Enteral feeding interventions for optimal fetal and neonatal health

Scientific and societal impact

This chapter reflects on the scientific and societal impact of this thesis, and on the ways the target audience can be informed of and engaged with the main results of this thesis, in order to promote future use of the acquired knowledge.

Research field and aims of this thesis

Each year, around 15 million babies are born prematurely, i.e., before 37 weeks of pregnancy[1]. Although more and more of these babies survive, premature birth remains the leading cause of death for infants below 5 years of age[1]. In addition, surviving children often have long-term health problems, especially if they are born extremely preterm (before 28 weeks of gestational age) or severely preterm (between 28 and 31 weeks of gestational age)[2]. An important reason for preterm birth is inflammation or infection of the membranes covering the inside of the uterus, which is called chorioamnionitis[3]. Chorioamnionitis is often accompanied by inflammation of the amniotic fluid and even of the fetal blood and tissues[4], which further increase the risk of death of the infant and major health concerns[5,6]. Importantly, since many diseases of premature babies are influenced by risk factors during pregnancy, such as chorioamnionitis, it is suggested that the development of such diseases may start before birth[6,7]. One of these diseases is necrotizing enterocolitis (NEC)[8]. NEC is characterized by severe inflammation of the intestines and, in later stages, death of intestinal tissue[9]. Why and how NEC develops is currently insufficiently clarified, but it is known that multiple factors such as loss of intestinal barrier function and altered intestinal motility contribute to NEC development[10]. NEC affects around 7% of infants who are treated at the neonatal intensive care unit (NICU). About 15-30% of infants with NEC die from it[10], and this number increases up to 50% in infants that need to be treated surgically[11]. In addition, children who do survive are at risk of long-term health consequences such as problems with digestion and absorption of nutrition[11] and disturbed brain development[10]. Importantly, besides being a major health issue, both premature birth and its sequelae pose a considerable economic burden on society. NICU care is expensive and a multitude of these costs are caused by the life-long care for individuals with preterm-birth-related long-term health consequences[12,13]. Direct health-care costs of NEC have been estimated to range from $70,000 to $180,000 per infant[14,15]. In addition, quality of life of both the infant and his or her parents (and further family) is negatively affected[16,17].

Despite having been studied for years, NEC treatment remains largely symptomatic and difficult to majorly improve, amongst others due to the rapid onset and aggressive disease course of NEC[9]. Consequently, the field of NEC research has, over the past decades, aimed at developing new preventative approaches[9,18]. In this context, breast milk and breast milk components are promising, since NEC cases are reduced by 10-fold when only human milk is fed to the infants, and by 3- to 5-fold when human milk feeding is combined with feeding of infant formula[19]. The protective role of breast milk can be explained by the large number of bioactive components present in breast milk that collectively act on several disease mechanisms involved in NEC development[20-22]. Unfortunately, mothers' own milk is not always available for infants treated at the NICU[23,24] and donor milk, which is increasingly being used, is not a full-fledged alternative[25,26]. Thus, the development of nutritional

interventions containing (combinations of) bioactive human breast milk components holds great promise for the prevention of NEC. As many years of research into feeding interventions have not majorly reduced NEC occurence yet, alternative research strategies are desired. Therefore, in the current thesis, we aimed to provide a framework for the improvement of preventative feeding intereventions for NEC, by applying novel strategies that specifically acknowledge the fact that NEC is caused by multiple factors and that NEC development may already start before birth.

Direct relevance of the key findings in this thesis

In this thesis, we provided an overview of the currrent state of evidence regarding feeding interventions for the prevention of NEC. This work provides (future) researchers with an interest in enteral feeding interventions for NEC, with a summary of what has already been done and which biological effects (such as reduction of intestinal inflammation and improved gut barrier function) were previously attributed to nutritional components. This information is also relevant for researchers looking into other diseases marked by similar disease mechanisms that may benefit from feeding interventions with such biological effects, such as inflammatory bowel disease (IBD)[27]. We demonstrated that although a large number of positive results were observed in experimental animal studies, only scarse interventions were succesful in clinical trials, and we identified various aspects that can explain this difference. Important reasons include that most studies start interventions postnatally, which may be (too) late, and the fact that the NEC development is incompletely understood. Moreover, most studies mainly looked at single component interventions, whereas, given the complex composition of breast milk and the multifactorial origin of NEC, interventions using combinations of nutritional components are more likely to be succesfull. These challenges were addressed in the studies incorporated in this thesis and this knowledge can be used to improve future (pre-)clinical studies in the field of neonatology and NEC and in other (bio)medical fields that face similar challenges.

We observed that alterations in the nervous system of the gut, which is, amongst others, responsible for bowel movements, and the intestinal mucus barrier, are found already before birth as a consequence of chorioamnionitis. Moreover, our studies provided indications on which disease mechanism could be involved. Since these alterations and potential mechanistic explanations correspond to findings in NEC, this knowledge contributes to a better understanding of NEC development and supports the concept that NEC development may start before birth. This could benefit researchers who are designing future studies to unravel NEC development, and strengthens the idea that feeding interventions before birth may be useful to improve outcomes after birth. In addition, scientists in other fields may benefit from the knowledge acquired, since the implicated disease mechanisms are generic for multiple diseases in neonates or in the gut, such as perinatal brain injury[28,29], IBD[30,31], and intestinal ischemia reperfusion injury (IRI)[32,33].

We reported, in a proof-of-concept study, that a prenatal feeding intervention with plant sterols (food components present in plant cell membranes) by administration in the amniotic fluid, reduced inflammation in the fetal blood and improved gut outcome. As far as we know, this is the first study to report that the fetus can be targetted perinatally with a

feeding intervention to improve gut health. This first study could pave the way for future studies addressing health problems of the prematurely born neonate at the earliest possible moment, namely before it is born, by modulation of the nutrition of pregnant women. Of note, although we performed this study in the context of gut inflammation and NEC, plant sterols and other interventions could also be relevant for other diseases associated with blood inflammation of the fetus, such as bronchopulmonary dysplasia (chronic lung disease)[5] and brain injury around birth[5]. In a broader context, our study once again shows that the composition of the amniotic fluid, which is partially determined by maternal diet, is important for the health of the fetus. This concept is vital for health care staff, such as obstetricians, midwives, neonatologists, and dieticians, but also for the mother/parents-to-be. Additionally, this information could be relevant for the government and health insurance companies, since they can promote a healthy lifestyle via legislation and/or public campaigns.

Last, we developed a human intestinal organoid model for the screening of nutritional interventions in the context of intestinal health and disease. In this model, we sucessfully tested the effect of hydrolyzed ('digested') and non-hydrolyzed ('non-digested') whey proteins as potential beneficial feeding intervention. Such a screening model is key for adjusting future research into preventative feeding interventions for NEC from single interventions, which have not been very succesful over the years, to combinations of interventions, of which more promising effects can be expected. In addition, this aproach can reduce the number of laboratory animals used. Given the findings in this thesis, plant sterols and whey protein are interesting candidates to be incorporated in such a combined nutritional intervention. The work described here forms a solid basis for further extending such organoid models, by creating organoid co-cultures with immune cells, microbes or cells from the nervous system of the gut in future studies. Such (extended) organoid models can be useful for other researchers developing (nutritional) interventions in the context of NEC and other gastrointestinal diseases such as IBD and IRI. In addition, techniques used in this model can be applied in other studies that use (human intestinal) organoids.

Potential long-term relevance of the key findings in this thesis

The work described in this thesis provides new scientific insights and adds points of departure for follow-up studies and future clinical trials. The knowledge acquired can, in the long run, contribute to the development of novel enteral feeding interventions that effectively prevent NEC as well as NEC-related death and long-term health consequences. Neonatal intensive care is very expensive, although it is much more cost effective compared to adult health care[13]. If enteral feeding interventions are effective in improving survival and reducing the duration of NICU stay, they are likely to reduce neonatal intensive care costs[13]. Importantly, environmental circumstances including nutrition in the first 1000 days of life are increasingly recognized as an important determinant for lifelong health[34,35] and more and more studies support interaction between development of the gut and other organs such as the brain and the lung[36-38]. In line with these findings, breastfeeding is, amongst others, linked to reduced risk of childhood obesity[39,40], type 2 diabetes[39,41], IBD[42], and

asthma[43]. This indicates that development of novel enteral feeding interventions can promote health beyond the gut and beyond the neonatal period.

Dissemination of knowledge to target groups

The direct impact of this thesis is mainly scientific; thus the main target group is the scientific community. Dissemination to this group has taken place and will take place in the future via scientific publications in international peer-reviewed journals and via presentations at (inter)national congresses. All published studies incorporated in this thesis have been published in open-access journals and are thereby freely available to the general public. A second target group that could benefit from the knowledge acquired in this thesis comprises health care staff, such as pediatricians and neonatologists, perinatologists and obstetricians, pediatric surgeons, midwives, and dieticians. Currently, this mainly concerns the general concept that NEC development may already start before birth, and that fetal and neonatal health are influenced by amniotic fluid composition. However, if the knowledge acquired supports better enteral feeding intervention studies for NEC in the future, it could in the long run lead to adjustment of clinical guidelines and improvement of neonatal intensive care. Healthcare staff can be updated about the content of this thesis via clinical congresses, presentations during teaching moments in the hospital, and during peer consultation.

A last target group is formed by (future) parents of premature neonates or infants with NEC, and their loved ones. In the first place, they may take comfort in the thought that efforts are being made to improve the outcome of their child and future children. Ultimately, this group directly benefits from this research by improved health care. This target group can be reached via patient organizations and platforms, such as the European Foundation for the Care of Newborn Infants (EFCNI), Care4Neo and Kleine Kanjers, and via (local) fundraisers in the field, such as Strong Babies and Kinderonderzoeksfonds Limburg.

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