Fetal programming in pregnancy-associated disorders
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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General discussion and future perspectives
General discussion

The early life or *in utero* environment has the ability to program the physiological functions throughout life and this field of research is widely known as ‘fetal programming’ or ‘the Developmental Origins of Health and Disease’. Hence, unfavorable conditions early in life may have long-lasting physiological consequences. It is now more apparent that certain diseases begin to develop in early life, and that some individuals are more susceptible than others. Moreover, different in utero exposures might be associated with a different health outcome. For that cause, we aimed in this thesis to develop models of human pregnancy-associated diseases that would be suitable for studying the underlying mechanisms of fetal programming.

To ensure optimal fetal growth and development and to prepare the fetus for postnatal life, normal pregnancy is associated with local and systemic maternal adaptations. Failure to achieve nutrient availability or placental functionality will lead to poor fetal growth and development of pregnancy-associated disorders. Among the most common pregnancy-associated disorders that contribute to a less optimal *in utero* development are preeclampsia, intrauterine growth restriction, and gestational diabetes. They are characterized by a complex etiology and can increase the risk of developing other pregnancy-associated disorders. Moreover, the exposed fetus is associated with an increased risk of developing cardiovascular and metabolic diseases in adulthood. However, which gestational factors are involved and to what extent this leads to fetal programming is still widely unknown.

In order to evaluate the effects of gestational factors on fetal programming, the development of comprehensive preclinical models is of major importance. The research described in this thesis focused on novel models for the most frequent pregnancy-associated disorders and its effects on the fetal health. Ultimately, the results as described in this thesis could help both clinical management and severity evaluation of the fetal programming.

Fetal growth restriction as a fetal programming indicator

Maternal health has an important role in fetal growth and development. Ideal conditions during pregnancy are associated with optimal fetal outcome parameters. As soon as the fetus does not receive the necessary nutrients and oxygen or is exposed to adverse signaling molecules or infection, it will fail to achieve its genetically predetermined size [1,2]. The majority of pregnancy-associated disorders, e.g. preeclampsia, placental insufficiency or diabetes, are accompanied with fetal growth restriction ([3,4] and **Chapter 2, 3, 4, 5, 6**) which is considered to have an immediate impact on fetal health. Nowadays, we are more aware of the long-term impact of these disorders on the offspring’s health ([5,6] and **Chapter 2, 5**).

Fetal growth restriction is considered as a medical condition on its own, however, based on the fetal programming principle, the importance of fetal growth
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Restriction is shifting towards a fetal programming ‘alarm’ for later neonatal outcome. It is now well established that growth restricted offspring not only have a higher mortality rate and an increased risk of cerebrovascular insults, but also the incidence of myocardial infarctions and metabolic syndrome in later life is increased [7]. Fetal growth restriction can be accompanied with sparing of the brain on the cost of the liver (Chapter 3, 4) leading to asymmetrical growth restriction, or can be characterized by a symmetrical growth reduction of both the liver and the brain (Chapter 4). Although the terms of asymmetrical and symmetrical growth restriction tend to be dispatched in medical terminology, they are still useful predictors of outcomes [8]. However, longitudinal studies on growth and health outcomes of growth-restricted fetuses, where the fetuses are stratified based on growth symmetry at birth, are scarce. Nevertheless, we report that experimental preeclampsia is followed by different patterns of growth restriction in males and females which in turn might be important in the explanation of sex-specific differences in fetal programming (Chapter 4). Correspondingly, not only the fetal sex, but also the level and the type of growth restriction might explain the different susceptibility to diseases in later life, which consequently needs to be evaluated.

The role of preeclampsia in fetal and adult health

The long-term health consequences of children exposed to preeclampsia were clearly shown in several epidemiological and experimental studies (Chapter 2), where the emphasis was on the cardiovascular and metabolic health of the offspring. Among the most frequent alterations, were reported hypertension, vascular stiffness and increased rate of cerebral stroke or myocardial infarction (Chapter 2). It should come as no surprise that preeclampsia is associated with increased risk of cardiovascular disorders in the offspring, when preeclampsia per se is a multifaceted vascular disorder of pregnancy [9]. However, during preeclampsia, the fetus is exposed to poor nutrition and to specific signaling molecules that in turn can affect metabolically active tissues such as the fetal liver [10]. Therefore, in chapter 3 we assessed the potential role of sFlt-1 in fetal programming, which was suggested as one of the most important pathophysiological factors in the development of preeclampsia [11,12]. We overexpressed the antiangiogenic factor sFlt-1 in pregnant dams and we compared fetal outcomes to that of controls. We showed that fetal growth restriction, as one of the most important fetal programming predictors, was present in our sFlt-1 overexpressed dams. Not the degree of maternal symptoms, but specifically circulating levels of sFlt-1 in the mother and the offspring were associated with fetal outcome, suggesting that high plasma sFlt-1 levels are associated with severe adverse fetal outcomes. Moreover, the fetuses exposed to high sFlt-1 concentrations had decreased liver weight and an altered molecular footprint with predominant alteration of the fatty acid metabolism genes and epigenetic changes of its master regulator Ppara (Chapter 3). These findings are compatible with previous data demonstrating that poor nutritional status in utero can affect DNA methylation marks of Ppara in the offspring’s liver [13]. Although the direct effects of sFlt-1 on Ppara
and fatty acid metabolism genes were not evaluated in our study, such analysis might be valuable to be carried out in future studies.

Overall, in addition to a considerable body of evidence for cardiovascular impairments due to in utero exposure to preeclampsia, our study shows new aspects of the effects of preeclamptic factors on fetal health including aberrant liver molecular patterns.

The ideal preclinical preeclamptic model to study fetal programming

To define a complex disease is a rather puzzling and challenging task, especially when the etiology is unknown. Moreover, it is even more provocative to design an optimal preclinical model, which will cover the full pathophysiological course of the disease. Preeclampsia is known as the disease of theories, where many pathophysiological mechanisms have been suggested, however, without firm knowledge which one is preceding the other [14,15]. Interestingly, each of these factors individually can mimic the clinical course of preeclampsia in animal models (e.g. hypertension and albuminuria during pregnancy) [16–19]. Nevertheless, in terms of fetal programming, we need to take into account the whole spectrum of possible pathophysiological mechanisms during gestation and their interaction, in order to obtain a better extrapolation of the human circumstances.

It is possible that the best approach in designing a novel model is to replicate the most common pathophysiological events. There are strong supporting data from several studies that increased inflammatory cytokines (inflammation) and antiangiogenic molecules such as sFlt-1 (angiogenic dysbalance) are increased in the majority of the preeclampsia cases [20–23]. In mice, acute systemic inflammation can be acquired by a single lipopolysaccharide injection [24] and increased sFlt-1 levels are induced by adenoviral overexpression of this factor [16]. Combination of these two techniques (described in Chapter 4) resulted in hypertension, albuminuria, a 2-fold increase in sFlt-1, and a specific metabolic footprint without major abnormalities of the placenta in pregnant dams. As a consequence, the fetuses showed growth restriction and a sex-specific metabolome pattern (Chapter 4) without obvious fetal malformations. Therefore, this experimental double hit preeclamptic model might serve as an indicator of sex-specific and symmetry based growth restriction, which can be illustrative in mere absence of human epidemiological data. The findings from our model may be of a clinical importance also for the offspring of preeclamptic pregnancies who are at higher risk for cardiovascular and metabolic diseases.
The role of placental insufficiency and intrauterine growth restriction in adult health

One of the best studied pregnancy-associated disorder in terms of fetal programming is intrauterine growth restriction. It is associated with a higher risk for development of diabetes, obesity and cardiovascular events in later life [25]. As presented in chapter 5, we designed a novel approach to study fetal programming by IUGR, by conditional deletion of one of the most important transcriptional factors for placenta development Tfap2c in the placenta. Due to our genetic manipulation, the junctional zone was reduced in size and smaller placentas were obtained. The junctional zone is important for nutrient transport (to a lesser extent when compared to the labyrinth zone) and for the endocrine function of the placenta [26]. The work described in chapter 5 provides novel insights into the long-term effects of placental insufficiency (and subsequent IUGR) into the metabolic perturbations without affecting maternal nutrition. The most evident difference between IUGR and control mice was a markedly decreased body weight at around one year of age. However, at the same point, only minor metabolic phenotypic differences were observed between the groups. This might be explained by our limited or no exposure to extra environmental stressors (e.g. nutrition, smoking, stress) of our animals during their postnatal life. However, certain molecular changes were observed in the aged IUGR females in terms of increased plasma free fatty acids, increased mRNA levels of fatty acid synthase and ER stress markers in white adipose tissue (Chapter 5). In turn, this might be an underlying process that can get exaggerated with aging or when second environmental stressors are present.

It is still intriguing why we observe these effects only in the aged IUGR females, as both sexes are equally exposed to the placental insufficiency in utero. One possible explanation could be that male and female fetuses have distinct growth patterns and because of that can be differently affected by the placental insufficiency [27,28]. Based on the findings obtained thus far, long-term effects of isolated placental insufficiency without maternal nutrient deprivation does not always lead to metabolic disorders, however, can affect certain molecular processes at least in the white adipose tissue.

The role of pre-existing diabetes in fetal programming

Prenatal exposure to an excess of nutrients or metabolic disturbances also influences the development of cardiovascular, metabolic and neurological functions in the offspring [29,30]. Moreover, many factors can potentially interfere with the genetically predetermined fetal growth. For example, metabolic alterations associated with diabetes can either stimulate fetal growth (via hyperglycemia) or restrict it (via maternal vasculopathy and subsequent placental insufficiency) [4,31]. Several studies reported that exposure of the fast-growing fetus to hyperglycemia and hyperinsulinemia can lead to either an increased or a decreased body weight, a disrupted immune system and metabolic alterations e.g. increased plasma triglycerides and cholesterol [6,32,33].
Pre-existing diabetes during pregnancy in most of the cases is represented by type 2 diabetes [34]. One important difference between gestational and pre-existing type 2 diabetes is that the pre-existing diabetes is already present during the first trimester of pregnancy which is also one of the most sensitive and critical periods of fetal growth and development [35]. Exposure to high levels of glucose and insulin during this period can lead to congenital abnormalities, but also to metabolic disturbances in the fetus and defective placental morphology [36]. Results presented in chapter 6 demonstrated that hyperglycemia, hyperinsulinemia and consequent hyperlipidemia of the mother lead to fetal growth restriction and cerebral changes, accompanied by epigenetic modifications of the transcriptional factor Srebf2. Moreover, Srebf2 has a major implication in cholesterol metabolism and it has been reported to be regulated by insulin [37], promoting it as a possible marker of fetal programming due to diabetes in pregnancy. Taking into consideration that children exposed to diabetes during pregnancy are at increased risk of neurological and cardiometabolic disorders, our data supported these observations by showing DNA methylation changes of Srebf2 promoter region in organs (such as liver and brain) that are dependent on cholesterol metabolism (Chapter 6). Improved control of hyperglycemia, hyperinsulinemia, and hyperlipidemia during early stages of pregnancy, should reduce the overall diabetic burden on fetal programming.

Pregnancy-associated disorders: do they differently affect the fetus?

One major characteristic of all described pregnancy-associated disorders in this thesis (Chapter 3, 4, 5) is an affected body weight of the fetus. The pathophysiological mechanisms of these disorders are distinctive, yet with a similar gross outcome. While preeclampsia in chapter 4 is characterized by inflammation and angiogenic dysbalance in the mother, in our IUGR model the placental insufficiency is the causative mechanism (Chapter 5). On the other side of the spectrum, during pre-existing diabetes (Chapter 6) there can be fetal growth restriction, even with maternal hyperglycemia and hyperinsulinemia. These all are characterized as gestational factors that can influence fetal growth, and possibly also to a different degree have an influence on fetal programming.

Another common mechanism where these factors can have their detrimental effects is via programming of the placenta. During preeclampsia, we observed smaller placentas (Chapter 4), and in our experimental IUGR model, the placenta was insufficient in the junctional zone (Chapter 5). During pre-existing diabetes, placental morphology is also heavily affected (unpublished data). This different degree of effects on the placental might, in turn, be important for the differences and the similarities between fetal programming by pregnancy-associated disorders. Based on our findings, long-term prospective studies should elucidate which effects are found and to what extent each of these gestational factors affects the offspring’s health.
Sex-specific differences in fetal programming

Sex differences are well-known factors for different outcomes in several cardiovascular, metabolic and neurodegenerative disorders. For example, while males are much more susceptible to diabetes, Parkinson disease and coronary heart disease [38–41], females are much more prone to develop obesity, metabolic syndrome or multiple sclerosis [42,43]. Although, the mechanisms behind these sex-specific differences in disease susceptibility are not well understood, at least in part they have been attributed to the fundamental differences in the metabolic homeostasis control, hormone production, inflammation capacities and genetics [40,44].

Sex-specific differences have been also reported in the terms of the fetal growth and development [28]. Male fetuses have a significantly higher growth rate in the last trimester of pregnancy [45,46] whereas when carrying a female fetus the concentration of placental growth factor is increased during the first trimester of pregnancy [47]. Also at birth, on average, male neonates are heavier than females. Moreover, fetal sex has a significant impact on pregnancy outcome, as e.g. preterm birth, gestational diabetes, and late-onset preeclampsia are more prevalent when carrying a male fetus [48,49].

It is well known that sex can serve as a significant predictor of fetal growth, development, and prevalence of diseases; and nowadays we are more aware of specific sex-differences in fetal programming [50]. When possible in our studies, we stratified the effects of pregnancy-associated disorders per sex (Chapter 3, 4). In these studies, we observed more profound alterations in female offspring exposed to preeclampsia (Chapter 3) or intrauterine growth restriction (Chapter 4). This is in line with previous observations showing that in utero insults that include placental modifications lead to fetal programming effects especially in female offspring [51,52].

While in general it is known that sex-differences affect disease prevalence, to what extent the pregnancy-associated disorders impact the progression to offspring diseases in function of sex is still unknown. For that cause, more experimental studies, stratified by fetal sex, in fetal programming are needed.

Future perspectives: Fetal programming as a drug target

Our studies revealed similar, but also distinct patterns of fetal programming in several pregnancy-associated disorders. All of the studied pregnancy-associated disorders resulted in fetal growth restriction and had distinct metabolic and sex-specific patterns in comparison to controls. These differences are triggered by exposure to different factors occurring during gestation such as inflammation, antiangiogenic mediators, placental insufficiency, hyperglycemia, and hyperinsulinemia. However, we were not able to perform experiments where we could abolish these gestational factors or ameliorate the fetal growth restriction in order to reverse the effect of fetal programming. In future, experiments that involve targeting of these factors may shift the gestational adverse effects.

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factors into balance creating optimal conditions for fetal growth and development. For example, usage of blockers against two of the most common pathophysiological mechanisms during preeclampsia, such as sFlt-1 antagonists in combination with nonsteroidal anti-inflammatory drugs (NSAID) [53], might counteract the clinical course of preeclampsia and alleviate the fetal programming effect. Sildenafil citrate was proposed as a potent vasodilator that might increase the blood flow during placental insufficiency and improve the fetal growth [54]. In addition, we showed that in adulthood, free fatty acids are increased in aged IUGR female offspring, so to a certain degree, a usage of fatty acid synthesis blockers such as the antifungal antibiotic cerulenin [55,56] might be effective in reversing this phenotype. To counteract the effects of a diabetic intrauterine environment, the most valuable effort would be early diagnosis and proper management (diet, exercise, PPAR agonists) of the maternal metabolic disturbances [57,58].

It is of extreme importance that such strategies are highly selective (including sex-specific) and properly timed, without further disruption of other maternal and fetal factors. A complete elucidation of mechanism important in fetal programming will contribute to an improved care of the pregnant women and the fetus as patients for the benefit of its future well-being.
References


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