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CESAREAN SECTION – IMPACTS ON CHILD HEALTH

Henriina Hermansson

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University of Turku

Faculty of Medicine
Department of Clinical Medicine
Paediatrics
Doctoral Programme in Clinical Medicine

Supervised by

Professor Erika Isolauri MD, PhD
Paediatrics and Adolescent Medicine
University of Turku
Turku, Finland

Professor, Samuli Rautava MD, PhD
Children's Hospital
University of Helsinki
Helsinki, Finland

Reviewed by

Professor Terhi Tapiainen MD, PhD
Paediatrics and Adolescent Medicine
PEDEGO Research Unit
University of Oulu
Oulu, Finland

Associate Professor Jakob Stokholm, MD, PhD
COPSAC
University of Copenhagen
Copenhagen, Denmark

Opponent

Professor Per Ashorn
Faculty of Medicine and Health Technology
Tampere University
Tampere, Finland

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To my family

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ABSTRACT

The prevalence of chronic immune non-communicable diseases (NCD) and obesity has increased during recent decades in Western societies. Epidemiologic studies suggest that birth by Cesarean section (CS) delivery increases the risk of the development of NCD and obesity later in life. The overall aim of this thesis was to assess the long-term impacts of the mode of delivery on child health and improve our understanding of the mediating mechanisms.

In this study, birth by CS delivery was associated with the development of asthma and allergic disease and obesity in early adulthood. The potential mechanism explaining the association may include aberrant microbial exposure at birth by CS. Moreover, this study revealed that CS delivery has an independent impact on breast milk microbiota composition at one month after delivery, suggesting that the aberrant microbial exposure related to birth by CS is not restricted to the perinatal period. Pregnancy is considered a pro-inflammatory state, and the composition of the maternal gut microbiota changes throughout pregnancy. In this study, the inflammatory-toned gut microbiota remained unchanged one month after delivery, and the concentrations of inflammatory cytokines continued to increase in the postpartum period, suggesting that the inflammatory tone continues beyond the perinatal period.

Birth by CS delivery is associated with the development of NCD and obesity later in life. Microbial depletion related to the delivery mode and aberrant breast milk microbiota composition, as well as increased exposure to antibiotics during early life, may be the mechanism in the developmental programming of child health. Future research should focus on supporting the development of a healthy gut microbiota composition in the child

KEYWORDS: Mode of delivery, non-communicable disease, breast milk microbiota, maternal gut microbiota

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TIIVISTELMÄ

Krooniset, immuunivälitteiset, tarttumattomat taudit sekä lihavuus ovat yleistyneet länsimaissa viimeisten vuosikymmenien aikana. Samanaikaisesti keisarileikkauksella syntyneiden lasten lukumäärä on kasvanut maailmanlaajuisesti. Epidemiologisissa tutkimuksissa keisarileikkauksella syntyneillä lapsilla on havaittu suurentunut riski sairastua kroonisiin ei-tarttuviin tauteihin ja lihavuuteen verrattuna alateitse syntyneisiin lapsiin. Tämän väitöstutkimuksen tavoitteena on selvittää syntymätavan vaikutusta lapsen myöhempään terveyteen sekä selvittää mahdollisia mekanismeja, joilla nämä vaikutukset välittyvät.

Tutkimuksessa havaittiin, että keisarileikkauksella syntyneillä oli enemmän astmaa ja allergisia sairauksia sekä lihavuutta 21 vuoden iässä. Lisäksi tutkimuksessa havaittiin keisarileikkauksella synnyttäneiden äitien rintamaidon mikrobiston koostumuksessa olevan eroja alateitse synnyttäneiden äitien rintamaidon mikrobistoon verrattuna. Erot rintamaidon mikrobistossa eivät selittyneet äidille synnytyksen yhteydessä annetulla antibioottihoidolla. Raskausaikana tiedetään, että äidin suoliston mikrobisto muuttuu ja tulehdusta lisäävien bakteerien suhteellinen osuus lisääntyy raskauden aikana. Tutkimuksessa todettiin, että nämä raskauden aikaiset muutokset äidin suoliston mikrobistossa säilyivät synnytyksen jälkeen ja äidin seerumista mitattujen sytokiinien pitoisuudet myös nousivat raskauden jälkeen ja ylläpitivät siten elimistön tulehduksellista tilaa raskauden päättymisen jälkeen.

Syntymä keisarileikkauksella lisää riskiä sairastua ei-tarttuviin tauteihin sekä lihavuuteen myöhemmällä iällä. Yhteys selittynee erilaisella varhaisella mikrobikontaktilla. Keisarileikkausten määrän pysyessä entisellään, tulisi tulevaisuudessa selvittää turvallisia keinoja tämän varhaisen mikrobikontaktin muokkaamiseen.

AVAINSANAT: Keisarileikkaus, ei-tarttuvat taudit, rintamaidon mikrobisto, äidin suoliston mikrobisto

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Abbreviations

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANOSIM	Analysis of similarities
CS	Cesarean section
DOHaD	Developmental origins of health and disease
GBS	Group B Streptococcus *(Streptococcus agalactiae)*
GDM	Gestational diabetes mellitus
IAP	Intrapartum antibiotics
IL-6	Interleukin -6
IL-8	Interleukin -8
MCP-1	Monocyte chemotactic protein -1
NICU	Neonatal intensive care unit
OTU	Operational taxonomical unit
PCoA	Principal coordinate analysis
qPCR	Quantitative polymerase chain reaction
RDA	Redundancy discrimination analysis
TNFα	Tumor necrosis factor alfa
VD	Vaginal delivery

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hermansson Henriina, Rautava Samuli, Löyttyniemi Eliisa, Salminen Seppo, Isolauri Erika. Pregnancy-induced proinflammatory immunological tone and gut microbiota profile are not reversed at the delivery. *Austin J Public Health Epidemiol 2022;9(4):1135*

II Hermansson Henriina, Hoppu Ulla, Isolauri Erika. Elective caesarean section is associated lower levels of adiponectin in cord blood. *Neonatology 2014;105(3):172–4.*

III Miettinen Reetta, Hermansson Henriina, Merikukka Marko, Gissler Mika, Isolauri Erika. Mode of delivery – impact on risk of noncommunicable diseases. *J Allergy Clin Immunol. 2015 Nov;136(5):1398–9*

IV Hermansson Henriina, Kumar Himanshu, Collado Maria Carmen, Salminen Seppo, Isolauri Erika, Rautava Samuli. Breast milk microbiota is shaped by mode of delivery and intrapartum antibiotic exposure. *Front Nutr. 2019 Feb 4;6:4*

1 Introduction

Delivering a human child is demanding compared with other primates and wild animals. The delivery is often prolonged, frequently requiring psychological, medical or surgical birth assistance. The head of the fetus is relatively large, and during birth, the fetus must undergo a series of rotations to reach the largest dimensions of the pelvic girdle in order to successfully pass the narrow bony birth canal (Wittman et al., 2007).

In 1960, Sherwood L. Washburn (Pavličev et al., 2020) presented this mismatch of the size of the fetal head and maternal pelvis as an obstetric dilemma – the female bony pelvis was considered an evolutionary compromise between the requirements of successful childbirth and bipedal locomotion. The evolution of human bipedalism began more than five million years ago, and the upright walking position led to the remodelling of the structure of the locomotor system, including the pelvis and pelvic girdle, where the relatively narrow bony birth canal is located. Further, the upright walking position enabled the development of the brain, and thus the size of the fetal head increased much later in human evolution. Notwithstanding these points, it has been argued that the narrow pelvis is disadvantageous to childbirth because it offers better support to carry the pregnancy until term (Pavličev et al., 2020).

The discrepancy in the size of the birth canal and fetal head makes child delivery complicated, creating a need for surgical assistance during birth, which is today known as Cesarean section (CS). Initially, CS was a hazardous surgical procedure in which the child's life was attempted to be saved at the cost of maternal health. During the development of modern medicine, CS delivery has become safer for both the mother and the child, but still, it remains unclear whether improved survival from the operation provides a guarantee of a later healthier life.

To date, CS is one of the most common surgical procedures in the world. In 2010–2018, a total of 21% of all children in the world were born by CS, although the frequency varies geographically, from 5% in sub-Saharan Africa to 43% in Latin America and the Caribbean (Betran et al., 2021; Boerma et al., 2018).

In parallel with the rising prevalence of CS deliveries, epidemiological studies show an association between CS delivery and the risk of developing asthma and allergic diseases and overweight and obesity (H. T. Li et al., 2013; Sevelsted et al.,

2015). This phenomenon has been explained by different microbial exposure during the birth. Current literature suggests that the human gut microbiome and its shift in composition early in life, dysbiosis, is also linked to the development of non-communicable diseases (NCD) and obesity (Gómez-Gallego et al., 2019). Currently, it is not known whether the connection is causal and, moreover, whether CS delivery induces dysbiosis and thereby a heightened risk of NCD.

2 Review of the Literature

2.1 Cesarean section – a historical perspective

The birth of a child through the opening of the mother's abdominal wall has been described in history ever since ancient times. According to Greek mythology, Asclepius, the god of medicine, was delivered by this method that we today know as Cesarean section (CS), performed by his father, Apollo, after his mother, Coronis, had died (Thomas et al., 2000). Another narrative of abdominal delivery was related to Scipio Africanus, born in 237 BC, the Roman general who defeated Hannibal (Thomas et al., 2000). Despite the name Cesarean section, the name does not originate from the birth of Julius Caesar (Van Dongen, 2009). Numa Pompilius, the second king of Rome, ordained the law *lex Regia* in 715 BC, in which he decreed that if a pregnant woman died, the child must be cut out of her abdomen. Later, the name of this law became *lex Caesarea* when Julius Caesar became the emperor of Rome (Thomas et al., 2000; Todman, 2007).

It has been speculated that the name CS may be derived from the Latin verb *caedare,* which means to cut, and Cesarean delivery means *delivery by cutting* (Van Dongen, 2009). Those born by CS in ancient times were referred to as *caesones,* probably to emphasise the importance of not having been born vaginally. Several ancient heroes were born by CS, but the actual numbers of children born by this method remain unknown, as does the future of the survivors (Lurie, 2005; Van Dongen, 2009).

In 1598, Jacques Guillimeau, a French surgeon, used the term *la section Caesarienne* for the first time in a book on midwifery, and gradually, this surgical procedure became known as CS (Todman, 2007). Before the Renaissance, CS delivery was performed when the mother was dead or dying to save the child. If the rescue of the child did not succeed, the mother and the child were buried separately. In some exceptions, CS was performed on living mothers, but it was highly unlikely that any woman could have survived this operation due to the circumstances of that time (Todman, 2007). The first successful CS, in which both mother and child survived, was performed in the 1500s in Switzerland. However, the context of this has been called into question, because the documentation of the event was inadequate (Lurie, 2005; Todman, 2007).

At the time of the Renaissance, knowledge of human anatomy was incomplete. In 1543, Andreas Vesalius published the book *De Corporis Humani Fabrica,* in which he described the female pelvic anatomy and abdominal structures, providing a theoretical basis for operative obstetrics (Todman, 2007). In the mid-to-late 1800s, dissection became an essential part of medical education, enabling more profound knowledge of anatomy, even though this practice was available exclusively to male students. Between the 17th and 18th centuries, the profession of man-midwife and obstetrician was established, while women were assigned as birth attendants (Todman, 2007).

The development of anaesthesia in the 19th century opened new possibilities for CS. Chloroform was the first anaesthetic successfully used during childbirth. However, for religious reasons, it was forbidden to use anaesthetics for pain relief during childbirth until Queen Victoria used chloroform during the birth of Prince Leopold in 1853 and Beatrice in 1857. After that, the use of chloroform became hastily popular for obstetric pain relief in the upper classes of society, and it was widely used in CS operations (Todman, 2007).

Despite the increasing knowledge of human anatomy and the development and use of anaesthesia during CS, women could not survive the operation because of complications such as haemorrhage and septicaemia (Todman, 2007). The survival of the neonates was also low: records from Berlin in 1864 reported that only three infants out of 147 survived after a postmortem CS (Lurie, 2005; Thomas et al., 2000). Suturing the uterine wall was generally believed to be unnecessary until 1870. In 1876, the Italian obstetrician Eduardo Porro performed a hysterectomy simultaneously with CS to avoid uterine haemorrhage and peritonitis. Using the Porro method, maternal mortality decreased, and newborn survival increased, although at the cost of maternal fertility (Todman, 2007).

The major development in operation techniques in CS was in 1882 when two German obstetricians, Max Sänger and Adolf Kehrer, discovered the method of suturing the uterine wound with silver wire (Todman, 2007). In the 19th century, it was noticed that asepsis, mainly handwashing, reduced puerperal fever. Carbolic lister was introduced in 1867; the spray was used during the operation to keep the air above the surgical site free from bacteria (Todman, 2007). Along with these improvements, CS became safer, which in turn led to the operation being performed before the mother's exhaustion. At the end of the 19th century, maternal mortality in the case of CS decreased dramatically, from 65–75% at the beginning of the century to 5–10% at the end of it (Todman, 2007).

At the beginning of the 20th century, the development of the surgical techniques of CS continued. Lawson Tait, a surgeon from Birmingham, United Kingdom, suggested for the first time the application of CS in the case of placenta praevia, the condition which today is one of the most common indications for elective CS.

However, the operation was often performed early in the third trimester, and due to the lack of neonatal care, many premature newborns did not survive (Todman, 2007). Other indications for CS included protracted labour, fetal distress, malpresentation of the fetus, previously scarred uterus and placental abnormalities associated with bleeding (Lurie, 2005; Todman, 2007). In point of fact, the indications have not changed, but their definitions have become more accurate thanks to the introduction of electrical monitoring of the fetus, improved neonatal care and the concept of evidence-based medicine. Improved surgical techniques, the use of blood transfusions, asepsis, including the use of antibiotics (sulphonamides in 1935 and penicillin in 1941) and the attitudes towards the safety of both mother and child further decreased the mortality in the case of CS, which was 0.1% in 1952 (Lurie, 2005).

In 1916, Edwin Craigin gave his presentation entitled *"Once a cesarean, always a cesarean"* to the New York Medical Society (Todman, 2007). At that time, the incidence of CS delivery was low, and the operation itself was hazardous, partly due to the incision technique. In the 1940s, the incision in the lower transversal segment was widely accepted since it prevented uterine ruptures in future pregnancies (Thomas et al., 2000). In 1949, the Twelfth Congress of Obstetrics and Gynaecology proclaimed that Cesarean section by this surgical technique is safe for both mothers and children, so consequently, the rates of CS deliveries started to increase worldwide, especially in Western societies (Todman, 2007).

2.2 Cesarean section – current perspective

There are several reasons for CS, both medical and non-medical. While the medical indications, such as protracted labour, malpresentation or fetal distress, have remained relatively unchanged over the decades, their definitions have transformed in parallel with the development of the operation technique and monitoring systems; for example, the development of fetal cardiotocography (Lewis et al., 2015). The number of repeated CSs has expanded in parallel with the increasing CS rates, although recent studies show that vaginal delivery is safe after previous CS (Morton et al., 2020; Zhang et al., 2011), challenging Edwin Craig's presentation from 1916. It is suggested that labour induction, mechanical or pharmacological attempts to initiate vaginal delivery, might be associated with increasing the rates of CS. Studies suggest that the risk of CS in labour induction is associated with maternal conditions such as higher maternal age and adverse maternal metabolic health (Morton et al., 2020; Tolcher et al., 2015).

Today more people are obese or overweight than underweight in all areas of the world except for sub-Saharan Africa and some regions in Asia (Bentham et al., 2017). Correspondingly, the prevalence of overweight and obesity has increased

among pregnant women (Di Cesare et al., 2016). Maternal overweight and obesity in pregnancy have various adverse effects on pregnancy outcomes (Catalano et al., 2017; Di Cesare et al., 2016). Overweight or obese pregnant women are at increased risk for congenital anomalies, preterm births and stillbirths (Patrick M. Catalano et al., 2017). Maternal overweight and obesity increase the risk of subclinical maternal metabolic dysfunction such as gestational diabetes mellitus (GDM) and pre-eclampsia, conditions that manifest during late pregnancy and may cause both infant macrosomia or large-for-gestational-age neonates or fetal growth restriction (Patrick M. Catalano et al., 2017). Maternal overweight and obesity may disturb spontaneous delivery, complicate both mechanical and pharmacological labour induction and, consequently, increase the risk of both elective and non-elective CS (Tolcher et al., 2015). Surgical complications related to CS, such as scar infections, are more common among overweight and obese mothers than among normal-weight mothers (Patrick M. Catalano et al., 2017).

To date, non-medical reasons for CS reportedly exceed medical reasons (Morton et al., 2020; Stjernholm et al., 2010) in middle- and high-income societies, although contradictory data have been presented (Mazzoni et al., 2011). Non-medical reasons and maternal requests for CS include a variety of psychosocial, political, financial, cultural and religious reasons. Psychosocial reasons such as fear of giving birth, previous negative maternal experience, misinformation regarding the safety of CS to the baby as well as the mother's autonomy on the decision of the delivery method have been cited as reasons to request CS (Eide et al., 2019; Stjernholm et al., 2010). Studies from Brazil and Argentina, where the rate of CS is high, report that only a few pregnant women actually prefer CS to vaginal delivery even though the number of elective CS in these countries is high (Mazzoni et al., 2016; Potter et al., 2008). The physicians' preference, as well as financial incentives for both physicians and delivery hospitals, may partly explain the increasing trend for CS (Chen et al., 2014). As one of the socioeconomic factors, studies show that women with private health insurance undergo CS more frequently than women with public health insurance (Hoxha et al., 2017). In Mexico, high rates of CS were seen in private hospitals and in women with higher socioeconomic status. National policies attempting to reduce maternal and infant mortality have led to the medicalisation of childbirth (Freyermuth et al., 2017). The cash transfer programme, in which families receive monetary incentives when participating in certain prenatal care visits, has increased the rates of CS in rural areas of Mexico, and the risk of CS has been correlated with the number of individuals participating in these visits (Barber, 2010; Freyermuth et al., 2017). In the US, the tax income system is more favourable for mothers who deliver a child by either labour induction or elective CS, with a greater benefit in December than in January (Schulkind et al., 2014). Schulkind and colleagues also demonstrated in the same study that the earlier timing of birth, even in full-term

deliveries, resulted in lower Apgar scores and increased risk of lower birth weight (Schulkind et al., 2014).

In Chinese culture, there is strong gender preference, and this may be seen already during pregnancy. Among Chinese women living in the US, it has been observed that male fetuses were more likely delivered by CS than female fetuses as compared with the situation of Japanese American pregnancies (Shen et al., 2020). In Chinese culture, there is a strong belief that being born on a certain auspicious day, according to the Chinese lunar calendar, will positively impact the child's future. Lo and colleagues noted that the rate of CS was significantly higher on auspicious dates compared with other days (Lo, 2003). Similarly, the number of CS deliveries was lower on the inauspicious dates, suggesting that culture plays a significant role in scheduling the birth (Lo, 2003). In addition, it was observed that among Chinese women living in the US, there are more births by CS than on other days on the auspicious dates of the Chinese calendar, and in fact, CS deliveries were less planned on known unlucky days. In the US, it seems that neither Chinese Americans nor White Americans are willing to deliver on the 13th of the month (Almond et al., 2015): the impact of specific birth dates is not restricted to Asian cultures. Moreover, in the US, the rate of CS increases on Valentine's Day, whereas a decreased rate of CS has been reported on Halloween (Levy et al., 2011). Similarly, the rate of CS deliveries is lower on February 29th as well as on April 1st, which are both considered unlucky dates (Gans et al., 2011).

Since the non-medical reasons for CS exceed its medical reasons, the question of their justification has been raised. In 1985, the WHO made a statement arguing that when it comes to the CS rate, "There is no justification for any region to have higher than 10–15%" (WHO 1985). This notion was partly based on the fact that in the Nordic countries, the rates of CS deliveries had not been increasing as rapidly as in the other middle- and high-income countries while the infant mortality had remained the lowest in the world. In Finland, the rate of CS has remained stable during the past decades, approximately 15% from 1987 to 2018 (THL 2022). However, the rate of CS has slightly increased from 2019, being highest in 2021 when 19.6% of deliveries were performed by CS. The ratio of CS performed for non-medical reasons is not available in Finnish birth registers, but according to current reports, the rise in CS deliveries is due to higher maternal age and maternal obesity (Kruit et al., 2022; THL, 2022). In Finland, perinatal mortality, defined as the number of stillbirths and deaths during the first week of life per 1,000 births, has decreased during the 21st century, being 5.8 per 1000 births at the beginning of the century and 3.4 in 2021 (THL 2022, 2022). The perinatal mortality rate in Finland is one of the lowest in the world.

Even though the WHO statement has been revised in recent years, according to recent studies, it still seems that higher rates of CS may decrease the benefits to

children and its risks are increasing, while geographical inequality still remains (Betrán et al., 2016; Molina et al., 2015; Xie et al., 2015; Ye et al., 2016).

2.3 Pregnancy related adaptations in the mother

2.3.1 Immunology

Pregnancy is associated with a unique inflammatory environment in which the maternal-fetal interface acts synergistically (Mor et al., 2017). Throughout pregnancy, the level of inflammation alternates between a pro-inflammatory state, in which specific cytokines increase inflammation, and an anti-inflammatory state, in which the active cytokine profile is the opposite. Placental implantation is considered a pro-inflammatory state, whereas mid-gestation, during which most fetal growth occurs, is considered an anti-inflammatory state (Mor et al., 2017). At the time of labour, pro-inflammatory activity increases again. High concentrations of neutrophils and macrophages have been detected in placental tissues, and the production of pro-inflammatory cytokines, mainly interleukin 6 (IL-6) and also interleukin 8 (IL-8), increases (Christiaens et al., 2008). A pro-inflammatory tone is present during labour in term and preterm deliveries in the absence of clinical infection (Chan et al., 2013). The differences in cytokine concentrations between venous and arterial blood samples suggest the fetal origins of specific pro-inflammatory cytokines (Chan et al., 2013). The pro-inflammatory stimuli initiating labour are absent in CS delivery, especially in elective CS. Several studies show that the cytokine profile in cord blood in newborns born by elective CS is different compared with vaginally born newborns (Keski-Nisula et al., 2010; Nandanan et al., 2019; Tadaki et al., 2009; Werlang et al., 2018). Recent studies have shown differences in the profile of cord blood immune cells and their functions in children born by elective CS compared to vaginally born children (Sundqvist et al., 2013; Thysen et al., 2015). Moreover, it has been reported that children born by CS delivery display reduced Th1 responses and lower levels of immunoglobulin-producing cells during the first years of life (Huurre et al., 2008; Jakobsson et al., 2014), and the aberrant Th1 maturation profile has been linked to later allergy development (Prescott et al., 1999). Current literature suggests that labour may act as a natural stimulus in fetal and neonatal immune system development and that the absence of this pro-inflammatory stimulation may have long-lasting effects on immune system development in the child.

2.3.2 Metabolic system

Pregnancy is associated with various metabolic and hormonal adaptations in women, all of which aim to ensure the normal growth and development of the fetus. Maternal glucose and lipid metabolism in particular change throughout pregnancy (Lain et al., 2007). During the first two trimesters of pregnancy, maternal subcutaneous fat increases to meet the increased maternal and fetal energy demands and to support lactation (Herrera et al., 2016). In the third trimester, insulin resistance increases, resulting in an increase in maternal blood glucose and free fatty acid concentrations ensuring fetal growth (Lain et al., 2007). In fact, pregnancy is considered a physiologically diabetogenic state, in which normal glucose metabolism and insulin sensitivity change dramatically. Moreover, maternal adipose tissue acts as an active endocrine organ during pregnancy by producing several cytokines that may increase insulin sensitivity, such as tumour necrosis factor alfa (TNF-a) or decrease insulin resistance, such as adiponectin (Catalano, 2014). Adverse maternal metabolic conditions, including pre-gravid overweight and obesity, high gestational weight gain and maternal gestational diabetes, may emphasise these metabolic changes (Herrera et al., 2016; Parrettini et al., 2020).

2.3.3 Maternal gut microbiota

Maternal microbiota, consisting of both vaginal and intestinal gut microbiota, change throughout pregnancy as a response to physiological adaptations. Maternal gut microbiota, assessed from faecal samples, changes dramatically from the first trimester of pregnancy towards the third trimester of pregnancy (Koren et al., 2012). Throughout a normal, healthy pregnancy, the relative abundances of potentially pro-inflammatory Proteobacteria and Actinobacteria phyla are increased while the abundance of the butyrate-producing anti-inflammatory bacteria Faecalibacterium is decreased (Koren et al., 2012). Similar pro-inflammatory trends in the gut microbiota have been observed in humans with metabolic syndrome, overweight or obesity, as well as states of insulin resistance and hypertension, diseases in which low-grade inflammation is present (Tremaroli et al., 2012). Current literature suggests that the human gut microbiota has a role in the development of obesity (Koren et al., 2012). Commensal gut microbiota impacts the host metabolic and nutritional status by affecting energy harvest from the host diet and energy storage as adipose tissue. Maternal prepregnancy weight and weight gain during pregnancy further shape gut microbial composition (Collado et al., 2008; Santacruz et al., 2010). Moreover, adverse metabolic states during pregnancy, such as maternal gestational diabetes mellitus, impact the maternal microbial composition (Crusell et al., 2018). In fact, changes in the maternal gut microbiota composition have been shown to precede the development of adverse glucose tolerance (Ma et al., 2020).

Maternal gut microbiota plays significant role in newborn gut colonization. According to current study, during the first week of life 72% of microbial population of newborn gut was transmitted from the mothers (Li et al., 2021). The majority of vertical transmission of microbes from mothers to infants originates from maternal gut microbiota, even though common bacterial strains has been found in maternal vaginal, skin and oral microbiota but to a lesser extent (Ferretti et al., 2018). Current literature suggest that maternal gut microbiota is the most important source for the newborn and infant gut microbiota due to the niche specificity of the microbes (Ferretti et al., 2018; Li et al., 2021).

2.4 Developmental origins of health and disease

Suboptimal environmental exposures during the critical window of fetal and infant programming have been suggested to be associated with long-term health development. This theory, formerly known as the "Barker hypothesis" after Charles Hales and David Barker, is based on the observation that fetal malnutrition is associated with hypertension later in life (Hales et al., 1992). Today, this theory is known as the Developmental Origins of Health and Disease (DOHaD) theory. The field of early microbiome was not originally part of DOHaD, although current literature suggests that early microbial exposures are considered to have a significant impact on immune development as described by the hygiene hypothesis of allergic diseases. The original hygiene hypothesis by J. Gerrard and colleagues in 1976 (Gerrard et al., 1976) and later by D. Strachan 1989 suggested that the lack of exposure to viral infections during childhood may predispose to the development of allergic and atopic diseases later in life (Strachan, 1989). Since then, this hypothesis has been revised, and current literature suggests that it is rather the lack of exposure to commensal, environmental microbes rather than infections which may predispose to altered immune modulation in the host and further the development of chronic inflammatory non-communicable diseases (Pfefferle et al., 2021).

According to current literature, birth by CS delivery may predispose a child to development of specific NCDs later in life. Epidemiological studies suggest that birth by CS delivery may increase risk of development of asthma and atopic diseases (Chu et al., 2017; Pistiner et al., 2008; Renz-Polster et al., 2005; Roduit et al., 2009; Thavagnanam et al., 2008), celiac disease and inflammatory bowel disease (Peter Bager et al., 2012; Decker et al., 2010; Mårild et al., 2012), diabetes (Cardwell et al., 2008) and rheumatoid arthritis (Sevelsted et al., 2015). According to current literature aberrant microbial exposure during perinatal period, such as CS delivery, intrapartum antibiotic exposure, short duration of breastfeeding, may link the birth by CS and the development of these NCDs later in life (Galazzo et al., 2020).

2.5 Cesarean section delivery from the child's perspective

2.5.1 Short term impacts on the newborn

CS can be a lifesaving procedure when performed in specific situations, for example, fetal distress. However, studies show that infants born by elective CS have a higher incidence of respiratory distress, hypoglycaemia and difficulties in maintaining body temperature (Karlström et al., 2013). The risk of neonatal admission to intensive care units (NICU) is higher in neonates born by elective CS compared with those born by vaginal delivery (Jastrow et al., 2008). In the case of elective CS, the lack of newborn stress reaction may disturb the initial adaptation after birth in the newborn. However, studies show that despite current obstetric guidelines, a considerable number of elective CSs are performed before 39 weeks of gestation, and thus the relative immaturity of the newborn at the time of the delivery may affect the initial adaptation processes to extrauterine life (Jastrow et al., 2008; Tita et al., 2009). Because of the higher incidence of NICU admissions, newborn and parental separation is more common in newborns born by both elective and non-elective operations. This may cause a variety of negative outcomes, such as anxiety and concern in the parents (Obeidat et al., 2009).

2.5.2 Cesarean section and breastfeeding

Breast milk is considered the gold standard of the newborn's nutrition. The WHO recommends the early initiation of breastfeeding within one hour of birth and exclusive breastfeeding for the first six months of life (WHO, 2019). According to current literature, breastfeeding is associated with several beneficial effects on both the mother and the child. Breastfeeding reduces the risk of breast and ovarian carcinoma and type 2 diabetes in the mother (Chowdhury et al., 2015). In children, exclusive breastfeeding is associated with lower morbidity and mortality to infectious diseases, dental malocclusions and higher intelligence compared with those who are not breastfed or breastfed only for a short time (Christensen et al., 2020; Peres et al., 2015; Sankar et al., 2015; Victora et al., 2015), whereas prolonged breastfeeding and nocturnal breastfeeding have been associated with dental caries (Peres et al., 2017).

Despite its nutritional and immunological benefits for newborns and long-term health benefits for both child and mother, only a minority of infants meet the WHO recommendations for breastfeeding exclusivity and duration, mainly in high-income countries (Victora et al., 2016). Several sociodemographic, socioeconomic lifestyle and maternal health factors exert an effect on breastfeeding initiation and

continuation. Tobacco smoking, low maternal education level and adverse maternal health factors, such as high prepregnancy body mass index (BMI), negatively affect breastfeeding (Wallwiener et al., 2016). Global epidemiological data suggest that breastfeeding is more common in low- and middle-income countries when compared with high-income countries. Moreover, in low- and middle-income countries, a longer duration of breastfeeding is seen among poor mothers, whereas in high-income countries, the opposite pattern is also seen (Victora et al., 2016).

According to the literature, CS delivery, both elective and non-elective, may have a negative effect on the initiation and total duration of breastfeeding (Hobbs et al., 2016; Wallwiener et al., 2016). Delivery by CS is associated with delayed initiation of breastfeeding and shorter duration of both exclusive and total breastfeeding (Cohen et al., 2018; Hobbs et al., 2016). Immediate skin-to-skin contact after delivery is considered important for lactogenesis, which may be delayed after CS deliveries (Lau et al., 2018). Newborns born by CS receive lower amounts of breast milk and show slower weight gain during the first week of life, suggesting that lactogenesis itself may be delayed due to the CS operation (Evans et al., 2003). However, a study conducted in Canada showed that women who deliver by elective CS had no intention to breastfeed or did not initiate breastfeeding as compared with vaginally delivered women, whereas women undergoing emergency CS had more breastfeeding difficulties and used more breastfeeding guidance before and after leaving the hospital compared with women with elective CS and vaginal delivery (Hobbs et al., 2016). In a Swedish study, birth emotional stress during pregnancy and CS delivery were associated with lower exclusive breastfeeding rates (Cato et al., 2017). Newborn admission to the NICU and maternal anxiety are considered to negatively impact breastfeeding. Regarding the total duration of breastfeeding, CS delivery and maternal anxiety or depressive symptoms have been reported to be associated with a shorter duration of exclusive breastfeeding and a shorter total duration of breastfeeding (Wallwiener et al., 2016).

The composition of breast milk varies throughout lactation. Several bioactive components of breast milk, including human milk oligosaccharides (HMO), promote the maturation of healthy gut microbiota and immune defence mechanisms (Victora et al., 2016). The HMOs in breast milk favour the growth of specific bifidobacteria in the newborn gut (Azad et al., 2013a; Fallani et al., 2010; Tannock et al., 2013). Exclusively breastfed newborns exhibit lower richness and less diverse gut microbiota composition than formula-fed newborns (Marcobal et al., 2012).

Recent studies show that breast milk harbors a unique microbial composition, and it is suggested that these bacteria may seed the infant gut microbiota (Pannaraj et al., 2017). The origin of milk bacteria remains unclear, however it has been suggested that there is an entero-mammary-pathway, which allows bacterial

translocation from the maternal gut to the mammary gland. Labor may act as a trigger to increase intestinal permeability, and thus enhance bacterial translocation in the maternal gut and consequently transfer bacteria to breast milk (Nagpal et al., 2017). Other theory suggest that breast milk microbiota originates from the aerola skin and infant oral cavity (Moossavi et al., 2020). In addition to the high interindividual variation seen in breast milk microbiota composition, there are several factors that may affect the microbial composition of breast milk such as maternal obesity, mode of delivery and exposure to intrapartum antibiotics, gestational age and parity, lactational stage and feeding method (Zimmermann et al., 2020). As intrapartum antibiotics are almost all cases administered during the CS delivery, it is not known whether the effect of CS delivery in the breast milk microbiota is mediated through exposure to intrapartum antibiotic procedure.

2.5.3 Cesarean section and the newborn gut microbiota

The mode of delivery is the most important driver of the initial microbial colonisation of the newborn. While passing through the maternal birth canal, the newborn is exposed to various microbes from the maternal gut and vaginal microbiota (Dominguez-Bello et al., 2010). Immediately after birth, the newborn is homogeneously colonised across the body sites by bacteria resembling those of the maternal vaginal microbiota. The very first microbial colonisers are *Lactobacillus, Prevotella Atopium* and *Sneathia* (Dominguez-Bello et al., 2010). After birth, *Bifidobacteria, Bacteroides* and *Escherichia* are the most abundant bacteria in newborn gut microbiota as assessed by faecal samples (C. J. Hill et al., 2017; Jakobsson et al., 2014; Reyman et al., 2019; Shao et al., 2020). These anaerobic bacteria do not grow outside the gut and thus are suggested to originate from the mother's gut microbiota (Dominguez-Bello et al., 2010). In fact, studies show that newborn gut microbiota composition resembles that of their mother, suggesting vertical mother-newborn transmission of intestinal gut bacteria during vaginal delivery (Bäckhed et al., 2015; Grönlund et al., 2011; Li et al., 2021). After birth, breast milk and its oligosaccharides modify neonatal and infant gut microbiota composition by favouring the growth of specific *Bifidobacterium* species (Marcobal et al., 2012). Infants receiving formula show differences in gut microbiota composition as compared to vaginally born, breastfed infants (Fallani et al., 2010).

In CS delivery, the newborn bypasses this vertical bacterial transmission from the mother, and the initial microbiota of the newborn consists of common bacteria originating from the hospital environment, such as *Enterococcus, Klebsiella* and *Staphylococcus epidermidis* (Shao et al., 2020; Shin et al., 2015). After that, the gut microbiota composition of CS-born infants shows higher abundances of *Klebsiella*

and *Enterococcus* and a delayed establishment of *Bacteroides* spp. (Jakobsson et al., 2014; Reyman et al., 2019; Shao et al., 2020), when compared to vaginally delivered newborns. *Klebsiella* and *Enterococcus* show potential as opportunistic bacteria and are often responsible for nosocomial infections (Shao et al., 2020). Studies suggest that the delay in initial bacterial colonisation in species such as those belonging to the genera *Bifidobacterium* and *Bacteroides* in infants born by CS may allow the growth of these opportunistic bacteria in the infant gut (Shao et al., 2020). After birth, breastfeeding restores some of the delivery-associated changes in bacterial composition in the infant gut microbiota, but there are studies suggesting that breastfeeding does not compensate for the lack of early *Bifidobacterium* colonisation in CS-delivered infants (Liu et al., 2019; Reyman et al., 2019).

Antibiotic prophylaxis is recommended for every woman undergoing CS delivery, both elective and non-elective CS. According to current obstetric guidelines, antibiotic prophylaxis should be given 60 minutes before the skin incision to achieve the appropriate concentrations in tissues to prevent surgical site infections (Yuan et al., 2016). Antibiotic prophylaxis in CS significantly reduces maternal wound infections, endometritis and severe infectious complications, but its effects on newborn gut microbiota composition and early immunological programming are less well-known (Caughey et al., 2018). Historically, antibiotics were given after cord clamping to reduce the potential harms of fetal exposure to the medicine, but since antibiotic prophylaxis seems to be more effective in preventing maternal infections complications when administered before the skin incision, recent guidelines recommend the antibiotic prophylaxis before the skin incision in CS (Kaimal et al., 2008; Smaill et al., 2014; Sullivan et al., 2007). Winther and colleagues have recently questioned the timing of antibiotic prophylaxis, because severe postpartum infections are rare, and the effects of antibiotic administration on the newborn gut microbiota are unknown; thus, they suggested the administration of antibiotic prophylaxis after cord clamping (Winther et al., 2020). However, Kamal and colleagues showed that the timing of prophylactic antibiotic administration in mothers undergoing CS was not associated with significant differences in infant gut microbiota composition at nine months old (Kamal et al., 2019). In addition prophylactic antibiotic administration in surgical procedures, intrapartum antibiotics are commonly used in vaginal deliveries on the suspicion of maternal infection but also to prevent early neonatal infections. Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a common colonizer in maternal vaginal microbiota. Vertical transmission of maternal GBS to newborn, may cause severe and fatal neonatal infection. Perinatal screening of GBS and the prophylactic antibiotic administration to mothers during the delivery has decreased neonatal GBS infections (Shane et al., 2017). Intrapartum antibiotics reportedly reduce the gut microbiota bacterial richness in infants until 1 year of age and it seems that the type of antibiotics administered

may further affect the microbiota composition (Di Renzo et al., 2015). Regardless of the delivery mode breast feeding may modify the effect of intrapartum antibiotics in CS delivered infant and vaginally born infants exposed to maternal intrapartum antibiotics (Azad et al., 2016).

3 Aims

The overall aim of this thesis was to uncover the long-term impacts of the mode of delivery on child health and improve our understanding of the mediating mechanisms. The principal aim of this study was:

> To assess, whether birth by CS delivery is associated with the risk of developing of chronic NCDs.

The targeted aims of the study are:

> To demonstrate whether birth by CS contributes to the development of obesity later in life and uncover potential mechanisms.
>
> To investigate how CS delivery impacts on the microbiota in the maternal gut and breast milk.
>
> To characterise how CS delivery contributes to systemic inflammation in the perinatal period.

4 Materials and Methods

4.1 Study I

4.1.1 Study population

The mothers in Study I were selected from an ongoing randomised controlled clinical trial assessing the impact of maternal probiotic supplementation on the occurrence of atopic dermatitis (Rautava et al., 2012). The mothers in the current study were selected based on blood and faecal sample availability. In the original study, a probiotic combination consisting of either *Lacticaseibacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 or *Lactobacillus paracasei* ST11 and *Bifidobacterium longum* BL999 or placebo was administered beginning two months before the expected delivery and continuing until two months after delivery. The inclusion criteria of the study were labour after 36 weeks of gestation after an uncomplicated pregnancy. Written informed consent was obtained from all participants. The Ethics Committee of the Hospital District of Southwest Finland approved the study.

4.1.2 Study design

Among participating women, 10 women who delivered by elective CS and 13 women with non-elective CS were selected for the nested case-control study. Women who had delivered vaginally (n=23) were chosen as controls. Pregnant women were matched for prepregnancy body mass index, probiotic intervention during pregnancy and antibiotic exposure during pregnancy and labour. Blood and faecal samples were collected in the third trimester of pregnancy and one month postpartum. The concentrations of the cytokines interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP-1) and tumour necrosis factor α (TNF-α) were measured from serum.

4.1.3 Statistical and microbial analysis

DNA extraction fecal samples was conducted with the use of the QIAamp DNA stool Mini kit (Qiagen) following the manufacturer's instructions, as described elsewhere

(Collado et al., 2008; Kumar et al., 2015).The composition of the gut microbiota was assessed by usin qPCR. PCR primers were used to target the *Bifidobacterium genus, B. longum, B. bifidum, B. adolescentis, B. catenulatum, Clostridium coccoides* group and *Clostridium leptum* group, *Bacteroides fragilis* group and *Akkermansia muciniphila*. PCR amplification and detection were performed on ABI PRISM 7300 PCR sequence detection system (Applied Biosystems, Foster City, CA). The cytokine levels and microbial data are presented as medians and ranges because of the skewness of the data The Wilcoxon signed-rank test was used to compare two-time points, and the Kruskal-Wallis test to compare delivery methods. The statistical analyses were performed using SAS Software Version 9.4.

4.2 Study II

4.2.1 Study population

The study subjects were consecutively selected from an ongoing nutritional intervention study involving 256 pregnant women during the first trimester of pregnancy. The mothers had been randomised as one control/placebo group and two dietary counselling groups. Those who were randomised into the dietary counselling groups received either probiotics (currently defined as *Lacticaseibacillus rhamnosus GG* and *Bifidobacterium lactis*) or a placebo in a double-blinded manner from the third trimester of pregnancy until one month postpartum (Aaltonen et al., 2011; Laitinen et al., 2009). Pregnant women with chronic diseases, except allergies, were excluded from the study. The mother-newborn pairs selected for the current study were based on their cord blood sample availability. All pregnancies were uncomplicated. Exclusion criteria for the study included birth weight below 2,500 g and gestational age less than 37 weeks. Written informed consent was obtained from the women, and the Ethics Committee of the Hospital District of Southwest Finland approved the study.

4.2.2 Data and sample collection

For the current study, maternal and birth data regarding delivery mode and maternal health status were collected from hospital records. Data were available in 159 mother-infant pairs. The infants' weight and length were measured in the maternity ward at birth. Cord blood samples were collected by the midwifery team at the delivery hospital. Serum was immediately separated, and the samples were initially stored at -20°C and then -70°C. Adiponectin concentrations were assayed in duplicate on a 1235 AutoDELFIA immunoassay system (Perkin Elmer Life Sciences, Boston, MA). All assays were in-house, two-step time-resolved fluorometric assays as previously described in more detail (Aaltonen et al., 2011; Semple et al., 2006).

4.2.3 Statistical analysis

The data were analysed using PASW Statistic 18.0. The data are presented as mean values (SD). Associations between the clinical characteristics of the infant and cord blood adiponectin were analysed using Pearson's correlation. Comparisons among the delivery groups (vaginal delivery, elective and non-elective CS) were made using ANOVA, Scheffé test and ANCOVA. Birth weight, gender, maternal weight and weight gain during pregnancy were used as covariates. $P < 0.05$ was considered statistically significant.

4.3 Study III

4.3.1 Study population

This study was a register study, in which the entire birth cohort of children born in 1987 in Finland was investigated. The original data regarding maternal health and diagnoses during pregnancy and perinatal data, including mode of delivery and birth outcomes, were based on the Finnish Medical Birth Register. The original data were complemented with follow-up information regarding births, delivery mode, deaths and health status, as well as the socioeconomic and educational status of the cohort members and their parents by linking several national registers.

4.3.2 Study design

The 1987 Finnish Birth Cohort comprised all 60,069 children born in Finland in 1987. Infants who were stillborn or died during the perinatal period were excluded from the study, and the remaining 59,476 children were included in the follow-up cohort for the years 1987 to 2008. Altogether 497 children had died and 557 had emigrated permanently during the study period. Thus the final study population was 58,430. Diagnoses of NCDs were identified from the Finnish Hospital Discharge Register, which includes all care episodes in all Finnish hospitals and all specialised-level outpatient visits. The diagnosis codes included in this study were based on the International Classification of Diseases (International Classification of Diseases, Ninth Revision from 1987 to 1995 and International Classification of Diseases, Tenth Revision from 1996 onwards).

4.3.3 Statistical analysis

The statistical analyses were performed using logistic regression and cross-tabulation. The results were adjusted for the mother's highest educational level, the child's sex, smoking during pregnancy, and the mother's socioeconomic status.

4.4 Study IV

4.4.1 Study population

In Study IV, mother-infant pairs were selected from an ongoing probiotic intervention study, based on maternal breast milk and faecal sample availability. The original study was a randomised clinical trial assessing the impact of maternal probiotic supplementation on the occurrence of atopic dermatitis (Rautava, Kainonen et al., 2012). In the original study, a probiotic combination consisting of either *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 or *Lactobacillus paracasei* ST11 and *Bifidobacterium longum* BL999 or a placebo was administered beginning two months before the expected delivery and continued until two months after delivery. All the women in the study delivered after 36 weeks of gestation after an uncomplicated pregnancy. Written informed consent was obtained from all participants. The Ethics Committee of the Hospital District of Southwest Finland approved the study.

4.4.2 Study design

In this study, the participating women were grouped based on the mode of delivery and exposure to intrapartum antibiotics. Altogether, 38 women who had delivered vaginally without intrapartum antibiotic exposure, 23 women who had delivered vaginally and received intrapartum antibiotics, 10 women who had delivered by elective CS without intrapartum antibiotic exposure, 6 women who had delivered by non-elective CS without intrapartum antibiotics and 7 women who had delivered by non-elective CS and received intrapartum antibiotics were selected for this study. Breast milk samples were collected at home one month after delivery. Mothers received written instructions for standardised self-collection of samples in the morning. Before sample collection, the breast was cleaned, and breast milk was collected manually, discarding the first drops, using a sterile milk collection unit. The milk samples were collected between August 2005 and April 2009 and stored at -20°C for later analyses.

4.4.3 Statistical and microbial analyses

The breast milk samples were processed for DNA isolation using previously established methods, as described in detail elsewhere (Kumar et al., 2016). Purified DNA was used for 16S rRNA gene amplification; multiplexing was carried out using the Nextera XT Index kit (Illumina, CA, USA). Primers targeting the V3-V4 region of the 16S rRNA gene were used for amplification according to previously described

methods using 2 × 300 bp paired-end run Illumina MiSeq platform (FISABIO sequencing service, Valencia, Spain) (Klindworth et al., 2013). Quality assessment was performed using the prinseq-lite program (min_length:50; trim_qual_right:20; trim_qual_type: mean; trim_qual_window:20) (Schmieder et al., 2011). Filtered and demultiplexed sequences were processed using open-source QIIME software (version 1.9.1, with default parameters) (Caporaso et al., 2010). The sequences were clustered to form OTUs tables (97% identity), and taxonomy classification was obtained at the phylum, family and genus levels using the Greengenes 13_8database. Alpha diversity indices (Shannon Index and richness index) were calculated. Calypso version 8.4 (http://cgenome.net/calypso/) was used with total sum normalisation (TSS) and square root-transformed for the multivariate analysis using OTU phylotype and to generate Venn diagrams showing the shared phylotypes at family level and Redundancy Analysis (RDA) using OUT phylotypes and mode of delivery as a factor. A p-value ≤ 0.05 was considered statistically significant.

Table 1. Charactheristics of the Studies I-IV.

Number	Objective of the Study	Study design	Study population	Study Exposure	Study Outcome
Study I	To determine the effect mode of delivery on the maternal gut microbiota composition and specific pro-inflammatory cytokine levels	Nested case-control study	Selected from a prospective intervention trial; elective CS n=10, non-elective CS n=13, controls/vaginal delivery n=23	CS delivery; elective CS, non-elective CS	The composition of maternal gut microbiota and proinflammatory cytokine levels one month postpartum
Study II	To assess the effect of CS on cord blood adiponectin concentration	Observational study	Selected from an ongoing nutritional study, n=159	CS delivery	Cord blood adiponectin concentration
Study III	To determine whether CS is associated with an increased risk of noncommunicable diseases later in life	Register study	The 1987 Finnish Birth Cohort (n=59476)	CS delivery	Diagnosis of any NCD by the year 2008 based on ICD-9 and ICD-10
Study IV	To determine the impact of the mode of delivery on the composition of breast milk microbiota	Comparative study	Selected from a prospective intervention study; vaginal delivery n=61, elective CS n=10, non-elective CS n=13	CS delivery; elective CS and non-elective CS	The composition of breast milk microbiota at one month postpartum

5 Results

5.1 Maternal gut microbiota composition and the cytokine profile in the pre- and postnatal period

To characterise how delivery by CS contributes to systemic inflammation in the perinatal period, the maternal serum concentrations of specific cytokines were measured. The concentrations of IL-8, MCP-1 and TNF-α were significantly higher one month postpartum compared with the third trimester of pregnancy, irrespective of the mode of delivery. The maternal gut microbiota composition had not changed from the third trimester of pregnancy to the situation one month after delivery. However, the numbers of *Clostridium coccoides* were higher one month postpartum in mothers who had delivered vaginally than those who had delivered by CS ($p=0.0001$).

Table 2. ICD-9 International Classification of Disease, Ninth Revision; ICD-10, International Classification of Diseases, Tenth Revision. Adjusted Odds ratios for mother's highest educational level, childs's sex, smoking during pregnancy and mother's socioeconomic status in 2009. values are n (%) except otherwise indicated. IBD, Inflammatory bowel disease. Table modified from the original publication I.

Disease	ICD-9	ICD-10	Vaginal delivery	Cesarean section	P-Value	Adjusted Odds ratio	95% CI
Neurodevelopmental disorders							
Autism	2990, 2998, 2999, 3017, 3308	F84.0.-F84.5, F84.9	154 (0.32)	34 (0.39)	0.410	1.18	0.79–1.77
Respiratory diseases							
Asthma	493	J45	2990 (6.22)	624 (7.19)	0.003	1.16	1.05–1.27
Allergy	477, 677	J30, L20	22219 (4.62)	448 (5.17)	0.148	1.09	0.97–1.22
Total			4497 (9.36)	925 (10.67)	0.005	1.12	1.04–1.22
Metabolic disorders							
Obesity	278	E66	371 (0.77)	98 (1.13)	0.001	1.51	1.20–1.92
Diabetes mellitus	250	E10-E14	484 (1.01)	91 (1.04)	0.715	1.05	0.82–1.34
Total			840 (1.75)	182 (2.10)	0.024	1.22	1.03–1.45
Bowel disorders							
Celiac disease	5790	K90.0	162 (0.32)	37 (0.43)	0.158	1.31	0.90–1.92
IBD	555, 556	K50, K51	238 (0.50)	41 (0.47)	0.420	0.86	0.59–1.24
Total			393 (0.82)	77 (0.89)	0.767	1.04	0.80–1.36
All diagnoses			**5698 (11.86)**	**1173 (13.52)**	**0.001**	**1.136**	**1.06–1.22**

5.2 Cesarean section and cord blood adiponectin

To detect the association between systemic inflammatory responsiveness and mode of delivery, cord blood adiponectin levels were analysed. The cord blood adiponectin levels were available in 159 infants, of whom 15 (9.4%) were born by nonelective CS and 13 (8.2%) by elective CS. The mean cord blood adiponectin concentration in the newborns was 21.0 μg/ml (5.0–45.9). Cord blood adiponectin levels were higher in female infants than males 22.1 μg/ ml, SD = 7.9 vs. 20.0 μg/ml, SD = 7.2; p = 0.075), respectively. Infant birth weight (p = 0.01), was positively correlated with cord blood adiponectin levels, but maternal weight gain during pregnancy was not (p= 0.4). Infants born by CS had lower adiponectin concentrations (18.0 μg/ml, SD = 8.1) than infants born by vaginal delivery (21.6 μg/ml, SD = 7.3; p = 0.022). This distinction was explained by the difference between infants born by elective CS and vaginal delivery in post hoc analysis (Scheffé; p = 0.015).

5.3 Cesarean section and the prevalence of non-communicable diseases and obesity

To determine the long-term health impacts of CS delivery on child and adolescent health, a register study based on the Finnish Birth Cohort 1987 was conducted. During the follow-up period of 21 years, NCDs were overexpressed among children and adolescents born by CS compared with those born by vaginal delivery among Finnish young adults born in 1987., The prevalence of asthma and obesity was higher among those born by CS than among those born by vaginal delivery (Table 1). The same observation was noticed for allergies, although it was statistically significant only in females, 5.6% in the CS group compared with 5.0% in vaginally delivered females (P = 0 .048, 95% Cl 0.81–0.95). The CS delivery rate was 14.5% in the 1987 birth cohort.

5.4 Mode of delivery and breast milk microbiota

To assess the effect of the mode of delivery on the breast milk microbiota composition, breast milk samples were collected one month after delivery and the microbial composition was analysed. The predominant bacterial phyla in breast milk samples one month after delivery were *Proteobacteria* and *Firmicutes* (Figure 1A). In contrast, *Streptococcaeae* and *Staphylococceae* were the most abundant bacterial families in the milk microbiota (Figure 1B).

The delivery mode and intrapartum antibiotic exposure were both associated with changes in the overall breast milk microbiota composition one month after delivery (Figure 2A). This finding was statistically significant as assessed by the analysis of similarities (ANOSIM) test (p = 0.001). The breast milk microbiota was

significantly distinct between mothers who had delivered vaginally and mothers who delivered by CS as assessed by Principal Coordinate Analysis (PCoA) (Figure 2B). In contrast, intrapartum antibiotic exposure, as the distinct variable, was not associated with statistically significant differences in breast milk microbiota composition (Figure 2C). There were no statistically significant differences at the bacterial phylum and family levels related to the mode of delivery or intrapartum antibiotic exposure.

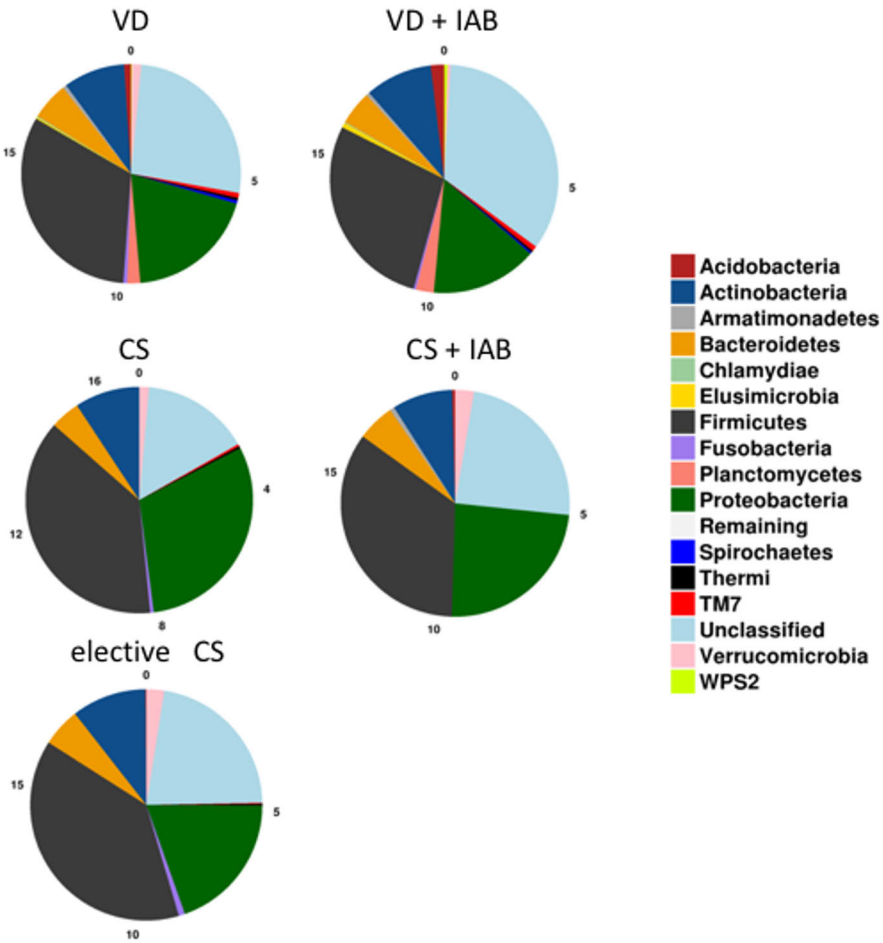

Figure 1A Composition of breast milk microbiota one month after delivery in mothers who delivered vaginally (VD) or by CS with or without exposure to intrapartum antibiotics (IAB). The relative abundances are on phylum the level. From the Original Publication IV.

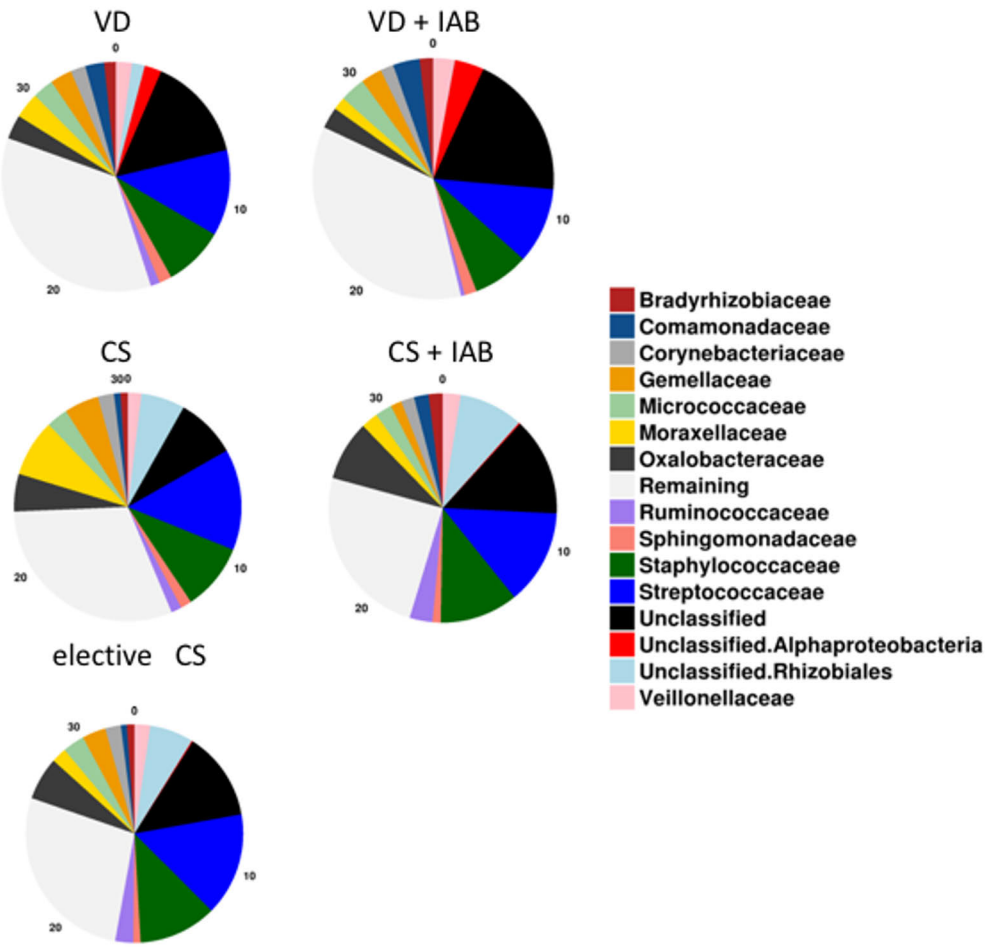

Figure 1B The 15 most abundant families in women who delivered vaginally or by CS with or without exposure to IAP. Figure from the Original Publication IV.

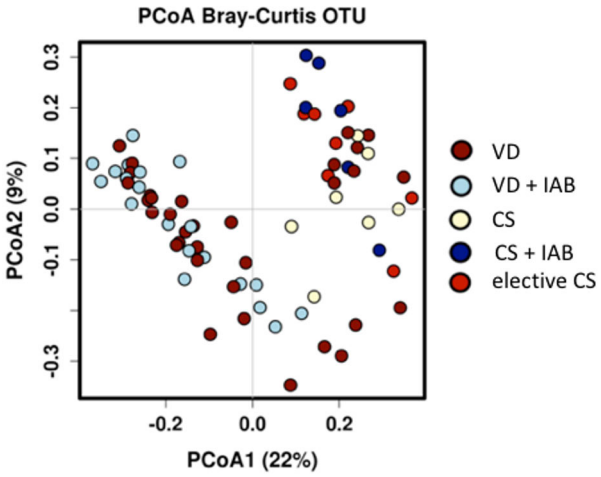

Figure 2A Significant difference in breast milk microbiota composition one month postpartum between in mothers who delivered vaginally or by CS and with or without exposure to intrapartum antibiotics was assessed by PCoA and ANOSIM test; p=0.001. Figure from the original publication IV.

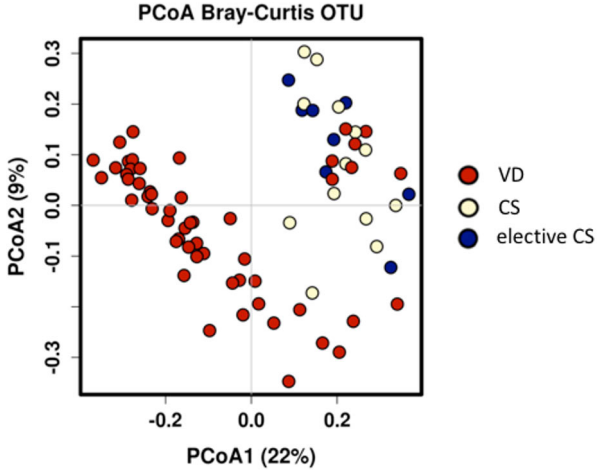

Figure 2B When mothers were grouped by only mode of delivery, VD mothers clustered differently from CS mothers. ANOSIM p = 0.001. Figure from the Original Publication IV.

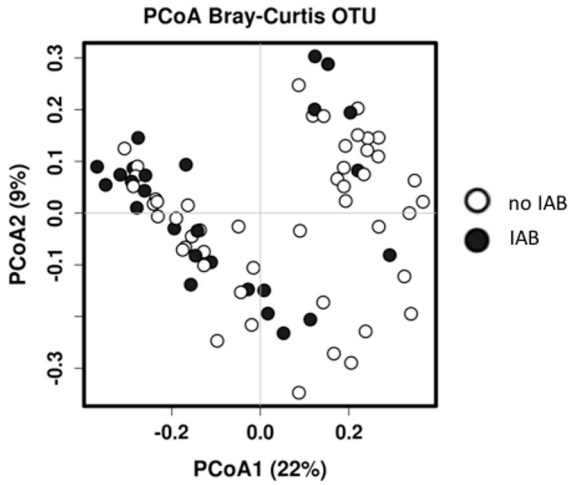

Figure 2C No statistically significant differences were detected when the mothers were grouped by IAB; ANOSIM p = 0.054. Figure from the Original Publication IV.

As per the obstetric guidelines at Turku University Hospital at the time of the study, none of the women who underwent elective CS received antibiotics. It is thus possible to distinguish between the effects of the CS delivery and the exposure in the breast milk microbiota. When the mode of delivery and intrapartum antibiotic exposure were considered simultaneously using Redundancy Discrimination Analysis (RDA), both modes of delivery (p = 0.001) and intrapartum antibiotic exposure (p = 0.015) were found to be independently associated with breast milk microbiota composition one month after delivery.

Breast milk microbiota richness was significantly higher in mothers who had delivered vaginally than in mothers who had delivered by CS (Figures 3A and B).

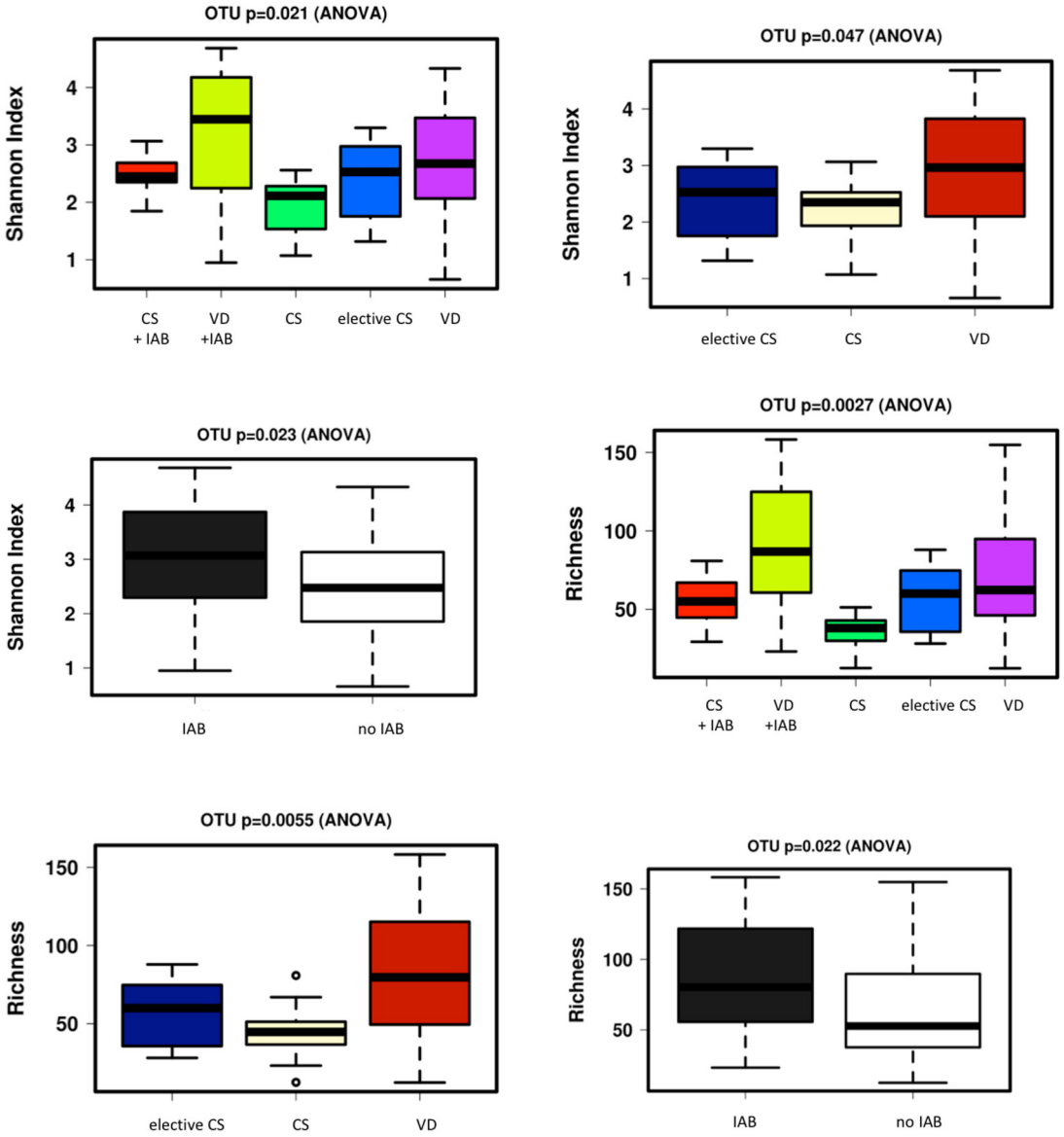

Figure 3. Significant differences in breast milk microbiota diversity one month after delivery as assessed by the Shannon Index between mothers who delivered vaginally VD or by cesarean section (CS) with or without exposure to intrapartum antibiotics (IAB) (**A**). VD mothers showed significantly higher diversity of breast milk microbiota than CS mothers (**B**). IAB was associated with higher microbiota diversity in breast milk microbiota diversity in breast milk microbiota diversity as compared to CS mother (**C**). The richness of the microbiota differed significantly depending on birth mode and exposure to IAB (**D**). VD mothers showed significantly showed higher richness in breast milk microbiota composition than CS mothers (**E**). IAB was associated with increased richness in breast milk microbiota composition (**F**). Figure from Original Publication IV.

A total of 18 bacterial families were shared between the mothers despite differences in the mode of delivery and intrapartum antibiotic exposure. The *Streptococcaea, Staphylococcaea and Bifidobacteriaceae* families were among the most common bacterial families detected. Of the 18 bacterial families, 16 were detected only in the breast milk of mothers who delivered vaginally whereas *Bifidobacterium* species were solely detected in the breast milk of mothers who did not receive intrapartum antibiotics Figure 4.

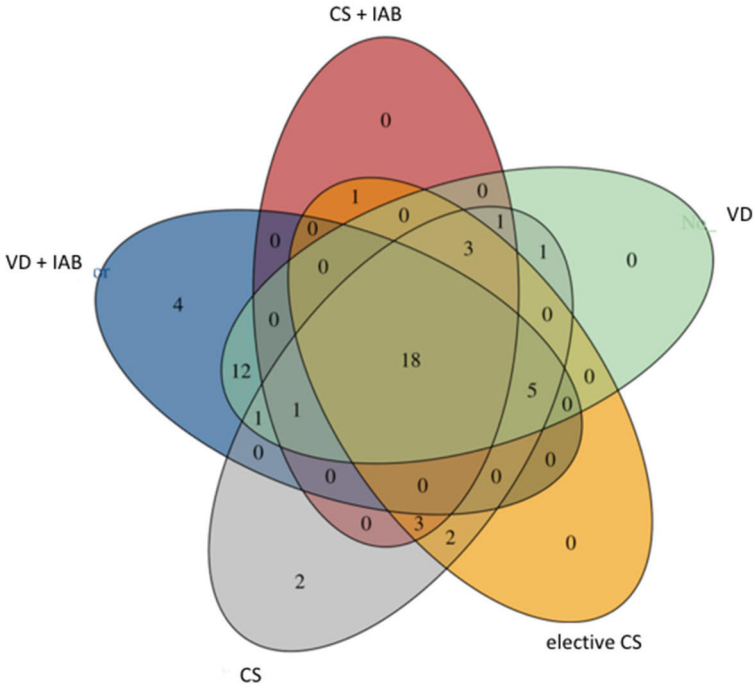

Figure 4. Venn diagram presenting shared bacterial families in breast milk microbiota in mothers who delivered vaginally (VD) or by cesarean section (CS) with or without exposure to intrapartum antibiotics (IAB). Picture from Original Publication IV.

6 Discussion

6.1 Compositional development of the gut microbiota in the child: pregnancy, birth and breastfeeding

6.1.1 When does the microbial colonisation begin?

For a long time, it has been considered that the first microbial contact occurs during birth and that the fetus develops in a sterile intrauterine environment. The presence of microbes in the placenta and amniotic membranes has been associated with adverse pregnancy outcomes, such as chorionamnionitis, preterm birth and severe maternal and neonatal infection. According to some reports, microbes have been isolated from healthy human placenta umbilical cord and meconium, challenging the old dogma of a sterile intrauterine environment and suggesting that human microbial colonisation begins as early as in utero (Golenberg et al., 2000; Jiménez et al., 2005; Aagaard et al., 2016). However, recent studies show no evidence of microbes in the placenta or amniotic fluid, whereas in newborn meconium, there are bacterial extracellular vesicles, cell-derived small particles known to carry various molecules, including microbial DNA, which are more likely to across biological barriers than whole-cell bacteria. This suggests that the first microbial contact during the perinatal period may be via bacterial extracellular vesicles rather than whole-cell bacteria (Turunen et al., 2022).

The maternal gut microbiota composition changes throughout pregnancy and exhibits an increased inflammatory tone later in the pregnancy (Koren et al., 2012). The Study I extends the previous data by documenting that the proinflammatory tone, as assessed by the gut microbiota composition of the gut microbiota and levels of specific maternal blood cytokines, seen during the pregnancy seems to continue beyond the perinatal period. In Study I, it was observed that the maternal gut microbiota composition, as assessed by qPCR-technique remained unchanged between the third trimester of pregnancy and one month postpartum, which is in line with the previous reports (Carrothers et al., 2015; Jost et al., 2014). In Study I, the delivery mode did not have significant effect to maternal gut microbiota composition, which could indicate that pro-inflammatory microbes are more stable

colonizers of the gut (Koren et al., 2012; Qin et al., 2021). This may partly explain the challenges of treating obesity and overweight.

In Study I, the abundance of *Clostridium coccoides* was higher in postpartum samples compared with the third trimester of pregnancy. *Clostridium coccoides* is part of the commensal intestinal microbiota, and may regulate the immune homeostasis of the gut (Mariat et al., 2009). It is not known how these pro-inflammatory changes in the maternal gut microbiota composition affect fetal development, but it might ensure the energy supplementation to the growing fetus. Indeed, the gut microbiota composition seen in the third trimester of pregnancy has obsenogenic properties, as such bacteria are associated with increased energy harvest capacity and adipose tissue storage (Bäckhed et al., 2015). The increased pro-inflammatory shift in gut microbiota seems to be physiological in pregnancy and considered important in shunting metabolic fuels to promote fetal growth, while the same profiles epitomize the metabolic syndrome in the non-pregnant situation. Adverse maternal metabolic conditions such as pre-pregnancy overweight and obesity, excessive weight gain during the pregnancy and impaired glucose tolerance all impact on the maternal gut microbiota composition, and it seems that aberrancies in gut microbiota composition precedes the development of the adverse metabolic conditions in pregnancy (Crusell et al., 2018; Pinto et al., 2023). However, there are opposing reports regarding maternal microbiota composition during pregnancy. DiGuilio and colleagues reported that maternal microbial composition remained stable over the course of pregnancy. They analyzed maternal microbial composition several times at multiple sites, such as vaginal, stool, saliva and gingival during the pregnancy and after delivery. The maternal microbial composition remained stable at multiple sites during pregnancy and after delivery (DiGiulio et al., 2015). DiGiulio and colleagues also found that minor differences in maternal vaginal microbial composition were observed in mothers who gave birth prematurely. Novel techniques allow us to know more about the microbial composition in human, but to know how different microbes impact the host immune and metabolic system, it is necessary to understand the microbial function and the interactions with the host.

6.1.2 Birth and breastfeeding directing the microbial colonisation in the child

The postnatal development of gut microbiota composition is probably the best characterised in the field of commensal microbiota development. During birth, the fetus is exposed to microbes from the maternal birth canal. The initial gut microbiota composition of the newborn consists of aerobic and facultative bacteria, such as streptococci and enterobacteria. In the following days, the oxygen level in the newborn gut decreases, and anaerobic bacteria, mainly *Bifidobacteria, Bacteroides,*

and *Clostridium* colonise the newborn's intestine (Martin et al., 2016). After birth, the feeding type further drives the bacterial colonisation of the newborn's gut. Newborns receiving exclusively breast milk have a higher abundance of *Bifidobacteria* in their gut microbiota than infants receiving formula feeding (Martin et al., 2016). Breast milk contains several bioactive components, such as human milk oligosaccharides, that serve as selective metabolic substrates and promotes the growth of specific bacteria. Exclusively breast fed newborns show lower richness and less diverse gut microbiota composition than formula fed newborns. (Marcobal et al., 2012). The gut microbiota of formula-fed newborn consists of several bacterial taxa, including *Lachnospirocae*, *Streptococcaceae*, *Bacteroides*, *Clostridium* and lactobacilli (Marcobal et al., 2012). However, the gut microbiota composition of infants who receive both breast milk and formula is considered more similar to exclusively formula fed infants (Madan et al., 2016). Interestingly, in a Japanese study, in which a most of infants studied received prebiotic enriched formulas, shows a higher abundance of bifidobacterial species at six months compared with exclusively breast fed infants (Madan et al., 2016). It seems that specific micronutrient and bioactive compounds of nutrition, both in breast milk and prebiotic-enriched infant formulas, selectively favour the growth of specific microbes in the infant gut.

The effect of delivery mode on the initial microbial colonisation of the infant gut is well-characterised, whereas less is known about how the delivery mode impacts breast milk microbiota composition. As mentioned above, nutritional bioactive components direct the compositional development of the infant gut microbiota. To improve our understanding of this important determinant of the compositional development of the child microbiota, Study IV aimed to establish whether delivery mode impacts the breast milk microbiota. Delivery by CS had a profound, independent impact on breast milk microbial composition one month after delivery. The breast milk of mothers delivered by CS showed reduced bacterial diversity and bacterial richness than that of mothers who had delivered vaginally. Apart from some potential contaminants from the maternal skin or environment (such as *Streptococceae* and *Staphylococceae*), the breast milk samples consisted mostly of anaerobic species, such as members of the family *Bifidobacterieae*, which are common bacteria found in the human gut. It is not fully understood where the breast milk bacteria originate, but it has been suggested that there is an entero-mammary pathway, which allows bacterial translocation from the maternal gut to the mammary gland.

Study IV, differences in breast milk microbiota composition were seen after elective or emergency CS. According to the local obstetric guidelines of that time, none of the studied mothers undergoing elective CS received prophylactic antibiotics before the operation. This suggests that CS itself has an independent effect on breast

milk microbiota composition. It has been estimated that in breastfed infants, more than a quarter of the intestinal gut microbes originate from breast milk (Pannaraj et al., 2017), and thus it seems that the effects of the mode of delivery in the development of gut microbiota composition are not restricted to the time of birth. CS delivery may impact the development of child gut microbiota composition via breast milk microbiota.

During the first two years of life, the child gut microbiota undergoes further compositional changes characterised by the increase or decrease of the relative abundance of specific microbial taxa, reaching an adult-like microbial composition by two years of age (Yatsunenko 2021). Breast milk modifies infant gut colonisation by favouring specific microbes, and it seems that it is rather the cessation of breastfeeding than the introduction of solid foods to the infant diet that modifies the gut microbiota maturation in infancy (Lyons et al., 2020). The number of bifidobacteria decreases in breastfed infants after weaning while the number of *Bacteroides* increases (Bäckhed et al., 2015). Infants who received formula feeding showed increased numbers of *Bifidobacteria* and *Ruminococcaecae* after the introduction of solid foods (Fallani et al., 2011; Thompson et al., 2015). The microbial diversity and richness of gut microbiota composition increase until three years of age, around which time the gut microbiota is considered mature, showing similar compositional shifts as in the adult gut microbiota. The effect of pre-weaning nutrition, breastfeeding or formula feeding, as well as the delivery mode, is still observed in infant gut microbiota composition after the introduction of solid food (Fallani et al., 2011; Thompson et al., 2015). Weaning causes rapid changes in gut microbiota composition, and these changes are more prevalent in formula-fed infants (Fallani et al., 2011).

6.2 Environmental impacts on child microbiota

Several lifestyle and environmental factors have attracted scientific interest in affecting the compositional development and maturation of child gut microbiota. While the mode of delivery and infant feeding practices are important factors influencing early infant gut microbiota composition, recent studies suggest that different environmental factors, such as the living environment, family size and exposure to pets, may further affect gut microbiota maturation in later infancy and childhood (Fallani et al., 2011). In addition, geographical location is associated with gut microbiota composition in infancy and childhood. For example, the gut microbiota composition in children living in rural Africa shows higher abundances of Bacteroidetes and lower abundances of Firmicutes, whereas children living in Europe showed higher abundances of Firmicutes (Tasnim et al., 2017). Similar compositional differences in gut microbiota according to geographical location have

been observed in European infants; infants living in Northern Europe show higher abundances of bifidobacteria, whereas *Bacteroides* and lactobacilli are more abundant in Southern Europe (De Filippo et al., 2010). The geographical differences in gut microbiota composition are explained mainly by local diet rather than genetics.

It is well-established that living in a farm environment protects the host against developing asthma and allergic diseases in childhood, although the exact mechanism behind this phenomenon is poorly understood (Fallani et al., 2011). Previous studies suggested that this might be due to horizontal transmission of environmental bacteria via the consumption of farm milk and contact with cows and straw (Ege et al., 2011). However, Depner and colleagues found that approximately 19% of the protective farm effect in the development of asthma could be explained by the maturation of gut microbiota composition in childhood and suggested that the environmental microbiota interacts with the gut microbiota (Depner et al., 2020). Family size has been suggested to affect the development and maturation of gut microbiota composition. Infants with older siblings show more diversity and higher richness in gut microbiota composition than firstborn infants, and these compositional differences have been observed throughout childhood (Laursen et al., 2015). Similarly, it is reported that day-care attendance is also associated with higher richness and diversity in infant gut microbiota composition (Azad et al., 2013b; Martin et al., 2016; Penders et al., 2006). Having furry household pets in infancy is associated with more diverse gut microbiota composition in infancy compared with no exposure to pets, and at least part of the differences in gut microbiota composition persist into adulthood (Thompson et al., 2015).

6.3 Evidence on the child microbiota and future disease risk

6.3.1 From hygiene hypothesis to microbial hypothesis

More than 30 years ago, D. Strachan and J. Gerrard postulated the Hygiene hypothesis, where they suggested that a lack of exposure to infections during childhood may predispose to the development of allergic and atopic diseases later in life. Since then, this hypothesis has been revised, and exposure to commensal, environmental microbes, rather than infections themselves, is considered part of healthy immune system maturation (Pfefferle et al., 2021). Indeed, there are signs that differences in gut microbiota composition may be associated with the development of atopic and allergic diseases later in life (Pfefferle et al., 2021). In a Dutch birth cohort study, infants' colonisation by *Clostridium difficile* at one month of age was associated with the development of atopic eczema and allergen sensitisation at two years old and wheezing and asthma at six to seven years old

(Galazzo et al., 2020). Low total diversity of gut microbiota and lower abundancies of *Bacteroides* and *Proteobacteria* in early infancy have been associated with the development of atopic eczema (Abrahamsson et al., 2012; Penders et al., 2007; Van Nimwegen et al., 2011). Similar differences in gut microbiota composition have been detected in other allergic manifestations. Azad and colleagues suggested that lower gut microbiota richness, lower abundance of the family *Bacteroidaceae* and higher abundance of the family *Enterobacteriaceae* at three months old and decreased abundance of *Ruminococcus* at 12 months old were associated with subsequent food sensitisation at 12 months old (Azad et al., 2015). In a Swedish longitudinal study, the underrepresentation of *Bacteroides*, *Coproccoccus* and *Prevotella* throughout childhood and the low abundance of *Ruminococcus* at six months of age were associated with the development of IgE-associated allergy at school age (Sjödin et al., 2019). Another longitudinal study showed that lower microbial diversity and early maturation of gut microbiota in infancy was associated with atopic and allergic manifestations later in childhood (Sjödin et al., 2019). The current literature supports the association between food allergy and gut microbiota composition, suggesting that gut bacteria may play a role in mucosal barrier systems. In addition, changes in gut microbiota composition may precede the resolution of the allergy (Galazzo et al., 2020).

While atopic eczema and food sensitisation often manifest in infancy and early childhood, the diagnosis of asthma is commonly made later in childhood and at school age (Bunyavanich et al., 2016). Similar to other atopic and allergic diseases, aberrancies in gut bacterial composition in early infancy have been associated with the development of asthma later in childhood. Lower gut microbiota diversity and richness in infancy reportedly precede the development of asthma at school age (Paller et al., 2019). Arrieta and colleagues demonstrated that gut bacterial dysbiosis during the first three months of life was associated with atopic wheezing during early childhood. They found that abundances of the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia* were significantly lower in children developing asthma by three years old (Arrieta et al., 2015). In other studies, the development of asthma has been linked to aberrancies in gut microbial maturation during the first year of life. Stokholm and colleagues suggested that delayed gut microbial maturation at 12 months old was associated with the development of asthma at five years old in infants with asthmatic mothers (Stokholm et al., 2018). Durack and colleagues suggested that the gut microbiota composition and maturation are at least partly modifiable by the administration of probiotics during the first six months of life, but no effects of probiotic supplementation were seen at 12 months old (Durack et al., 2018). The gut microbial aberrancies may not be restricted to early infancy and childhood, since recent studies have also suggested that there are

differences in gut microbiota composition in asthmatic adults compared to those without asthma (Durack et al. 2018).

The disappearing microbiota hypothesis rationalises the emergence of NCD by the depletion of microbial contact in the modern industrialised world resulting from changes in hygiene, diet, lifestyle and living conditions (Wang et al., 2018). The earliest and most massive source of microbial exposure is associated with the establishment of the gut microbiota, with a strong stimulatory effect on the maturation of the gut-associated lymphoid tissue and, through the common mucosal immune system, the total immunologic capacity of the host supports the microbiota hypothesis of NCD.

6.3.2 The Cesarean section delivery – a novel example of the microbial hypothesis

CS delivery is a modern example of microbial depletion during birth and early life.. Indeed, in Study III, CS delivery was associated with the development of asthma and allergy later in life. These findings are in line with previous studies (P. Bager et al., 2008; Baumfeld et al., 2018; Black et al., 2015; Kero et al., 2002; Sevelsted et al., 2015), although inconsistency exists among the previous reports (Robson et al., 2020; Yu et al., 2015). It seems that the risk of developing NCDs is highlighted in elective CS rather than unscheduled or emergency CSs, in which the labour process has begun. The rupture of amniotic membranes may also allow microbes from the maternal birth canal and maternal gut to reach the fetus and thus initiate the microbial colonisation and immunologic maturation also in the case of emergency CS (Chu et al., 2017; Kolokotroni et al., 2012; Sevelsted et al., 2016).

The link between the birth by CS and the development of asthma may be mediated by microbes. Indeed, in CS delivery the initial microbial colonization of newborn gut is aberrant and delayed when compared with those born by vaginal delivery as the initial microbial colonizers in vaginal delivery originate from maternal gut microbiota. The early colonizers in CS are originated from birth the environment, mainly in hospital environment in high-income societies, and maternal skin. It seems that when CS related microbial changes in infant gut microbiota remained at the 1 year of age, the risk for asthma development was increased the later in the childhood (Stokholm et al., 2020). Moreover, CS is associated with delayed breast feeding, increased newborn exposure to antibiotics both of which influence the gut colonization and thus the later disease risk.

The human gut microbiota has also been linked to the development of overweight and obesity. Commensal gut microbiota has several impacts on the host nutritional status by affecting nutrient absorption, the production of micronutrients and vitamins and the regulation of energy homeostasis (Sevelsted et al., 2016). Gut

microbes mediate the digestion of non-digestible carbohydrates into more absorbable forms by hydrolysing and fermenting them (Connor, 2013). Some gut microbes show increased capability for energy harvest from the host diet and increased energy storage as adipose tissue (Bouhnik et al., 2004). In fact, the gut microbiota composition differs among lean and obese individuals. Generally, bacterial taxa belonging to Firmicutes are linked to obesity, whereas Bacteroides are more abundant in lean individuals, and weight loss in obese individuals is associated with subsequent change in the Firmicutes and Bacteroides ratio (Bäckhed et al., 2004). The causal mechanism between gut microbiota composition and obesity was suggested in an experimental mouse model, in which a faecal microbiota transplant with obesity-associated gut microbiota increased energy harvest and adipose tissue content in germ-free mice compared with the colonisation of normal, lean microbiota (Ley et al., 2005, 2006). Similar obsenogenic shifts in gut microbiota composition have been observed in late pregnancy. The colonisation of germ-free mice with gut microbiota from pregnant women in the 3rd trimester of pregnancy resulted in the development of obesity in the mice when compared with the colonisation of gut microbiota in the 1st trimester of pregnancy (Bäckhed et al., 2004; Turnbaugh et al., 2006). Although nutrition plays a significant role in determining gut microbial composition, recent studies support causality between gut microbiota and obesity.

During recent decades, overweight and obesity have become more common in children and adolescents, and similar shifts in gut microbiota composition between normal-weight and overweight children have been shown (Koren et al., 2012). In fact, it is suggested that differences in gut microbiota composition may be seen already in infancy and early childhood in children who later develop overweight and obesity (Mbakwa et al., 2019; Riva et al., 2017). Similar findings have been reported in Norwegian children, suggesting that the gut microbiota composition at age two predicts overweight and obesity at age eight (Kalliomäki et al., 2008).

In Study III, CS delivery was associated with the development of obesity in early adulthood, which is in line with most previous studies, although discrepancies exist among previous studies (Stanislawski et al., 2018). Due to the obesity pandemic, maternal overweight and obesity have increased and may thus be considered a novel obstetric dilemma since they increase the risk of obstetric complications, including CS deliveries (Ahlqvist et al., 2019; Blustein et al., 2013; Goldani et al., 2013; H. T. Li et al., 2013). It has been speculated whether the development of offspring obesity in CS-born children is due to the maternal conditions rather than the delivery mode itself. However, CS has been shown to be associated with the development of obesity after adjustment for maternal prepregnancy weight status (Carroza Escobar et al., 2021). In families, siblings born by discordant modes of delivery have an increased risk of becoming obese later in life (Mueller et al., 2017). In the register based study setting the maternal characteristics, such as pre-pregnancy BMI, pregnancy

complications such preeclampsia and perinatal infections, and infant birth data e.g. gestational age and birth weight, all of which are potential contributing factors in development of obesity later in life.

Due to routine antibiotic administration in CS deliveries, it is challenging to determine whether the disturbances in child microbiota composition are due to the perinatal antibiotic exposure or the operation itself. In Study IV, the mothers undergoing elective CS did not receive antibiotic prophylaxis according to the obstetric guidelines of the time, unlike those undergoing non-elective CS. The breast milk microbiota composition was different between the elective and non-elective CS delivery groups, which may reflect the idea that lack of physiological stress during labour may impact the breast milk microbiota. This is a unique finding, as the prophylactic antibiotic in CS is understandably a common routine in CS deliveries.

In Study IV, intrapartum antibiotic exposure at the time of delivery had an independent but relatively modest impact on breast milk microbiota composition at 1 month after delivery. As the microbial composition changes over the course of lactation, the intrapartum antibiotics may have had a more significant effect on breast milk microbiota in the beginning of lactation.

The risk of developing NCD seems, to at least some extent, to be associated with the type of CS delivery (Thysen et al., 2015). In unplanned CS, the labour process has usually begun, causing a physiological stress reaction in the fetus, unlike in planned, elective CS, in which the physiological stress reaction in the fetus is absent. The cord blood concentrations of cytokines and immune cells are different in different delivery modes, and thus, these immunologic differences may affect the development of the immune system (Thysen et al., 2015). In Study II, cord blood adiponectin was lower in elective but not in non-elective CS delivery, suggesting that lack of physiological stress reaction may modify early immune responses in the newborn.

6.4 Future aspects on the interaction between child health and the gut microbiota

Birth by CS increases the risk of the development of NCDs and obesity later in childhood and early adulthood by depleting the child's microbial contact during birth and breastfeeding and by exposing the newborn to antimicrobial medications during the perinatal period. The association between CS delivery and later risk of developing NCD is of particular concern in situations in which the CS is performed for non-medical reasons. Since the rate of CS deliveries is increasing worldwide, the focus of future research should be aimed at reducing the risk of the development of NCD in the child by optimal microbiota modulation, which has been scientifically proven to be safe and effective. By definition, probiotics are "live microorganisms

which, when administered in adequate amounts, confer a health benefit on the host" (Ly et al., 2006), whereas prebiotics are substrates "that are selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). Both probiotics and prebiotics have beneficial effects on human gut microbiota.

Breast milk shows both probiotic and prebiotic properties, and it seems to potentially impact the compositional development of the infant gut microbiota. Thus, breastfeeding should also be considered the gold standard of feeding for gut microbes. In high-income countries, the rates of breastfeeding initiation have been increasing during recent decades, but only a few meet the WHO recommendations for the duration of breastfeeding (Gibson et al., 2017). Future research should focus on promoting breastfeeding.

Probiotic supplementation during pregnancy, lactation and infancy is considered safe and effective and may impact gut microbiota composition. Probiotic administration to newborns born by CS and exposed to antibiotics during birth has been reported to correct the microbial aberrancies associated with CS delivery and antibiotic exposure during birth (Vaz et al., 2021). There are data indicating that probiotic interventions during pregnancy and the perinatal period reduce the risk of developing atopic and allergic diseases in childhood (Korpela et al., 2018). However, the mechanisms of action of probiotics are not completely understood, and nor are the possible intrinsic factors of the host that may affect the effect of administered probiotics (Kalliomäki et al., 2003; Simpson et al., 2015). Future studies should be aimed at clarifying the optimal timing and duration of the use of probiotics, as well as the specific probiotic strains.

The aberrant microbial contact in a newborn during birth by CS is well-characterised, and there has been scientific interest in introducing the maternal microbiota to the newborn. Restoring the maternal microbiota to a CS-born newborn by transplanting the maternal faecal microbiota orally via breast milk or seeding the newborn maternal vaginal fluid have been under investigation (Dominguez-Bello et al., 2016). Notably, microbes from the maternal vaginal microbiome do not occur in the gut microbiome (Avershina et al., 2017). Maternal faecal transplantation via breast milk after CS has been shown to alter the gut microbiota composition of a newborn towards a composition resembling that of neonates delivered vaginally and CS-born newborns who received microbial transplantation from the mother (Korpela et al., 2020). However, as described above, the development of child microbiota during early life is a stepwise process compared to faecal transplantation, where a newborn receives adult-like, maternal –possible inflammatory-shifted faecal microbiota.

6.5 Strengths and limitations of the study

The present study adds to current knowledge about how the mode of delivery impacts a child's future health and offers some possible mechanisms. However, some limitations should be taken into account. Study III was a register study based on a large Finnish Birth Cohort from 1987. The follow-up period in this study was sufficiently long to reliably diagnose the specific NCDs studied. The diagnoses, especially asthma and allergic status, were based on the Hospital Discharge Register which has been proven to be accurate in the diagnosis of NCD. Data on medication purchases would have been valuable, but these data were not available in the Finnish Birth Cohort 1987. Since the type of CS may affect the risk of development of NCDs later in life, it would have been interesting to see the effect of non-elective versus elective CS on the risk of the developing the NCDs studied. Unfortunately, the type of CS was not available in the medical birth registers until 1990.

The number of participating mothers was relatively low in Studies I, II and IV. In Study II, the concentrations of adipose tissue-derived anti-inflammatory hormone, adiponectin, were measured in cord blood. It would also have been valuable to measure some other inflammatory markers in cord blood to gain a more comprehensive view of the inflammatory response during the CS. In Study I, the maternal microbial composition and inflammatory cytokine levels were assessed during the third trimester of pregnancy and once after delivery, and more time points would have increased the value of the study.

In the Study I the maternal microbial composition was assessed by qPCR method which quantifies the DNA of the specific targeted primers. As a limitation of the Study I, the qPCR method focused only specific microbes in maternal gut microbiota whereas 16S rRNA gene amplification, which was used in Study IV to assess the composition of breast milk microbiota, would have offer more comprehensive view of maternal gut microbiota profile.

In Study IV, none of the mothers undergoing elective CS received antibiotic prophylaxis before the CS delivery as per the obstetric guidelines of that time, allowing assessment of the impacts of the type of CS delivery on breast milk microbiota. This makes the study population unique, as the current practice guidelines recommend antibiotic prophylaxis before CS delivery in all instances. The breast milk microbiota was assessed only at one time point, and serial analyses would have been valuable. However, the effect of other environmental factors on maternal breast milk would possibly have more impact on breast milk microbiota at later ages.

7 Summary

The present study shows that birth by CS is associated with an increased risk of developing certain NCDs in childhood and adolescence. The link between the mode of delivery and the later risk of development of disease is explained by the microbial depletion that occurs during birth by CS. In addition to the aberrant microbial contact during birth, birth by CS has an indirect impact on the compositional development of the child gut microbiota via breast milk microbes and exposure to antimicrobial medications.

In the present study, birth by CS was associated with the development of obesity later in childhood and adolescence. In addition to aberrant microbial exposure during birth and the perinatal period, elective CS delivery was associated with lower adiponectin levels in cord blood samples. Lower adiponectin levels have been documented in association with obesity in children and adults.

Throughout pregnancy, the maternal gut microbiota shows pro-inflammatory properties. In this study, the maternal gut microbiota remained relatively stable one month after delivery, suggesting that inflammatory bacteria in the gut microbiota may be more stable colonisers.

CS delivery had an independent effect on breast milk microbiota composition one month after delivery. Women undergoing elective CS without receiving antibiotic prophylaxis before the operation showed a distinct microbial composition in their breast milk one month after delivery.

Regardless of the delivery mode, an elevated pro-inflammatory tone was observed in pro-inflammatory cytokine levels in mothers during the perinatal period.

As the rate of CS deliveries is continuously high in Western societies, future research should be focused on diminishing the impacts of CS on the development of infant gut microbiota composition. Probiotic interventions during pregnancy and the perinatal period are proven to be safe and have shown positive impacts on the compositional development of infant gut microbiota. Nevertheless, the secret of successful programming may be timing – primary and secondary preventive measures should be undertaken during pregnancy and in early infancy.

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