Early-life endocrine regulation and neurodevelopmental outcomes

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Summary

BACKGROUND

This thesis is centered around the hypothesis that events in early-life can affect health and disease at an older age.

Infants who are born preterm (with a gestational age of less than 37 weeks), form an interesting group within this hypothesis. Where normally they would still be safe in the womb, they are suddenly exposed to disturbances, like medications, procedural pain related to operations and blood draws, and infections. Meanwhile, they have trouble breathing unassisted and their organs are still immature.

In 2017, 11.978 children were born prematurely in the Netherlands, of which 2.274 were very preterm (gestational age <32 weeks). Due to improved care, an increasing number of preterm born infants survive, and focus is therefore shifting towards the long-term effects of prematurity; previous studies have shown that children who were born preterm are at an increased risk for neurodevelopmental problems, cardiovascular diseases and deviating growth. It is important to research which factors contribute to this increased risk, and it is equally important to study the factors that can improve or prevent the adverse consequences of preterm birth. Treatment of preterm infants is likely to be most successful when normal physiology is pursued, and a comprehensive understanding of these processes is therefore crucial.

The work presented in this thesis aimed to elucidate both normal physiology, as well as some of the factors contributing to or preventing adverse outcomes in preterm infants, with a focus on early-life endocrine regulation.

SUMMARY OF THE RESULTS

Part 1: Early-life glucocorticoid regulation

In **chapter 2** we explored cortisol and cortisone (glucocorticoids; GCs) concentrations measured in hair of neonates. We found that with an increasing gestational age, concentrations of GCs increased as well. This is in line with the hypothesis that a spike in GC concentrations might be a part of the mechanism behind the induction of labor. In **chapter 3** we researched the association between hair GC concentrations and experienced maternal distress during pregnancy. The infants' hair contained lower GC concentrations when their mothers experienced elevated levels of distress during pregnancy, while the mothers themselves had higher hair GC concentrations. The results of these two studies suggest that GC levels measured in hair could aid in the understanding of glucocorticoid regulation in utero.

Infants are exposed to maternal GCs in the womb, but after birth they can still be exposed to small amounts of maternal GCs through breastmilk. In **chapter 4** we have

reviewed all the available research on the effects of breastmilk GCs on the offspring thus far. However, we concluded that most studies were not performed optimally. Our research group has previously shown that breastmilk GCs follow the maternal GC rhythm that is present in blood: GC concentrations are high in the (early) morning, and are low during the evening and night. None of the studies assessing the effects of breastmilk GCs on offspring took this rhythm into account. Moreover, laboratory analyses to measure GC levels were not optimal either. We therefore recommended that future research should take breastmilk GC rhythmicity into account and determine GC levels with a sensitive method.

We performed research ourselves with regard to the effect of breastmilk rhythmicity on the offspring. First, we assessed whether there was an association between milk macronutrients (carbohydrates, protein and fat) and GCs in breastmilk in **chapter 5**, which we did not find. Next, in **chapter 6**, we studied the association between GC rhythmicity in breastmilk and in the infants' saliva at the age of 1 month. There are some indications that such an association is present, and that breastmilk GC rhythmicity could play a role in the development of a GC rhythm in infants, but the associations are quite weak. We did find that on a group level the infants displayed a different GC rhythm compared to the rhythm seen in adults: the GC rhythm in infants appeared to be biphasic, with a peak in both the morning and evening. This rhythm might be a reflection of the development towards an adult-like glucocorticoid regulation out of fetal-type glucocorticoid regulation. Subsequently, in **chapters 7 and 8**, we studied the associations between breastmilk GC rhythmicity and body composition, behavior and sleep of the infants at the age of 3 months. We did not find any associations, which might indicate that GCs in breastmilk do not significantly influence outcomes in the infants. The lack of associations might also be explained by the small sample size and relatively short follow-up period of our study.

Part 2: Glucocorticoid regulation and sex

It is known that sex differences in glucocorticoid regulation are present in adults. However, sex differences in (clinical) outcomes are already present in preterm born infants; for example, mortality risk is higher in boys compared to girls. In **chapters 9 and 10** we assessed whether sex differences with regard to basal GC concentrations as well as after stress tests are already present during childhood. To do this, we reviewed the existing literature systematically, and we also performed a meta-analysis. We discovered that sex differences are already present during childhood, both with regard to basal GC concentrations as well as after stress tests. These differences appeared to change under the influence of puberty, at least for the basal GC concentrations. The presence of sex differences in glucocorticoid regulation might partly explain the observed differences in (clinical) outcomes seen after preterm birth. It could also be a part of the mechanism

behind the different disease risk profiles between adult men and women. However, comparing the study results – especially with regard to GC concentrations after stress tests – was hampered by the many different protocols which were used, as well as the lack of a uniform presentation of the data. We have therefore recommended to use standardized stress tests as well as a standardized presentation of results for future studies on (sex differences in) glucocorticoid regulation.

Part 3: Early-life thyroid regulation in preterm infants

The organs of preterm born infants are still immature. This is also the case for the thyroid gland: it is possible that a temporary dip in thyroid hormone concentrations occurs in preterm born infants. Thyroid hormones are important for the development of the brain; children with congenital hypothyroidism or infants whose mothers had low thyroid hormone concentrations during pregnancy are at an increased risk for neurodevelopmental delays. Previous studies have shown that the temporary dip in thyroid hormones is also associated with adverse neurodevelopmental outcomes in childhood. In **chapters 11 and 12** we studied whether these associations are still present at the age of 19 years. No differences were found in IQ and motor performance between preterm-born children who did and did not have a temporary dip in thyroid hormones. Some differences in behavioral outcomes were found: adolescents were more likely to have internalizing behavior when a dip in thyroid hormones had occurred. However, we did not consider this to be clinically significant enough to recommend standard screening for the temporary thyroid hormone dip in preterm infants.

Part 4: Early-life growth and neurodevelopment

Infants can be admitted to the neonatal intensive care unit (NICU) for a multitude of reasons: for example, they were born prematurely, or their birth weight was too low. Many studies on prematurity include study subjects based on gestational age. However, some studies (also) include infants based on birth weight. Two often-used terms in studies concerning prematurity are Very Preterm (VP; gestational age <32 weeks) and Very Low Birth Weight (VLBW; birth weight <1500 grams). It is already known from previous research that the short-term outcomes are different between these two terms. We have studied whether these two terms also lead to different long-term outcomes in **chapters 13 and 14**. We discovered that infants born VP had a different growth pattern and final height compared to infants born with a VLBW. Additionally, these infants also differed with regard to neurodevelopmental outcomes: they had a different IQ-score as well as different behavioral outcomes, although no differences were found regarding education level and occupation. We have therefore recommended that future studies in preterm infants should include subjects based on gestational age rather than birth weight, at least in countries where gestational age can be reliably assessed in utero. This way, a

representative study population can be created and the true impact of preterm birth can be studied.

In the past decades, treatment strategies in the NICU have been improved significantly. We studied whether this has also led to different growth and neurodevelopmental outcomes in **chapter 15**. We found that an adverse growth pattern (appropriate-for-gestational-age with postnatal growth retardation, or small-for-gestational-age without catch-up growth) occurred less frequently in a cohort established in 2003 compared to a cohort established in 1983. However, the associations between adverse growth patterns and neurodevelopmental outcomes did not change in those 20 years. Therefore, it is important to remain focused on achieving optimal growth in preterm infants.

CONCLUSION

In this thesis, we explored glucocorticoid regulation and its development. We found that GCs measured in hair of neonates reflects glucocorticoid regulation in utero. Hair GC levels are mostly affected by gestational age, while maternal distress during pregnancy also has its influences. After birth, GC levels in 1-month-old infants showed a biphasic diurnal rhythm, possibly reflecting the development of an adult-like glucocorticoid regulation. Breastmilk GC rhythmicity might influence the infant's glucocorticoid regulation at age 1 month, but it was not associated with other neurodevelopmental and growth outcomes in the infants at age 3 months. Neurodevelopment at age 19 was associated with intra- and extra-uterine growth, but not with thyroid functioning in preterm infants. Lastly, in order to improve research and the comparison of studies, we concluded that methodological standardization with regard to inclusion criteria as well as testing protocols should be encouraged.

These new insights form a foundation and a framework for future studies, particularly with regard to glucocorticoid regulation in preterm infants and its effects on long-term neurodevelopmental outcomes.