The fetal origins of adult disease, the evidence and mechanisms
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Summary and discussion
This thesis focuses on the fetal origins hypothesis, which states that undernutrition during important periods of growth and development can lead to permanent changes in the physiology and metabolism of the body. Since it was formulated by David Barker in the late 1980’s, a large body of evidence has been assembled to support the hypothesis; varying from direct evidence from experiments in animal models to evidence from large observational human cohort studies. In recent years, underlying mechanisms have been investigated and the relevance of the fetal origins of health and disease for current pregnancies has been further explored. The research reported in this thesis further adds to this field in science.

Chapters 2 and 3 are systematic reviews that summarize the available evidence from animal experiments of the effects of prenatal undernutrition on blood pressure and glucose metabolism. Chapter 2 focusses on the effects of prenatal undernutrition on glucose and insulin metabolism and beta cell mass. The meta-analysis showed that despite significant heterogeneity in the studies - prenatal protein restriction increased plasma glucose concentrations in later life (0.42 mmol/l (95% CI 0.07 to 0.77)). Both general undernutrition and protein restriction reduced plasma insulin concentrations in later life (general undernutrition: -0.03 nmol/l (95%CI -0.04 to -0.01), protein restricted: -0.04 nmol/l (95%CI -0.08 to 0.00)) and beta cell mass (general undernutrition: -1.24 mg (95% CI -1.88 to -1.60), protein restriction: -0.99 mg (95% CI -1.67 to -0.31)). Chapter 3 presents a systematic review and meta-analysis on the effects of prenatal undernutrition on blood pressure. We concluded that both maternal general and protein undernutrition increased systolic blood pressure in later life (general undernutrition: 14.5 mmHg, 95% CI 10.8 to 18.3; protein undernutrition: 18.9 mmHg, 95% CI 16.1 to 21.8) and mean arterial pressure (general undernutrition: 5.0 mmHg, 95% CI 1.4 to 8.6; protein undernutrition: 10.5 mmHg, 95% CI 6.7 to 14.2). Here also, there was substantial heterogeneity in the results. Diastolic blood pressure was increased by protein undernutrition (9.5 mmHg, 95% CI 2.6 to 16.3), while general undernutrition had no significant effect. Both reviews have shown that evidence from experiments in different species has shown that prenatal undernutrition results in increased glucose, reduced insulin concentrations, reduced beta cell mass and generally increased blood pressure in later life, therefore generally supporting the fetal origins hypothesis.

Chapter 4 describes the effect of prenatal exposure to the Dutch famine on adult hand grip strength. Previous studies have shown that those exposed to the Dutch famine during gestation have worse health than those unexposed to famine during gestation. Since hand grip strength is a marker of adult health, our hypothesis was that hand grip strength might be affected after prenatal exposure to the Dutch famine. Contrary to our expectations, we found that men exposed during early gestation had greater hand grip strength compared to unexposed men. This could however largely be explained by adult height.

In Chapter 5 we describe whether epigenetic changes may play a role in the programming effects of prenatal exposure to famine on adult health as they have been found in the Dutch famine birth cohort study. Epigenetics, and specifically methylation, is thought to be a likely mechanism that stores early life experiences. For our study, we considered four candidate genes, all with
cardiovascular and metabolic relevance, and measured methylation at the proximal promoter sites. We found no evidence of effects of prenatal exposure to famine on the methylation status of these four genes. This means that the phenotypic effects we have found are unlikely to be mediated by altered methylation levels in these genes. We also studied at methylation levels and markers of adult health and lifestyle, and observed that methylation of GR and PPARγ were associated with lifestyle factors such as smoking, BMI and exercise, suggesting that postnatal factors also influence methylation patterns.

Chapter 6 described the association between GR methylation and stress response in a subsample of the Dutch famine birth cohort. We found that a decrease in methylation of the GR1-C promoter was associated with a decrease in stress activity, indicated by lower cortisol levels and lower heart rate activity. These associations could be largely explained by differences in lifestyle and education.

In Chapter 7 we investigated whether the offspring of people who were exposed to famine prenatally were less healthy than the offspring of unexposed people, in other words, whether the effects of prenatal famine exposure pass down generations. In this young population, we found that children of prenatally exposed men were heavier and had a higher BMI than children of unexposed men. In women, we found no transgenerational effects. We found no evidence of transgenerational effects of prenatal famine exposure on health in this relatively young F2 sample, but the increased adiposity in the offspring of prenatally undersupplied fathers may lead to increased chronic disease rates in the future.

In Chapter 8 we report on a study describing the consequences of a clinical condition that resembles the nutritional condition a fetus experienced during the Dutch famine; hyperemesis gravidarum (HG). Studies concerning this severe form of nausea and vomiting during early pregnancy mainly focus on treatment of the pregnant women, but little is known about the effects for the offspring. We preformed a systematic review and meta-analysis to summarize the evidence on short and long term outcomes of pregnancies complicated by HG. We found that HG is associated with a higher female/male ratio in the offspring and a higher incidence of low birth weight, being born small for gestational age and prematurity. Apart from a case control study describing an association between HG and testicular cancer in the offspring, little is known about the long term consequences of HG on the health of the offspring.

DISCUSSION

Animal studies
Animal experiments have contributed much to our understanding of disease. Not only in the field of Developmental Origins of Adult Disease (DOHaD), but also in the development of medical therapies. The majority of medical therapies in use today has initially been developed and tested in animals. In a systematic review evaluating how often highly cited animal studies translate to
successful human research, the authors conclude that none of the highly cited studies were negative\(^1\). Less than half of the studies were of good methodological quality and that few included random allocation of animals or blinding of outcome assessment, while the median citation count of these studies was 889. For these animal studies the results of only one-third could be replicated in human randomized trials and just one tenth of the interventions were subsequently approved for use in patients\(^1\).

There are differences between animal studies and the situation in humans that make comparison difficult. For reasons of cost and efficiency, most animal experiments are done in very young and healthy animals, not reflecting the conditions in many human diseases. Many animal studies are limited to male animals, restricting the generalizability of the study to female animals, let alone the human situation. Generalizability might also be impaired by the fact that animals are kept under extremely controlled circumstances. Also, pathophysiology in animals and humans is not always comparable.

It appears self-evident that the quality of the design of an animal experiment will affect its scientific validity, but this had received little attention in the field of translational medicine. The existing evidence shows that these issues are crucial, just like in human studies\(^2\). Animal studies reporting on interventions in emergency medicine were three times more likely to report a positive result if the publication did not report randomization of blinding\(^3\). A systematic review on treatment of acute ischemic stroke showed larger benefit of treatment with lower study quality\(^4,5\). One review found large overstated reporting on the reduction of infarct volume in animal stroke studies without randomization or blinding compared to randomized or blinded studies\(^5\). Human studies support these facts; clinical trials that did not report blinding, concealment of allocation or (double) blinding report larger treatment effects than trials that did report on these methodological issues\(^6-8\). Publication bias is another factor that plays an important role in animal studies. Again in the field of experimental stroke, a meta-analysis was conducted including 525 publications. Of these, only ten (2\%) did not report at least one significant effect on the outcome measures\(^9\). This suggests that negative or neutral animal studies are published much less frequently than positive studies.

In this thesis, we have systematically reviewed the evidence from animal studies for the fetal origins hypothesis regarding glucose metabolism and hypertension. Experiments to study the effects of maternal malnutrition during gestation on the health of the human offspring would be unethical, for obvious reasons. We therefore rely on animal experiments to gain insight in the effect of changes in the maternal diet for the offspring. We conclude that the methodological quality as well as the reporting of these studies is poor. In the field of prenatal undernutrition, different experimental diets are used, which makes comparison difficult. It has been described that exposure to low protein diets in fetal life does not in itself determine the development of hypertension in adult life. The balance of the nutrients within the maternal diets appeared to play a critical role in the programming effect in the offspring\(^10\). There is also a need for improving the methodological quality of animal studies. Reporting should be done according to standards
similar to those applied in human studies. In order to avoid publication bias, animal studies could be registered comparable to the registration of clinical trials. These measures will help reducing publication bias while improving reliability and reproducibility of animal studies.

**Epigenetics**

Until recently, organisms were thought to convey their properties to their offspring by genetic inheritance. The mechanisms leading to the effects of maternal undernutrition during gestation on the health of the offspring, however, are not sufficiently explained by this model.

The term epigenetics was first proposed by developmental biologist Conrad Waddington in the 1940s, who used it to explain how a multicellular organism could develop from one genome. Later, others have defined epigenetics as “the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence.”

The first molecular factor that was identified was DNA methylation in the 1970s followed by histone modification in the 1990s. In DNA methylation, methylation of the 5’ position of cytosine suppresses gene expression by modulating the access of the transcription machinery to the chromatin or by recruiting methyl-binding proteins. Unlike genetic information, which is extremely stable, epigenetic marks are reversible, responding to endogenous and environmental signals.

Epigenetic modifications are a likely explanation for how influences in the early environment can lead to permanent changes in metabolism thereby changing long-term disease risks. It is no surprise then, that many studies have been conducted to elucidate the role of epigenetics in the field of DOHaD.

Animal experiments have shown how maternal dietary restriction resulted in altered methylation patterns of genes in the offspring. These methylation patterns appeared to be stable, as they were present in both juvenile and adult offspring, and were passed down to the next generation. Methylation then seems to act as a memory of an event long after the exposure has ceased. This apparent stability is in sharp contrast with another characterization of epigenetics; reversibility which is necessary for the dynamic epigenome to interact with the environment.

DNA methylation is the only epigenetic mechanism directly affecting the DNA molecule. CpG dinucleotides are the main target for DNA methylation. CpG islands, DNA sequences where CpG dinucleotides are clustered, are found at the 5’ promoter regions of housekeeping genes. A large body of evidence suggests that CpG island methylation of these promoter regions affects transcription. Recently, however, also non-promoter CpG islands are identified (UMR’s, unmethylated regions). These UMR’s can become methylated and potentially modify gene expression. Non-CpG methylation has also been described. In this case, methylation does not involve protein-binding sites, but the gene body is methylated. These findings illustrate the complexity of DNA methylation and the importance of investigating regions outside CpG islands and promoters in future studies. To make matters even more complex, DNA methylation acts in
concert with histone modifications, mediated by methyl- or histone-binding proteins influencing gene expression\textsuperscript{20}. Future studies should not focus on one epigenetic mechanism, but investigate combinations of the mechanisms leading to a better understanding of the epigenetic process.

The changes in methylation levels that are found in humans as a result of changes in maternal diet appear to be much smaller than those found in animal studies. A study measuring 5 CpG’s in the \textit{IGF2} DMR found that children whose mother did and did not take 400 ugram of folic acid per day in the periconceptional period has whole blood methylation levels of 49.5\% and 47.4\% respectively\textsuperscript{21}. The first study describing altered methylation levels after prenatal exposure to the Dutch famine found a methylation difference of 5\% in people exposed to famine prenatally compared to their unexposed siblings\textsuperscript{22}. In contrast, in animals differences of up to 200\% (relative to the control group) are being described\textsuperscript{23}. Furthermore, it is unknown whether this change in methylation is associated with alterations in expression nor whether it is linked to disease in humans.

Other considerations that must be made in future epigenetic studies are the animal model or tissue to use, the technique used to measure (either genome-wide or gene-specific), the timing of measurement (perhaps measuring methylation levels longitudinally), which could help understand the sequence of events and the stability (or plasticity) in time.

**Implications for future research**

Years of research in the field of fetal origins and on the Dutch famine have shown that maternal undernutrition during fetal development has lasting negative consequences for the offspring’s health. Extrapolating this to current pregnancies implies that the environment of the developing embryo is of importance. There are studies showing that undernutrition during the earliest phases of pregnancy, even before implantation, can permanently increase the offspring’s blood pressure\textsuperscript{24}. This suggests that even the very early pre-implantation embryo environment may have long term consequences for the health of the individual. An increasing body of evidence suggests that this is the case for assisted reproductive techniques (ART). Randomly assigning embryos to two different commercially available IVF culture media showed a difference in mean birth weight of 200 grams\textsuperscript{25}. Long term follow up of children born after assisted reproductive techniques has demonstrated that IVF children have higher systolic and diastolic blood pressure and higher fasting glucose levels compared to controls conceived spontaneously\textsuperscript{26}. Total body fat also seems to be increased\textsuperscript{27}. Girls born after ICSI pregnancies are more adipose at puberty compared to girls born after spontaneous pregnancies\textsuperscript{28}. Recently, alterations in vessel wall properties have been shown among ART children\textsuperscript{29}.

In this thesis we discussed the outcomes of pregnancies complicated by hyperemesis gravidarum. Recently, the first long term consequences of this condition have been described. Offspring exposed to hyperemesis gravidarum in utero was significantly more likely to have a psychological and behavioral disorder compared to unexposed siblings. Depression, bipolar disorder and anxiety were the most frequent reported disorders\textsuperscript{30}. This finding strengthens our
conclusion that children born after pregnancies that are complicated by hyperemesis should be followed up to document the long term consequences of the condition.

Not only in the field of ART or hyperemesis gravidarum, but more in general for all studies comparing interventions during pregnancy to improve neonatal health, the focus should expand to outcomes later in life. Follow up studies should investigate whether children born after these pregnancies have an altered cardiometabolic risk profile due to influences during the crucial phases in early life.
REFERENCE LIST

Summary and discussion


