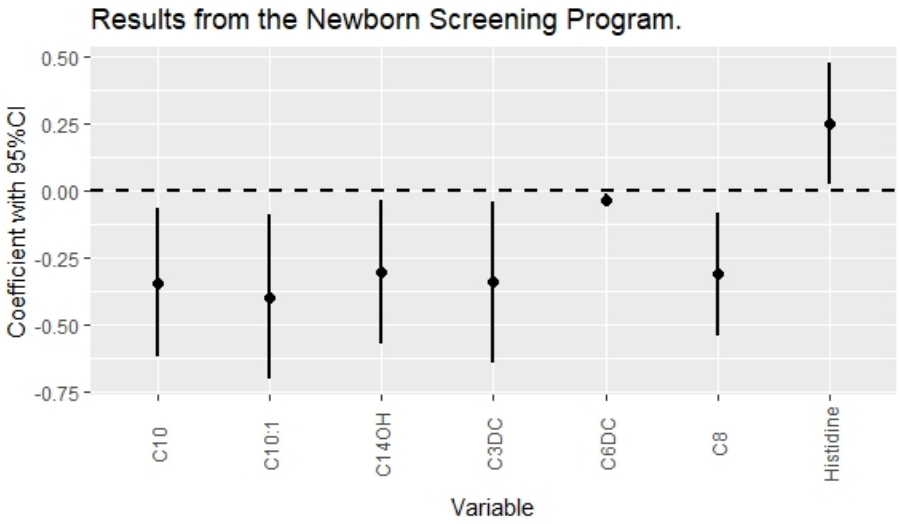

Figure 6. Differences in metabolomic profile in results from the NBS, across mode of delivery.
Adjusted mean differences (in %) in amino acids and acylcarnitines concentrations from the NBS with 95% CI among newborns born by VD compared to ECS (N 115, 83 VD and 32 ECS). The mean difference was adjusted for gestational age, birth weight, neonatal gender, age of mother, parity, Apgar score at one and five minutes, weight change and age in hours of newborn from birth to newborn screening. Of the 21 amino acids and 35 carnitines examined only those who reached statistical significance are presented (see otherwise Table 15). None remained significant after adjustment for multiple comparisons using the Benjamini-Hochberg procedure.

Ratios between maternal and cord blood samples at birth (maternal/cord blood) are shown in Figure 7A for amino acids and Figure 8A for acylcarnitines. Shown as well is the comparison across delivery modes, where ECS is set as a reference and adjusted for gestational age, birth weight, newborn gender, maternal age, parity and Apgar scores at one and five minutes.

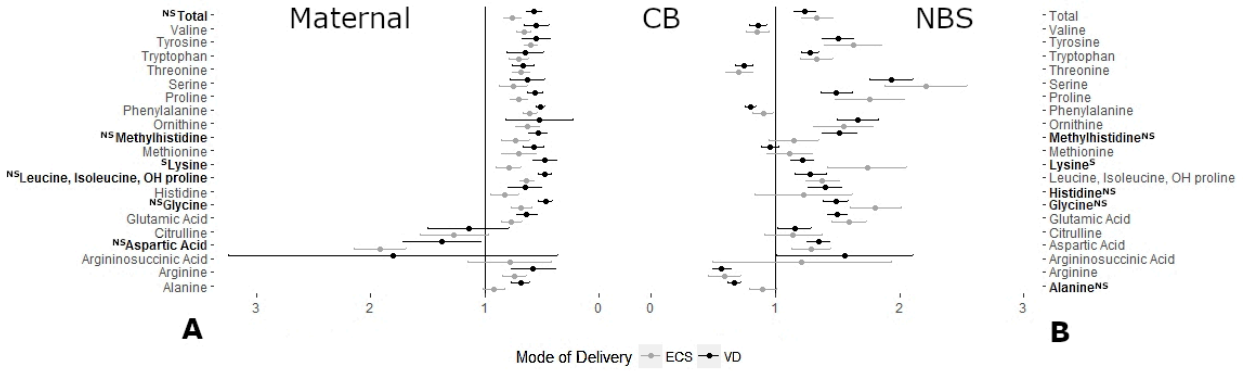

Figure 7. Differences between sample groups for amino acid concentrations shown as ratio of mean values on maternal vs cord blood (A) and NBS and cord blood (B), across modes of delivery.
Ratio of mean values on amino acids between maternal blood and cord blood (M/CB, A, x-scale is reversed) and between NBS results and cord blood (NBS/CB, B), with 95% CI. If mean values were higher in CB (ratio <1), the mean ratio with 95% CI lies in the middle of the figure, otherwise on either side. The ratios shown are both for VD (black) and ECS (grey). Variables with statistical significance, from the comparison across delivery mode using linear model with t-test adjusted for possible confounding factors (A and B), are shown in bold letters. A adjusted for gestational age, birth weight, gender, age of mother, parity, Apgar score at one and five minute and B adjusted for gestational age, birth weight, gender, age of mother, parity, Apgar score at one and five minute, weight change and age of neonate at the time of newborn screening. Correction for multiple testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as ^{NS} and those who were significant after correction are marked with ^{S}.

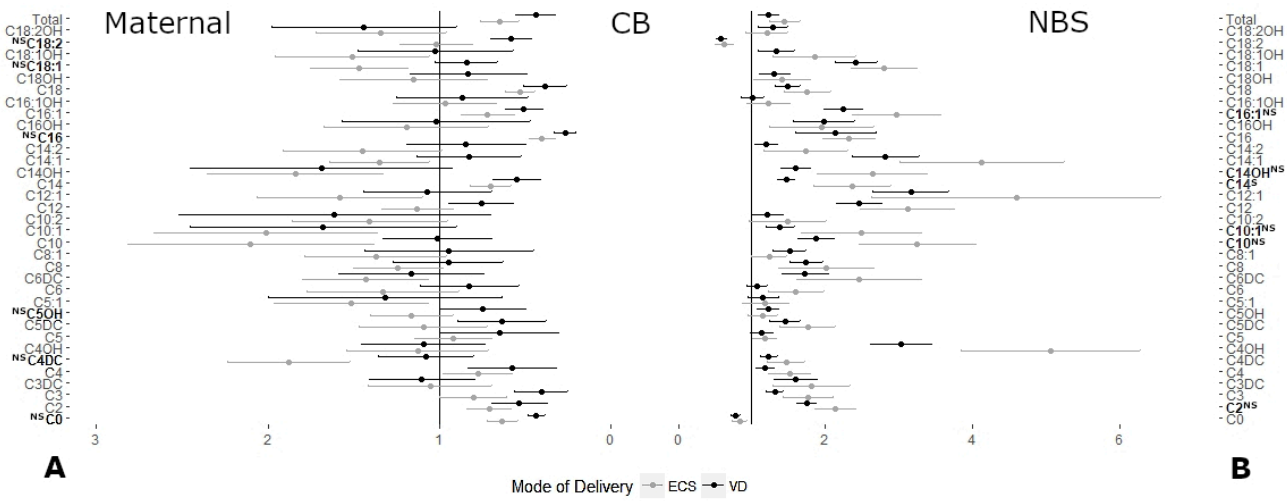

Figure 8. Differences between sample groups for acylcarnitine concentrations shown as ratio of mean values on maternal vs cord blood (A) and NBS and cord blood (B), across modes of delivery. Ratio of mean values on acylcarnitines between maternal blood and cord blood (M/CB, A, x-scale is reversed) and between NBS results and cord blood (NBS/CB, B), with 95% CI. If mean values were higher in CB (ratio <1), the mean ratio with 95% CI lies in the middle of the figure, otherwise on either side. The ratios shown are both for VD (black) and ECS (grey). Variables with statistical significance, from the comparison across delivery mode using linear model with t-test adjusted for possible confounding factors (A and B), are shown in bold letters. A adjusted for gestational age, birth weight, gender, age of mother, parity, Apgar score at one and five minute and B adjusted for gestational age, birth weight, gender, age of mother, parity, Apgar score at one and five minute, weight change and age of neonate at the time of newborn screening. Correction for multiple testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as ^{NS} and those who were significant after correction are marked with ^{S}.

In general, the ratios suggest that amino acids were higher in cord blood samples compared to maternal blood, with the exception of argininosuccinic acid, aspartic acid and citrulline. Regarding acylcarnitines, the ratios suggest that short-chain acylcarnitines, i.e. C0, C2, C3, C4, as well as C14, C16, C16:1, C18, were higher in cord blood. In all comparisons across delivery modes between the maternal and cord blood samples, only lysine showed a significant difference after correction for multiple testing (Benjamini-Hochberg procedure) where neonates born by VD had a lower ratio.

The ratios between cord blood and NBS (NBS/cord blood) are shown in Figure 7B and 8B for amino acids and acylcarnitines, respectively. The comparison across delivery modes is shown there as well, with ECS as the reference and adjusted for gestational age, birth weight, newborn gender, maternal age, parity, Apgar score at one and five minutes, neonatal weight

change and age at the time of NBS. The ratios generally suggest that amino acids have higher values at the NBS compared to cord blood, with the exception of alanine, arginine, phenylalanine, valine and threonine. Regarding the acylcarnitines, the ratios suggest that both those born by VD and ECS had higher values at the time of NBS compared to cord blood, with the exception of C0 and C18:2, which were higher in cord blood samples. Across delivery modes only lysine and C14 were significant after correction for multiple testing where both showed higher ratio for those born by ECS.

Table 13. Maternal blood. Median concentration with 10th – 90th percentile (µmol/L) in amino acids and acylcarnitines from maternal blood and precentage differences between modes of delivery. ECS were used as a reference. The differences presented were adjusted for gestational age, birth weight, gender, maternal age and parity.

	Concentrations (µmol/L) Median (10 – 90 percentile)			**Mean difference in concentrations for VD relative to ECS**	
	All (N 37)	**VD (N 11)**	**ECS (N 26)**	**% with 95%CI**	**p-value**
Amino acids					
Alanine	160 (113-219)	161 (115-190)	159 (115-221)	-8% (-24, 12%)	0.44
Arginine	10 (6-15)	8 (5-14)	11 (7-15)	-22% (-37, 6%)	0.15
Argininosuccinic acid	0.4 (0.1-1.1)	0.7 (0.1-1.3)	0.4 (0.1-0.9)	35% (-41, 210%)	0.49
Aspartic acid	62 (30-90)	50 (31-71)	64 (32-93)	-30% (-47, -8%)	**0.02**^{NS}
Citrulline	14 (8-22)	12 (8-24)	15 (9-21)	-4% (-28, 28%)	0.78
Glutamic acid	191 (133-238)	189 (134-209)	192 (140-250)	-2% (-18, 16%)	0.79
Glycine	135 (97-199)	130 (97-156)	138 (115-211)	-15% (-29, 3%)	0.11
Histidine	36 (20-53)	32 (19-43)	41 (23-59)	-25% (-42, -4%)	**0.03**^{NS}
Leucine, isoleucin, hydroxyproline	64 (44-84)	59 (47-71)	68 (44-87)	-11% (-26, 6%)	0.19
Lysine	89 (65-111)	79 (69-101)	98 (65-113)	-18% (-31, -4%)	**0.03**^{NS}
Methionine	12 (7-17)	12 (6-16)	12 (9-18)	-11% (-32, 14%)	0.37
Methylhistidine	2.5 (1.0-4.6)	1.9 (1.1-3.9)	3.0 (1.0-4.6)	-31% (-54, 3%)	0.08
Ornithine	31 (12-50)	21 (13-47)	34 (14-52)	-25% (-46, 4%)	0.09
Phenylalanine	35 (26-46)	35 (32-41)	35 (26-46)	-3% (-17, 13%)	0.67
Proline	122 (89-155)	123 (103-167)	118 (89-152)	4% (-12, 23%)	0.62
Serine	21 (16-26)	19 (16-24)	21 (17-27)	-11% (-27, 8%)	0.24

Threonine	17 (10-23)	16 (11-20)	18 (11-23)	-15% (-32, 7%)	0.18
Tryptophan	9 (5-12)	9 (8-11)	9 (5-12)	9% (-13, 36%)	0.45
Tyrosine	26 (17-34)	26 (18-34)	26 (18-34)	-3% (-19, 16%)	0.71
Valine	74 (45-95)	72 (48-88)	76 (45-96)	0.05% (-17, 20%)	1.00
Total amino acids	1104 (927-1357)	1025 (914-1229)	1164 (999-1421)	-13% (-25, 0.7%)	0.06
Acylcarnitines					
C0	12 (8-16)	11 (8-14)	13 (8-17)	-2% (-19, 19%)	0.83
C2	8 (6-12)	8 (6-11)	8 (6-12)	-7% (-24, 14%)	0.51
C3	0.7 (0.3-1.3)	0.7 (0.3-1.0)	0.8 (0.3-1.3)	-9% (-36, 30%)	0.62
C3DC	0.05 (0.02-0.07)	0.05 (0.02-0.08)	0.05 (0.02-0.07)	3% (-29, 49%)	0.87
C4	0.13 (0.06-0.27)	0.14 (0.06-0.18)	0.12 (0.06-0.28)	-10% (-38, 30%)	0.57
C4DC	0.22 (0.11-0.41)	0.22 (0.12-0.34)	0.24 (0.12-0.42)	-8% (-33, 28%)	0.65
C4OH	0.05 (0.02-0.11)	0.07 (0.02-0.11)	0.04 (0.02-0.12)	41% (-13, 125%)	0.18
C5	0.09 (0.01-0.14)	0.07 (0.01-0.1)	0.1 (0.04-0.15)	-40% (-62, -5%)	**0.04**^{NS}
C5DC	0.02 (0.01-0.04)	0.02 (0.01-0.03)	0.03 (0.01-0.04)	-7% (-42, 49%)	0.77
C5OH	0.13 (0.05-0.22)	0.11 (0.05-0.26)	0.14 (0.06-0.2)	-8% (-38, 36%)	0.70
C5:1	0.09 (0.03-0.16)	0.07 (0.04-0.12)	0.1 (0.03-0.18)	-17% (-46, 27%)	0.41
C6	0.06 (0.01-0.11)	0.06 (0.02-0.1)	0.06 (0.01-0.11)	25% (-27, 112%)	0.43
C6DC	0.05 (0.01-0.1)	0.05 (0.02-0.07)	0.05 (0.01-0.11)	0.3% (-2, 2%)	0.82
C8	0.04 (0.01-0.06)	0.04 (0.01-0.06)	0.03 (0.01-0.05)	6% (-37, 79%)	0.82
C8:1	0.06 (0.02-0.09)	0.05 (0.02-0.07)	0.06 (0.02-0.1)	-8% (-49, 68%)	0.79
C10	0.04 (0.01-0.06)	0.04 (0.02-0.06)	0.03 (0.01-0.06)	-6% (-38, 45%)	0.80
C10:1	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	12% (-24, 63%)	0.59
C10:2	0.08 (0.03-0.16)	0.08 (0.05-0.15)	0.08 (0.03-0.15)	41% (-6, 110%)	0.11
C12	0.04 (0.01-0.08)	0.04 (0.04-0.06)	0.04 (0.01-0.08)	-4% (-34, 39%)	0.84
C12:1	0.03 (0.01-0.05)	0.04 (0.01-0.05)	0.03 (0.01-0.05)	13% (-30, 80%)	0.62
C14	0.09 (0.04-0.13)	0.09 (0.06-0.12)	0.09 (0.04-0.14)	17% (-15, 62%)	0.34
C14:1	0.04 (0.01-0.08)	0.04 (0.01-0.06)	0.05 (0.02-0.08)	-25% (-52, 17%)	0.21
C14:2	0.03 (0.01-0.04)	0.02 (0.01-0.03)	0.03 (0.01-0.04)	-8% (-41, 43%)	0.73
C14OH	0.03 (0.01-0.05)	0.03 (0.01-0.06)	0.03 (0.01-0.04)	2% (-38, 67%)	0.94

C16	0.52 (0.29-0.74)	0.56 (0.28-0.66)	0.49 (0.34-0.74)	-11% (-30, 12%)	0.30
C16OH	0.02 (0.01-0.04)	0.03 (0.02-0.04)	0.02 (0.01-0.04)	15% (-21, 67%)	0.47
C16:1	0.06 (0.02-0.08)	0.06 (0.04-0.07)	0.06 (0.02-0.08)	1% (-27, 42%)	0.94
C16:1OH	0.04 (0.01-0.06)	0.04 (0.02-0.08)	0.05 (0.01-0.06)	45% (-7, 125%)	0.11
C18	0.26 (0.1-0.38)	0.26 (0.16-0.33)	0.26 (0.09-0.39)	15% (-17, 62%)	0.40
C18OH	0.02 (0.01-0.03)	0.02 (0.01-0.02)	0.02 (0.01-0.03)	-5% (-36, 42%)	0.81
C18:1	0.5 (0.34-0.75)	0.47 (0.38-0.6)	0.5 (0.34-0.76)	7% (-13, 32%)	0.53
C18:1OH	0.02 (0.01-0.04)	0.02 (0.01-0.02)	0.02 (0.01-0.04)	-24% (-49, 14%)	0.19
C18:2	0.12 (0.07-0.18)	0.11 (0.07-0.18)	0.12 (0.08-0.18)	-12% (-32, 13%)	0.30
C18:2OH	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	17% (-27, 86%)	0.51
Total acylcarnitines	25 (18-29)	21 (18-25)	26 (18-30)	-10% (-27, 11%)	0.29

[NS] or [S] Correction for multible testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as NS and those who were significant after correction are marked with S.

Table 14. Neonatal cord blood. Median concentrations with 10th – 90th percentile (µmol/L) in amino acids and acylcarnitines from cord blood and precentage differences between modes of delivery. ECS were used as a reference. The differences presented were adjusted for gestational age, birth weight, gender, maternal age, parity and Apgar at one and five minutes.

	Concentrations (µmol/L) Median (10 – 90 percentile)			**Mean difference in concentrations for VD relative to ECS**	
	All (N 114)	**VD (N 83)**	**ECS (N 31)**	**% with 95%CI**	**p-value**
Amino acids					
Alanine	235 (125-335)	254 (155-346)	181 (124-267)	27% (13, 42%)	**0.0001[S]**
Arginine	15 (5-23)	15 (6-22)	16 (5-26)	-6% (-23, 15%)	0.53
Argininosuccinic acid	0.48 (0.05-0.88)	0.45 (0.04-0.85)	0.63 (0.07-1.01)	-29% (-52, 5%)	0.09
Aspartic acid	36 (21-50)	37 (21-53)	35 (22-40)	11% (-3, 26%)	0.13
Citrulline	13 (5-19)	13 (5-18)	13 (6-19)	-6% (-20, 12%)	0.49
Glutamic acid	280 (183-369)	289 (179-373)	277 (184-341)	6% (-4, 19%)	0.21
Glycine	257 (165-323)	261 (176-355)	237 (169-300)	11% (1, 21%)	**0.04[NS]**
Histidine	49 (24-73)	49 (23-70)	47 (30-76)	-11% (-24, 2%)	0.10
Leucine, isoleucin, hydroxyproline	122 (77-146)	130 (75-150)	113 (79-133)	11% (1, 21%)	**0.03[NS]**

Lysine	146 (88-200)	157 (93-203)	129 (87-184)	17% (6, 30%)	**0.002^{S}**
Methionine	21 (12-29)	21 (11-29)	20 (13-29)	3% (-9, 15%)	0.64
Methylhistidine	4 (2-6)	4 (2-6)	5 (2-6)	-12% (-26, 5%)	0.16
Ornithine	57 (29-82)	57 (29-80)	58 (31-83)	-5% (-18, 11%)	0.53
Phenylalanine	70 (42-91)	72 (53-92)	64 (43-82)	14% (4, 25%)	**0.004^{S}**
Proline	220 (120-293)	230 (142-297)	183 (114-234)	26% (13, 41%)	**0.0001^{S}**
Serine	34 (19-42)	35 (19-42)	32 (21-39)	6% (-4, 17%)	0.22
Threonine	25 (15-33)	25 (14-33)	27 (19-35)	-10% (-20, 2%)	0.11
Tryptophan	16 (9-20)	16 (10-20)	14 (9-17)	11% (-1, 22%)	0.07
Tyrosine	48 (33-61)	49 (33-61)	44 (32-56)	7% (-3, 19%)	0.18
Valine	130 (66-166)	138 (74-169)	117 (70-143)	15% (4, 28%)	**0.009^{S}**
Total amino acids	1845 (1241-2190)	1892 (1266-2244)	1677 (1257-1976)	11% (2, 21%)	**0.02^{NS}**
Acylcarnitines					
C0	26 (12-38)	27 (13-39)	23 (12-34)	23% (5, 45%)	**0.01^{NS}**
C2	17 (6-26)	17 (10-27)	15 (6-19)	28% (9, 51%)	**0.003^{NS}**
C3	1.5 (0.6-2.5)	1.6 (0.8-2.7)	1.4 (0.5-1.8)	48% (23, 79%)	**<0.0001^{S}**
C3DC	0.05 (0.02-0.08)	0.05 (0.02-0.08)	0.06 (0.02-0.1)	-2% (-22, 23%)	0.85
C4	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	14% (-9, 42%)	0.24
C4DC	0.21 (0.08-0.31)	0.23 (0.1-0.31)	0.18 (0.08-0.27)	39% (16, 65%)	**0.0004^{S}**
C4OH	0.06 (0.02-0.11)	0.07 (0.03-0.12)	0.06 (0.02-0.08)	23% (-1, 55%)	0.06
C5	0.12 (0.05-0.19)	0.12 (0.05-0.19)	0.12 (0.08-0.2)	-2% (-17, 17%)	0.82
C5DC	0.03 (0.01-0.04)	0.03 (0.01-0.04)	0.03 (0.02-0.04)	-3% (-21, 19%)	0.74
C5OH	0.14 (0.06-0.22)	0.15 (0.06-0.22)	0.13 (0.06-0.2)	4% (-14, 26%)	0.66
C5:1	0.07 (0.02-0.14)	0.07 (0.02-0.14)	0.08 (0.03-0.12)	-10% (-32, 19%)	0.45
C6	0.07 (0.02-0.12)	0.07 (0.03-0.13)	0.06 (0.02-0.09)	27% (3, 58%)	**0.03^{NS}**
C6DC	0.05 (0.01-0.08)	0.05 (0.01-0.08)	0.04 (0.01-0.06)	0.3% (-1, 2%)	0.65
C8	0.05 (0.01-0.09)	0.05 (0.02-0.08)	0.05 (0.02-0.09)	3% (-18, 30%)	0.81
C8:1	0.07 (0.02-0.12)	0.07 (0.02-0.12)	0.09 (0.04-0.12)	-18% (-35, 4%)	0.10
C10	0.04 (0.01-0.07)	0.05 (0.01-0.07)	0.03 (0.01-0.06)	15% (-8, 43%)	0.22
C10:1	0.03 (0.01-0.05)	0.03 (0.01-0.05)	0.03 (0.01-0.05)	23% (-5, 60%)	0.12

C10:2	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	-11% (-33, 19%)	0.42
C12	0.06 (0.02-0.09)	0.06 (0.02-0.09)	0.05 (0.02-0.07)	26% (3, 54%)	**0.03^{NS}**
C12:1	0.02 (0.01-0.04)	0.02 (0.01-0.05)	0.03 (0.01-0.03)	21% (-4, 54%)	0.11
C14	0.16 (0.06-0.25)	0.17 (0.08-0.25)	0.14 (0.05-0.21)	42% (20, 68%)	**0.0001^{S}**
C14OH	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	34% (4, 72%)	**0.03^{NS}**
C14:1	0.05 (0.02-0.08)	0.05 (0.02-0.07)	0.04 (0.02-0.08)	17% (-5, 46%)	0.15
C14:2	0.03 (0.01-0.05)	0.03 (0.01-0.05)	0.03 (0.01-0.04)	23% (-5, 60%)	0.11
C16	1.9 (0.6-2.6)	2 (1-2.7)	1.6 (0.7-2.2)	36% (16, 60%)	**0.0002^{S}**
C16OH	0.03 (0.01-0.05)	0.03 (0.01-0.05)	0.03 (0.01-0.05)	5% (-20, 36%)	0.73
C16:1	0.1 (0.04-0.17)	0.11 (0.05-0.18)	0.09 (0.04-0.14)	36% (12, 65%)	**0.003^{NS}**
C16:1OH	0.06 (0.02-0.1)	0.06 (0.02-0.1)	0.05 (0.02-0.09)	13% (-9, 41%)	0.26
C18	0.7 (0.3-1)	0.7 (0.3-1)	0.5 (0.3-0.9)	36% (16, 60%)	**0.0003^{S}**
C18OH	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	4% (-20, 35%)	0.77
C18:1	0.6 (0.2-0.8)	0.6 (0.3-0.8)	0.5 (0.2-0.7)	43% (21, 70%)	**<0.0001^{S}**
C18:1OH	0.02 (0.01-0.04)	0.03 (0.01-0.04)	0.02 (0.01-0.04)	12% (-12, 42%)	0.36
C18:2	0.21 (0.07-0.31)	0.22 (0.08-0.31)	0.17 (0.07-0.27)	36% (13, 63%)	**0.002^{NS}**
C18:2OH	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	-4% (-26, 25%)	0.78
Total acylcarnitines	49 (25-70)	54 (28-73)	42 (24-54)	30% (12, 52%)	**0.001^{S}**

^{NS} or ^{S} Correction for multible testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as NS and those who were significant after correction are marked with S.

Table 15. NBS results. Median concentrations with 10th – 90th percentile (µmol/L) in amino acids and acylcarnitines from the NBS and precentage differences between modes of delivery. ECS was used as a reference. The differences presented were adjusted for gestational age, birth weight, gender, maternal age, parity, Apgar at one and five minutes, weight change and age in hours of the newborn at the time of newborn screening.

	Concentration (µmol/L) Median (10 – 90 percentile)			**Mean difference in concentration for VD relative to ECS**	
	All (N 115)	**VD (N 83)**	**ECS (N 32)**	**% with 95%CI**	**p-value**
Amino acids					
Alanine	160 (84-227)	160 (76-243)	160 (103-202)	2% (-16, 22%)	0.86
Arginine	7 (3-12)	7 (3-12)	8 (3-12)	4% (-20, 34%)	0.79

Argininosuccinic acid	0.4 (0.1-0.7)	0.4 (0.1-0.7)	0.4 (0.1-0.7)	11% (-16, 45%)	0.47
Aspartic acid	45 (22-67)	46 (29-67)	42 (19-62)	16% (-3, 38%)	0.11
Citrulline	13 (6-22)	13 (6-22)	13 (8-23)	-4% (-24, 22%)	0.76
Glutamic acid	431 (239-521)	441 (228-521)	413 (314-517)	-0.1% (-9, 9%)	0.98
Glycine	382 (214-524)	378 (207-523)	403 (290-553)	-7% (-21, 9%)	0.40
Histidine	60 (26-94)	64 (29-95)	51 (26-90)	28% (3, 60%)	**0.03**[NS]
Leucine, isoleucin, hydroxyproline	148 (89-194)	148 (85-211)	148 (95-187)	1% (-18, 19%)	0.89
Lysine	176 (89-285)	172 (89-256)	190 (99-326)	-14% (-28%, 3%)	0.10
Methionine	19 (11-29)	19 (10-28)	19 (12-29)	-10% (-26, 7%)	0.24
Methylhistidine	5 (2-8)	6 (3-8)	5 (2-6)	11% (-9, 34%)	0.31
Ornithine	85 (43-113)	85 (43-114)	83 (48-111)	12% (-8, 34%)	0.26
Phenylalanine	58 (26-73)	59 (25-75)	54 (44-68)	9% (-3, 25%)	0.16
Proline	307 (159-440)	312 (189-478)	302 (131-406)	16% (-3, 38%)	0.12
Serine	62 (28-97)	62 (27-97)	64 (38-95)	-8% (-25, 13%)	0.43
Threonine	17 (10 -24)	16 (10-25)	18 (10-22)	-1% (-16, 16%)	0.90
Tryptophan	19 (12-25)	20 (11-25)	17 (13-23)	7% (-5, 21%)	0.26
Tyrosine	68 (36-108)	68 (36-108)	69 (44-98)	6% (-12, 30%)	0.54
Valine	103 (58-157)	105 (53-161)	95 (68-120)	19% (-1, 42%)	0.08
Total amino acids	2176 (1648-2796)	2124 (1704-2811)	2194 (1581-2650)	-5% (-17, 8%)	0.43
Acylcarnitines					
C0	18 (9-31)	19 (10-32)	18 (9-23)	12% (-10, 38%)	0.31
C2	29 (15-42)	29 (14-44)	27 (16-41)	5% (-14, 27%)	0.63
C3	2.0 (0.9-3.0)	2.1 (0.9-3.3)	1.8 (0.9-2.8)	9% (-13, 38%)	0.45
C3DC	0.07 (0.02-0.11)	0.06 (0.02-0.1)	0.08 (0.03-0.15)	-29% (-47, -5%)	**0.03**[NS]
C4	0.27 (0.1-0.4)	0.24 (0.1-0.43)	0.3 (0.12-0.44)	4% (-20, 34%)	0.77
C4DC	0.25 (0.09-0.38)	0.25 (0.13-0.39)	0.25 (0.08-0.35)	12% (-11, 41%)	0.36
C4OH	0.19 (0.06-0.32)	0.18 (0.06-0.3)	0.24 (0.07-0.43)	-13% (-33, 13%)	0.27
C5	0.12 (0.05-0.19)	0.12 (0.05-0.19)	0.13 (0.06-0.19)	-3% (-22, 22%)	0.82
C5DC	0.04 (0.01-0.07)	0.04 (0.01-0.06)	0.04 (0.02-0.08)	-24% (-43, 1%)	0.07
C5OH	0.15 (0.06-0.24)	0.16 (0.07-0.24)	0.13 (0.06-0.22)	3% (-18, 30%)	0.80

C5:1	0.06 (0.02-0.13)	0.06 (0.02-0.12)	0.07 (0.02-0.14)	-10% (-36, 25%)	0.51
C6	0.07 (0.02-0.1)	0.07 (0.02-0.1)	0.08 (0.02-0.11)	3% (-20, 32%)	0.83
C6DC	0.06 (0.02-0.12)	0.06 (0.02-0.09)	0.08 (0.02-0.14)	-4% (-6, -1%)	**0.002**[NS]
C8	0.08 (0.03-0.12)	0.08 (0.03-0.12)	0.1 (0.03-0.13)	-27% (-42, -9%)	**0.008**[NS]
C8:1	0.08 (0.03-0.15)	0.08 (0.03-0.15)	0.08 (0.03-0.15)	-11% (-33, 17%)	0.40
C10	0.07 (0.02-0.14)	0.07 (0.03-0.11)	0.1 (0.02-0.18)	-29% (-46, -7%)	**0.02**[NS]
C10:1	0.03 (0.01-0.06)	0.03 (0.01-0.05)	0.04 (0.01-0.09)	-33% (-50, -10%)	**0.01**[NS]
C10:2	0.02 (0.01-0.04)	0.02 (0.01-0.03)	0.02 (0.01-0.04)	-24% (-43, 4%)	0.09
C12	0.13 (0.05-0.2)	0.13 (0.06-0.2)	0.14 (0.05-0.2)	-1% (-21, 22%)	0.91
C12:1	0.07 (0.02-0.15)	0.06 (0.02-0.14)	0.08 (0.02-0.15)	-12% (-38, 22%)	0.44
C14	0.25 (0.13-0.37)	0.24 (0.13-0.35)	0.27 (0.17-0.43)	-11% (-27, 8%)	0.23
C14OH	0.03 (0.01-0.06)	0.03 (0.01-0.06)	0.04 (0.01-0.08)	-27% (-43, -4%)	**0.03**[NS]
C14:1	0.12 (0.04-0.21)	0.11 (0.04-0.19)	0.14 (0.05-0.22)	-10% (-33, 20%)	0.46
C14:2	0.03 (0.01-0.05)	0.03 (0.01-0.04)	0.03 (0.01-0.06)	-4% (-29, 30%)	0.79
C16	3.28 (1.52-4.87)	3.45 (1.45-5.89)	3.10 (1.79-4.24)	12% (-15, 48%)	0.42
C16OH	0.04 (0.01-0.06)	0.04 (0.01-0.06)	0.04 (0.01-0.07)	-4% (-29, 30%)	0.79
C16:1	0.22 (0.08-0.37)	0.22 (0.09-0.37)	0.21 (0.09-0.36)	-3% (-23, 22%)	0.83
C16:1OH	0.05 (0.02-0.09)	0.05 (0.02-0.1)	0.05 (0.03-0.09)	2% (-23, 35%)	0.91
C18	0.9 (0.46-1.42)	0.9 (0.46-1.5)	0.88 (0.46-1.18)	15% (-7, 42%)	0.20
C18OH	0.02 (0.01-0.04)	0.02 (0.01-0.03)	0.02 (0.01-0.05)	-19% (-40, 8%)	0.16
C18:1	1.23 (0.59-1.91)	1.25 (0.79-1.93)	1.13 (0.56-1.56)	19% (-2, 43%)	0.09
C18:1OH	0.03 (0.01-0.05)	0.03 (0.01-0.05)	0.03 (0.01-0.05)	-20% (-39, 6%)	0.12
C18:2	0.10 (0.03-0.19)	0.11 (0.03-0.19)	0.09 (0.04-0.15)	25% (-0.7, 55%)	0.06
C18:2OH	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	13% (-17, 52%)	0.46
Total acylcarnitines	59 (33-90)	61 (37-97)	56 (32-72)	8% (-16, 41%)	0.52

[NS] or [S] Correction for multible testing was done using the Benjamini-Hochberg procedure. Those p-values <0.05 that were not siginificant after correction for multiple testing are marked as NS and those who were significant after correction are marked with S.

5 Discussion

5.1 Obstetric and neonatal risks among extremely macrosomic babies and their mothers (paper I)

The ratio of extremely macrosomic babies was high in this population, 0.8%, compared to 0.1% in the USA (Joyce A. Martin, 2002). Data from the United Arab Emirates and Sweden show a ratio of 0.24% (Anoon et al., 2003) and 0.45% (Mollberg et al., 2005b), respectively. Why Icelandic babies are born heavier is not known. Most studies show that births of macrosomic babies are getting more frequent (Alberman, 1991; Henriksen, 2008; Koyanagi et al., 2013). Data from Sweden show that in 1987 the ratio of births of babies ≥4000 g was 17.3 %, but by 1997 this had reached 19.5% (Mollberg et al., 2005a), compared to 23.8% in 1982-85 (G Jonsdottir et al., 1989) and 28.7% in 1998-2001 ("Live births and late fetal deaths by sex 1951-2018," 2019) in Iceland. The male/female ratio of 2:1 and nulli- and multiparous ratio of 1:4 in our material was, however, comparable to other studies (Alsunnari et al., 2005; Anoon et al., 2003).

The difference in BMI and weight gain between the mothers indicates that the mothers of macrosomic babies tended to be heavier and gain more weight in pregnancy than mothers of babies within normal weight range. This was mirrored in the higher PI of the macrosomic babies, again similar to other studies (Anoon et al., 2003; Clausen et al., 2005; Thorsdottir et al., 2002). The connection between weight gain during pregnancy and birth weight is well described (Ehrenberg et al., 2004; Stotland et al., 2004; Thorsdottir et al., 2002). Age was not related to the occurrence of macrosomia, except inversely for the oldest age group of the mothers. This may be explained by changing demographics with regards to higher age at first delivery and hypertensive complications related to this and resulting in growth restriction (Montan, 2007). No difference between the mother-groups regarding gestational diabetes was surprising. Only two women had pre-existing diabetes, a known risk factor for macrosomia (Alsunnari et al., 2005; Anoon et al., 2003; Ehrenberg et al., 2004). Diabetic pregnancy care is centralised and tight in the relatively small Icelandic population and birth is usually induced at 38-40 gestational weeks.

Most of the deliveries among the mothers of extremely macrosomic babies were vaginal in spite of the large fetal size, but not without dangers. These large babies and their mothers were more likely to suffer birth trauma, of which

one of the most serious was shoulder dystocia, which was nearly 27 times more common, with a greatly increased chance of Erb´s palsy (large confidence interval makes exact assessment difficult). In real terms, 46 shoulder dystocias among 334 extremely large babies, or 14%, is substantial, even though most came through this without lasting problems (35/46). For comparison, a study in Ireland of 182 extremely macrosomic babies born between 2008-2012, showed that for 14.2% of those born vaginally the delivery was complicated by shoulder dystocia (Hehir et al., 2015). In our material, clavicular and humerus fractures can be added to this. Hypoxia at birth and respiratory disorders were not more common in macrosomic births, which differs from other studies (Bjorstad et al., 2010; Oral et al., 2001), but this may be related to the study size and lack of statistical power of the material to detect such occurrences, which are relatively rare. Hypoglycemia was four times more common among the extremely macrosomic babies, but for the same reason this was not statistically significant. A follow-up to assess later development of the children was, however, not part of this study. Watkins et al showed an association between maternal obesity and congenital malformations (Watkins et al., 2003). In our study we corrected for maternal body mass index and there was still an increased risk for congenital malformation among the exposed cohort. It is conceivable that many of the women had a borderline diabetic state, as there is a link between rising blood sugar values and birth weight (Metzger et al., 2008), even at the start of pregnancy, and with accompanying metabolic changes. A second trimester glucose tolerance test for all obese women should be of value, even though this may not identify but a proportion of all extremely macrosomic babies.

Alsunnari et al showed that only 44% of extremely macrosomic babies were born by VD (Alsunnari et al., 2005), considerably lower than the 70% among exposed mothers in our study and 66% reported by Hehir et al (Hehir et al., 2015). Still the section rate of 30% in our study was almost twice the national average (G. Jonsdottir et al., 2009). The ratio in our study between emergency and elective cesarean sections was 4:1, which contrasts with other studies where emergency section rates were less frequent with a ratio of 2:3 (Alsunnari et al., 2005), 9:10 (Anoon et al., 2003) and 2:1 (Hehir et al., 2015). While there was no increase in ECS in our population, where women wish in general to attempt VD, there was a five-fold increase in emergency sections for the exposed compared to the non-exposed cohort. Clinical guidelines for handling possible macrosomia seem with reference to our results to be justified and should include both a high suspicion index for EM and liberal resort to cesarean delivery.

Instrumental delivery rates were not different between exposed and non-exposed groups in our material. Anoon et al showed similar results but concluded that such deliveries should be more common because of the high rates of shoulder dystocia and possible cephalopelvic mismatch (Anoon et al., 2003). There was also a doubling of the incidence of perineal trauma for the exposed mothers compared to non-exposed. This included both somewhat more frequent 4th degree tears, but also episiotomies, presumably done to protect from possible greater injury. Risk factors for shoulder dystocia, such as augmentation of labor, induction and impending instrumental delivery should constitute a reason for caution and a more liberal resort to cesarean section.

ECS is protective against trauma for the baby and the mother, although this is not without exceptions. A method is needed to identify these extremely large babies before labor. In our material ultrasound estimation was not carried out systematically or with the sufficient frequency to allow this to be studied, and the common clinical method of abdominal palpation showed that the size of these babies is greatly underestimated. Clinical size evaluation is thus not to be relied upon, although the practice of assessing fetal size has its firm and justified place in practice. Chauhan et al (Chauhan et al., 1998) showed that neither ultrasound nor clinical estimation were adequate methods to determine whether a baby was macrosomic or not.

Our study had the strength of a comparatively large material and the comparison mother-newborn pairs were chosen in order to minimize the confounding effects of changes in obstetric practice over the study period. We used country-wide data adjusted for major risk factors. Weaknesses include the registration by midwives and/or medical practitioners of soft data such as perineal trauma or even difficulties in delivering the shoulders. It may be a difficult decision to link an otherwise normal delivery to a possible serious complication for the baby, such as shoulder dystocia, where under- rather than over-registration may dominate.

An increasing prevalence of taller and heavier mothers in many middle and high income countries and excess caloric intake will result in excess availability of maternal substrates and thus fetal hyperinsulinism (Metzger et al., 2008). At term the proportion of large and even extremely large babies will therefore be greater. From a clinical and public health perspective it is necessary to find ways to better predict when the risk for damage during delivery is real and to devise ways of handling this, both during antenatal care and at the time of delivery.

5.2 Does metabolomic profile differ with regards to birth weight? (paper II)

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. However, when applying the Benjamini–Hochberg procedure two out 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 the NBS 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 (Biering et al., 1985). In our study population all neonates meeting the inclusion criteria for AGA were classified as AGA, to minimize the risk of selection bias. 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 (Magi et al., 2019; Walker & van der Donk, 2016). 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 glucose (Adeva-Andany et al., 2016; Rui, 2014). Why threonine was increased in those born LBW is more difficult to explain.

EM newborns, with their excess adipose tissue (Hammami et al., 2001; Lee et al., 2012), are probably able to 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 (Adeva-Andany et al., 2016) and fatty acids are released for energy metabolism in the liver, creating ketones at the same time (Rui, 2014). Glucose and ketone bodies are particulary important in the newborn period as energy substrates for 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 in a catabolic state which is normal for 3-4 days old neonates adapting to extrauterine life. They were being fed regularly, but not yet receiving sufficient amount of nutrients to reach an anabolic state. Over the first week the nutritional intake of a newborn increases gradually and most reach an anabolic state after their first week of life. This applies also to infants who get supplementary feeding with infant formula.

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 infant formulas are rich in long-chain fatty acids. They are also rich in C0, 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 NBS 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 (Williams, 1997; Wilson et al., 2014). Kadakia et al. took many of the major factors into account in their study on the correlation between newborn adiposity and metabolites (Kadakia et al., 2018). They were, however, looking at cord blood values which may have been affected by both maternal and placental metabolism and therefore difficult to compare to our results. This has to be taken into account since

amino acids move across the placenta from the mother by active transport (Holm et al., 2017). Gucciardi et al. 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 (Gucciardi et al., 2015). Wright and Baker only looked at samples taken between 48 to 72 hours, but were not able to take gestational age into account (Wright & Baker, 2020). Since gestational age and birth weight are strongly correlated it is difficult to compare this to our results.

Ryckman et al. and Yang et al. reported changes in BCAA and AAA (Ryckman et al., 2013; Yang et al., 2018), as seen in older obese children and adults, as well as adults with type 2 diabetes and insulin resistance (Butte et al., 2015; Guasch-Ferré et al., 2016; Menni et al., 2013; Zhao et al., 2016). 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, and corrected for gestational age and the time of sampling. There is, however, a possibility that differences in BCAA and AAA were not detected due to the small sample size of the sub-groups 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 (Sanchez-Pintos et al., 2017; Wright & Baker, 2020; Yang et al., 2018) with an additional correlation to newborn adiposity regarding C2, C3, C4DC/Ci4DC and C4OH (Kadakia et al., 2018). Both macrosomic and SGA neonates have been shown to have an increased risk for obesity and metabolic syndrome later in life (Barker et al., 1993; Cnattingius et al., 2012; Kain et al., 2009; Sparano et al., 2013; Yu et al., 2011) 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 NBS. Furthermore, the study provides information on newborn metabolism and shows that being at either end of the birth weight range gives it´s mark in the first days of life.

5.3 Mode of delivery was associated with transient changes in the metabolomic profile of neonates (paper III)

The main results of this study showed that there were transient yet distinctive differences in the newborn metabolomic profiles across delivery modes in cord blood, suggesting that neonates born vaginally have higher values of several amino acids and acylcarnitines at the time of birth compared to those delivered by ECS.

The differences observed across modes of delivery in cord blood could be explained by normal stress effects during uncomplicated VD. These effects are mediated by stress hormones, i.e. adrenalin/noradrenalin and cortisol, and also by uterine contractions that momentarily reduce the otherwise mostly constant flow of oxygen and nutrients to the fetus (Hillman et al., 2012; Lagercrantz & Slotkin, 1986; Williams, 1997). The full term fetus is capable of responding to the stress and to a decrease of umbilical and/or placental flow by activating metabolic pathways that provide energy supply in a catabolic state (Şengül & Dede, 2014). Our results suggest that both glycogenic and ketogenic amino acids are increased at birth in neonates born vaginally compared to ECS where alanine showed the most increase. Alanine has a vital role in gluconeogenesis (Adeva-Andany et al., 2016; Williams, 1997) even though, in most cases, it is not fully initiated at birth (S. C. Kalhan et al., 2001). The neonate is most likely mainly using the liver glycogen stores. Many of the acylcarnitines were also increased after VD compared to ECS which could indicate increased mobilization and breakdown of fatty acids for producing ketone bodies, partly due to effects of stress hormone release (Bahnsen et al., 1984; Herrera & Amusquivar, 2000).

The differences observed across modes of delivery in the NBS (samples taken at 48-72 hours of age) showed only a significant difference for one amino acid, i.e. an increase in histidine among babies born vaginally compared to ECS. Furthermore, a decreased difference in a few of the medium- and long-chain acylcarnitines was observed in the vaginally delivered babies compared to those born by ECS. However, these results need to be interpretated with caution as none remained significant after adjustment for multiple comparison using the Benjanmini-Hochberg procedure.

The same applies to the differences across delivery modes observed in the maternal samples since they became nonsignificant after correction for multiple testing. There might be differences in the maternal metabolomic profiles which were obscured in our material due to the limited number of samples from mothers delivering vaginally. Alternatively the effects of

increased stress hormones and the complex mixture of the hormones that induce and accelerate labor (Irestedt et al., 1982; Vannuccini et al., 2016; Vogl et al., 2006) could be similar to the effects of fasting (Kerndt et al., 1982). Furthermore, it needs to be acknowledged that the VD group represented women in need of intravenous access in an otherwise normal vaginal delivery. We have no reason to suspect that they differed from other women having an uncomplicated vaginal delivery since those factors potentially of significance for the study results had been excluded.

Our results on the change in ratios in amino acids between maternal and neonatal cord blood showed that newborns have higher amounts of most amino acids in cord blood, both after VD and ECS. That is in line with previous research since amino acids and various forms of lipids are transported actively from the mother to the fetus via the placenta (Battaglia & Regnault, 2001; Herrera & Amusquivar, 2000; Holm et al., 2017; Regnault et al., 2002).

Both amino acids and acylcarnitines, mostly observed in short-chain acylcarnitines, showed a greater difference and higher amount of those substrates in babies born by VD than ECS in comparison with maternal samples. The amounts of catecholamines are higher after VD, or after a delivery where birth asphyxia occurs, than at any other time of life (Lagercrantz & Slotkin, 1986), which could partly explain the differences. Catecholamines increase after ECS (Irestedt et al., 1982; Vogl et al., 2006) could explain that such neonates tend to have higher values of short-chain acylcarnitines than their mothers. Across delivery modes, only the increased difference in lysine among VD remained significant after adjustment for multiple comparisons.

Looking at the ratios between cord blood and NBS, almost all amino acids and acylcarnitines were increased in NBS, apart from alanine, phenylalanine, threonine, valine and C0. Most studies on those substrates show that they increase in the first days after birth as the energy metabolism shifts from primary using glucose in utero to using other substrates such as proteins and fat, until the maternal milk production has met the nutritional requirements of the newborn (Vieira Neto et al., 2012; Walter et al., 2009). Why some amino acids, such as alanine, and C0 are higher after birth might relate to that gluconeogenesis and fatty acid oxidation is not fully established at birth (S. C. Kalhan et al., 2001), even though the substrates have been mobilized through effects of catecholamines and cortisol release (Hillman et al., 2012; Williams, 1997). Regarding comparison across delivery modes, only lysine and C14 remained significant after correction for multiple testing, whereas neonates born by ECS had increased differences compared to those born vaginally.

To our knowledge, a comparison on neonatal and maternal metabolomic factors comparing VD to ECS has not been done previously. Thus, it is difficult to compare our results to other studies on metabolomic profiles. Those studies show methodological diversities in how they were done. That concerns both which factors were taken into account and how the samples were taken, as samples from plasma differ from DBS, f.ex. due to the attachment of long-chain acylcarnitines to red blood cells (de Sain-van der Velden et al., 2013). Our study had a relatively small sample size, adressed many variables and three different sampling sites were used; venous in mothers, mixed arterial and venous blood from umbilical cord and heel prick in neonates. Nonetheless, all blood samples were placed directly onto DBS. Then, there were no further analyses on the measured variables, for example to differentiate C4DC from methylmalonylcarnitine (Rizzo et al., 2014) or C5 from pivaloylcarnitine (Abdenur et al., 1998). Given the number of comparisons made, false positive values would be expected. To address this concern, we used the Benjamini-Hochberg procedure to account for multiple testing. Still this method may result in too conservative corrections eliminating some differences that were not chance findings. However, several comparisons in cord blood from the newborns remained significant after applying this correction procedure.

In conclusion, our results on neonatal metabolomic profile across delivery modes showed distinct differences between newborns directly after birth, where neonates born by VD had transiently higher values of many amino acids and acylcarnitines than neonates born by ECS. At the time of newborn screening there were small differences, if any, and that might suggest that the mode of delivery does not affect the results of NBS.

6 Conclusions

Paper I: Risk for EM is correlated to high maternal BMI and excess maternal weight gain over the gestation. The risk of shoulder dystocia for extremely macrosomic babies is markedly raised, as are minor complications, while for mothers the main risk is emergency cesarean section.

Paper II: There are distinctive differences in the metabolomic profiles of LBW and EM neonates born at term compared to term AGA newborns.

Paper III: There are distinct differences in the metabolomic profiles between newborns across delivery modes directly after birth, where neonates born by VD show transiently higher values of many amino acids and acylcarnitines than neonates born by ECS. At the time of newborn screening the differences are small, if any. That might suggest that in comparison between normal vaginal birth and ECS, the mode of delivery does not affect the results of NBS.

6.1 Future perspectives

In the past two decades there has been increasing interest in studying macrosomia and EM since there is a marked increase in birth weight world-wide, a trend which is connected to the increase in obesity. The risks that follow EM are now largely established as far as birth is concerned, but there is a need to define better how prevention is best organized before and during pregnancy in order to minimize maternal and neonatal complications. Furthermore, it is not straightforward how to choose the appropriate time and mode of delivery when EM is suspected. In addition it will be necessary to follow up the babies with regard to childhood obesity and metabolic disturbances and establish how this may extend into adulthood, affecting endocrine, cardiovascular, musculoskeletal and even psychological risks in the later years of these individuals.

Since there are increased risks for obesity, heart- and vascular complications and metabolic syndrome including insulin-resistant diabetes that follows being EM at birth, there is a need for further understanding the dynamics of neonatal metabolic and nutritional adaptation, considering the nutrition given. This might help in understanding the possible changes in epigenetic factors that affect metabolism in those babies that are born extremely macrosomic. This will be likely to contribute to a better understanding of what may prevent later childhood- and adult obesity and related illnesses.

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Original publications

Paper I

Paper II

Paper III

Appendix 1

Distribution of amino acid and acylcarnitine biomarkers from Paper II, „Does metabolomic profile differ with regards to birth weight?“ presented as mean and SD and median with 10th - 90th percentile in Table 1 and 2.

Table 1 . Distribution of amino acid biomarkers in µmol/L, total and for birth weight groups, shown as mean (standard deviation, first line) and median (10th to 90th percentile, second line).

Amino acids	Total N: 6131	LBW N: 36	AGA N: 6058	EM N: 37
Alanine	201 ±59 191 (104-279)	220 ±48 222 (133-269)	201 ±59 191 (104-279)	199 ±51 189 (123-271)
Arginine	11 ±7 10 (3-19)	11 ±5 11 (4-18)	11 ±7 10 (3-19)	12 ±5 12 (4-19)
Argininosuccinic acid	0.74 ±0.84 0.55 (0.22-1.36)	0.66 ±0.57 0.58 (0.04-1.08)	0.74 ±0.84 0.55 (0.04-1.35)	1.05 ±1.25 0.66 (0.12-1.89)
Aspartic acid	60 ±23 55 (28-88)	63 ±16 61 (32-82)	60 ±23 55 (28-88)	60 ±16 58 (40-81)
Citrulline	12 ±4 12 (6-18)	13 ±5 13 (5-20)	12 ±4 12 (6-18)	13 ±6 11 (7-19)
Glutamic acid	434 ±89 424 (271-550)	472 ±100 456 (304-579)	434 ±89 424 (271-549)	469 ±98 458 (286-604)
Glycine	389 ±108 369 (221-525)	398 ±122 349 (207-591)	389 ±108 369 (222-524)	424 ±98 414 (286-540)
Histidine	76 ±35 69 (28-117)	73 ±32 65 (30-117)	76 ±35 69 (28-117)	83 ±28 78 (38-125)
Lysine	208 ±70 199 (80-298)	234 ±83 234 (96-337)	207 ±69 199 (80-298)	197 ±70 190 (96-301)
Methionine	20 ±6 20 (11-27)	20 ±7 18 (8-29)	20 ±6 20 (11-27)	20 ±5 19 (13-27)
Methylhistidine	5 ±3 5 (2-8)	5 ±1 5 (3-7)	5 ±3 5 (2-8)	6 ±3 5 (2-9)
Ornithine	103 ±38 96 (45-149)	103 ±37 90 (42-163)	103 ±38 96 (45-149)	101 ±31 93 (60-136)
Phenylalanine	59 ±12 57 (37-75)	63 ±15 62 (37-82)	59 ±12 57 (37-75)	61 ±13 58 (41-77)
Proline	394 ±126 373 (194-557)	416 ±136 388 (223-563)	394 ±126 373 (193-557)	356 ±92 329 (229-476)
Serine	96 ±40 87 (41-142)	92 ±31 83 (40-138)	96 ±40 87 (41-142)	105 ±32 96 (63-155)
Threonine	25 ±8 23 (12-35)	30 ±8 30 (15-40)	25 ±8 23 (12-34)	26 ±7 25 (14-34)
Tryptophan	19 ±5 18 (10-25)	20 ±4 19 (12-25)	19 ±5 18 (10-25)	20 ±5 19 (12-26)
Tyrosine	85 ±33 79 (39-123)	91 ±36 79 (42-150)	85 ±33 79 (39-123)	75 ±26 73 (32-94)
Valine	132 ±30 128 (78-171)	126 ±29 120 (88-162)	132 ±30 128 (78-172)	127 ±22 124 (81-157)

Total	1332 ±306 1281 (820-1730)	1384 ±292 1296 (874-1776)	1332 ±306 1281 (820-1729)	1335 ±261 1251 (1024-1716)

AGA = appropriate-for-gestational age (birth weight between 10th to 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)

Table 2. Distribution of acylcarnitine biomarkers in µmol/L, total and for birth weight groups, shown as mean (standard deviation, first line) and median (10th to 90th percentile, second line).

Acylcarnitines	**Total** **N: 6131**	**LBW** **N: 36**	**AGA** **N: 6058**	**EM** **N: 37**
C0	23 ±9 22 (14-34)	30 ±9 27 (22-40)	23 ±9 22 (14-34)	24 ±9 22 (13-35)
C2	31 ±11 30 (20-45)	36 ±14 33 (21-54)	31 ±11 30 (20-45)	38 ±12 37 (24-53)
C3	2.1 ±0.9 1.9 (1.2-3.2)	2.0 ±0.7 1.9 (1.2-2.9)	2.1 ±0.9 1.9 (1.2-3.2)	2.5 ±1.1 2.0 (1.3-3.8)
C3DC	0.08 ±0.03 0.08 (0.04-0.12)	0.08 ±0.03 0.08 (0.05-0.13)	0.08 ±0.03 0.08 (0.04-0.12)	0.09 ±0.03 0.08 (0.06-0.15)
C4	0.31 ±0.13 0.28 (0.17-0.47)	0.31 ±0.14 0.27 (0.17-0.52)	0.30 ±0.13 0.28 (0.17-0.47)	0.33 ±0.14 0.28 (0.18-0.49)
C4DC	0.32 ±0.12 0.31 (0.19-0.48)	0.32 ±0.12 0.3 (0.2-0.49)	0.32 ±0.12 0.31 (0.19-0.48)	0.29 ±0.09 0.28 (0.19-0.41)
C4OH	0.19 ±0.09 0.18 (0.1-0.31)	0.14 ±0.06 0.14 (0.07-0.21)	0.2 ±0.09 0.18 (0.1-0.31)	0.23 ±0.1 0.21 (0.12-0.33)
C5	0.15 ±0.09 0.13 (0.08-0.21)	0.16 ±0.06 0.15 (0.08-0.25)	0.15 ±0.09 0.13 (0.08-0.21)	0.14 ±0.05 0.13 (0.07-0.22)
C5DC	0.04 ±0.02 0.04 (0.02-0.07)	0.05 ±0.02 0.04 (0.02-0.07)	0.04 ±0.02 0.04 (0.02-0.07)	0.04 ±0.02 0.04 (0.02-0.06)
C5:1	0.09 ±0.05 0.08 (0.04-0.15)	0.10 ±0.06 0.09 (0.05-0.17)	0.09 ±0.05 0.08 (0.04-0.15)	0.09 ±0.03 0.09 (0.05-0.12)
C5OH	0.2 ±0.08 0.19 (0.12-0.31)	0.22 ±0.07 0.2 (0.15-0.32)	0.2 ±0.08 0.19 (0.12-0.3)	0.21 ±0.08 0.21 (0.13-0.32)
C6	0.09 ±0.04 0.08 (0.05-0.13)	0.1 ±0.03 0.09 (0.06-0.15)	0.09 ±0.04 0.08 (0.05-0.13)	0.1 ±0.04 0.09 (0.05-0.15)
C6DC	0.07 ±0.06 0.07 (0.04-0.11)	0.06 ±0.03 0.06 (0.03-0.09)	0.07 ±0.06 0.07 (0.04-0.11)	0.08 ±0.03 0.08 (0.04-0.12)
C8	0.1 ±0.04 0.09 (0.06-0.15)	0.11 ±0.05 0.1 (0.07-0.16)	0.1 ±0.04 0.09 (0.06-0.15)	0.09 ±0.03 0.09 (0.06-0.11)
C8:1	0.13 ±0.06 0.12 (0.07-0.2)	0.18 ±0.07 0.18 (0.1-0.27)	0.13 ±0.06 0.12 (0.07-0.2)	0.12 ±0.04 0.13 (0.07-0.16)
C10	0.09 ±0.04 0.09 (0.05-0.14)	0.1 ±0.04 0.11 (0.06-0.16)	0.09 ±0.04 0.09 (0.05-0.14)	0.1 ±0.04 0.09 (0.06-0.15)
C10:1	0.05 ±0.02 0.05 (0.03-0.08)	0.06 ±0.02 0.06 (0.04-0.08)	0.05 ±0.02 0.05 (0.03-0.08)	0.05 ±0.02 0.05 (0.03-0.08)
C10:2	0.03 ±0.01 0.02 (0.01-0.04)	0.03 ±0.02 0.02 (0.01-0.06)	0.03 ±0.01 0.02 (0.01-0.04)	0.03 ±0.01 0.03 (0.01-0.04)
C12	0.13 ±0.05 0.12 (0.08-0.2)	0.14 ±0.05 0.14 (0.07-0.2)	0.13 ±0.05 0.12 (0.08-0.2)	0.14 ±0.06 0.13 (0.08-0.19)
C12:1	0.06 ±0.03 0.06 (0.03-0.1)	0.06 ±0.03 0.05 (0.03-0.09)	0.06 ±0.03 0.06 (0.03-0.1)	0.07 ±0.04 0.06 (0.03-0.11)
C14	0.29 ±0.09 0.27 (0.18-0.4)	0.3 ±0.1 0.28 (0.2-0.45)	0.29 ±0.09 0.27 (0.18-0.4)	0.31 ±0.12 0.28 (0.17-0.42)
C14OH	0.04 ±0.02 0.03 (0.02-0.06)	0.03 ±0.01 0.03 (0.02-0.05)	0.04 ±0.02 0.03 (0.02-0.06)	0.04 ±0.02 0.03 (0.02-0.06)

C14:1	0.11 ±0.05	0.11 ±0.05	0.11 ±0.05	0.12 ±0.06
	0.1 (0.06-0.17)	0.11 (0.05-0.18)	0.1 (0.06-0.17)	0.11 (0.06-0.19)
C14:2	0.04 ±0.02	0.05 ±0.02	0.04 ±0.02	0.04 ±0.01
	0.03 (0.02-0.06)	0.04 (0.03-0.07)	0.03 (0.02-0.06)	0.04 (0.02-0.05)
C16	3.6 ±1.2	3.3 ±1.4	3.6 ±1.2	3.9 ±1.3
	3.4 (2.2-5.2)	3.2 (1.7-5.1)	3.4 (2.2-5.2)	3.9 (2.2-5.7)
C16OH	0.04 ±0.02	0.04 ±0.02	0.04 ±0.02	0.04 ±0.02
	0.04 (0.02-0.06)	0.03 (0.01-0.05)	0.04 (0.02-0.06)	0.04 (0.03-0.07)
C16:1	0.22 ±0.08	0.2 ±0.1	0.22 ±0.08	0.26 ±0.1
	0.2 (0.12-0.33)	0.18 (0.09-0.34)	0.2 (0.12-0.33)	0.25 (0.14-0.37)
C16:1OH	0.07 ±0.03	0.07 ±0.02	0.07 ±0.03	0.06 ±0.03
	0.06 (0.04- 0.1)	0.07 (0.05-0.1)	0.06 (0.04-0.1)	0.06 (0.04-0.11)
C18	1.1 ±0.3	1.0 ±0.3	1.1 ±0.3	1.1 ±0.4
	1.0 (0.7-1.5)	1.0 (0.6-1.5)	1.0 (0.7-1.5)	1.0 (0.7-1.6)
C18OH	0.02 ±0.01	0.02 ±0.02	0.02 ±0.01	0.03 ±0.01
	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)
C18:1	1.5 ±0.4	1.7 ±0.5	1.5 ±0.4	1.6 ±0.4
	1.4 (1.0-2.1)	1.7 (1.0-2.3)	1.4 (1.0-2.1)	1.5 (1.1-2.2)
C18:1OH	0.03 ±0.01	0.04 ±0.01	0.03 ±0.01	0.03 ±0.01
	0.03 (0.02-0.05)	0.03 (0.02-0.05)	0.03 (0.02-0.05)	0.03 (0.02-0.04)
C18:2	0.17 ±0.08	0.32 ±0.15	0.17 ±0.08	0.16 ±0.07
	0.16 (0.09-0.28)	0.28 (0.14-0.51)	0.16 (0.09-0.28)	0.16 (0.08-0.27)
C18:2OH	0.03 ±0.01	0.03 ±0.02	0.03 ±0.01	0.02 ±0.01
	0.02 (0.01-0.04)	0.03 (0.02-0.05)	0.02 (0.01-0.04)	0.02 (0.01-0.04)
Total	57 ±19	69 ±23	57 ±19	66 ±21
	54 (37-82)	63 (45-96)	54 (37-81)	61 (37-93)

AGA = appropriate-for-gestational age (birth weight between 10th to 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, C3DC: Malonylcarnitine, C4: Butanoylcarnitine, C4DC: Succinylcarnitine, C4OH: Hydroxybutyrylcarnitine, C5: Isovalerylcarnitine, C5DC: Glutarylcarnitine, C5OH: Hydroxyisovalerylcarnitine, C5:1: Tiglylcarnitine, C6: Hexanoylcarnitine, C6DC: Methylglutarylcarnitine, C8: Octanoylcarnitine, C8:1: Octenoylcarnitine, C10: Decanoylcarnitine, C10:1: Decenoylcarnitine, C10:2: Decadienoylcarnitine, C12: Dodecanoylcarnitine, C12:1: Dodecenoylcarnitine, C14: Tetradecanoylcarnitine, C14OH: 3-hydroxytetradecanoylcarnitine, C14:1: Tetradecenoylcarnitine, C14:2: Tetradecadienoylcarnitine, C16: Hexadecanoylcarnitine, C16OH: Hydroxyhexadecanoylcarnitine, C16:1: Hexadecenoylcarnitine, C16:1OH: 3-hydroxyhexadecenoycarnitine, C18: Octadecanoylcarnitine, C18OH: Hydroxyoctadecanoylcarnitine, C18:1: Oleylcarnitine, C18:1OH: 3-OH-oleylcarnitine, C18:2: Linoleoylcarnitine, C18:2OH: 3-OH-linoleoylcarnitine.