From preeclampsia to renal disease
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Chapter 8

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GENERAL DISCUSSION

Preeclampsia is a syndrome affecting maternal and fetal health during gestation that also has an impact on the long-term health of women. Formerly preeclamptic women are more susceptible to cardiovascular and renal disease later in life as compared to women with a history of a healthy pregnancy. Whether it is preeclampsia per se or shared risk factors for preeclampsia and cardiovascular and renal disease inducing this increased risk is unknown. In addition, the mechanisms behind this increased susceptibility remain to be elucidated. Both questions were addressed in this thesis.

We hypothesized that increased angiotensin II (ang II) sensitivity and endothelial dysfunction might be involved. We first validated a rat model for preeclampsia for these specific features assumed to be involved in the postpartum increased risk for cardiovascular and renal disease (Chapter 2). Thereafter, we performed postpartum studies in formerly preeclamptic women and formerly experimental preeclamptic rats (Chapter 3-7). We aimed to assess whether disturbances seen during preeclampsia, i.e. altered renal hemodynamics, increased ang II sensitivity, and endothelial dysfunction, persisted postpartum in order to elucidate whether these mechanisms might be involved in the increased susceptibility to cardiovascular and renal disease (Figure 1). Elucidating these mechanisms will not only help to explain why formerly preeclamptic women are more susceptible for cardiovascular and renal disease, but will also be an important step towards structured follow-up and specific preventive treatment of these women.

The angiotensin II sensitivity and endothelial function in experimental preeclampsia

In this thesis, we used the low-dose endotoxin rat model for preeclampsia. This model is based on the increased inflammatory status seen in human preeclampsia and is pregnancy specific 1. In this model, the two main preeclampsia-like features, hypertension and proteinuria, have been described extensively by our group and others 1-5. Previous studies found glomerular inflammation 6 and increased activation of circulating monocytes and granulocytes 7, 8. In addition, fetal and placental involvement have been shown 4-5.

In Chapter 2 we studied ang II sensitivity and endothelial function in pregnant rats with low-dose endotoxin induced preeclampsia 1, both characteristics of the multifactorial disease of preeclampsia 9-11. In healthy pregnancy, sensitivity to ang II is decreased 12, while during preeclampsia, ang II sensitivity is increased 9, 10. In addition, healthy pregnancy is characterized by an altered regulation of the endothelial-derived vasodilating factors leading to increased vasodilation, while in preeclampsia endothelial dysfunction occurs, leading to impaired vasodilation 11. To assess ang II sensitivity and endothelial (dys)function, ex-vivo isotonic contraction experiments in the thoracic aorta were performed.

Similar to the human situation, decreased ang II sensitivity was seen during healthy pregnancy in the rat and sensitivity to ang II was increased during experimental preeclampsia. Interestingly, the experimental preeclamptic rats responded similarly to ang II as non-pregnant rats, suggesting maladaptation to pregnancy in experimental preeclampsia. In human pregnancy, the maladaptation in ang II sensitivity seen in preeclampsia, is already present during the first trimester of
pregnancy\textsuperscript{9}. Since endotoxin was infused on day 14 of rat pregnancy (i.e. second half of pregnancy) and ang II sensitivity was assessed at the end of gestation, it can be suggested that pregnancy-specific adaptations are reversed in experimental preeclampsia. Increased ang II sensitivity may cause generalized vasoconstriction in the vascular system and therefore contribute to the development of hypertension seen in preeclampsia\textsuperscript{11}. Moreover, the increased ang II sensitivity in this model might also contribute to the development of other preeclampsia-like features since blocking the ang II type I receptor (AT1-R) ameliorated hypertension, proteinuria, and fetal outcome in this model\textsuperscript{5}.

Although several mechanisms have been proposed to be involved in the changes in ang II sensitivity, in Chapter 2 we mainly focused on the role of the AT1-R and the ang II type II receptor (AT2-R) in the ang II sensitivity. The AT1-R mediated vascular constriction and AT2-R mediated vascular relaxation were increased in experimental preeclampsia as compared to healthy pregnancy and an increased AT1-R/AT2-R mRNA balance in the thoracic aorta of the experimental preeclamptic rat was found. The recently suggested phenotypic change of the AT2-R towards a constrictor receptor during preeclampsia\textsuperscript{10}, seemed not to be present in our animal model for preeclampsia. We found increased AT2-R mediated vasodilation in experimental preeclampsia as compared to the healthy pregnant rats. Since experimental preeclampsia was induced in the second half of pregnancy and ang II sensitivity was studied at day 19 of gestation, studying in-vivo and ex-vivo responses of ang II via the AT2-R, at different time points during gestation, is of interest. In rat the uterus\textsuperscript{14,15} and the rat kidney\textsuperscript{16} for example, it has been shown that ang 1-7, ACE2, and AT2-R expression varied throughout gestation. Therefore, it can be suggested that in order to adapt to the pregnancy-induced maternal hemodynamic changes, the magnitude and site of expression of the ang II receptors varies during gestation, thereby affecting ang II responses and receptor function. During preeclampsia this ingenious RAAS regulation might then be disturbed via for instance endothelial dysfunction, secreted factors from the stressed placenta directly influencing the RAAS (i.e. soluble fms-like tyrosine kinase-1 (sFlt1)) or via AT1-R auto-antibodies (AT1-AA) influencing receptor activity and expression.

In Chapter 2, in addition to the role of the RAAS, the role of endothelial (dys)function on vascular tone was assessed during pregnancy and in experimental preeclampsia. The increased role of NO in the pregnancy-induced vasodilation\textsuperscript{17} was not seen in our study. This coincides with the lack of differences in eNOS and iNOS mRNA expression found in our study. The absence of the increased role for NO in vasodilation suggests that in these rats, NO is of less importance in the pregnancy-induced vasodilation. To study this in more detail, NO metabolites (nitrate, nitrate) and NO activity (cGMP) should be assessed in future studies. In addition, the role of NO in the vasodilation of mesenteric arteries can be studied in order to elucidate whether pregnancy-induced changes are vascular bed dependent and is different in restrictive vessels. Whereas the role of NO was not affected by pregnancy, we did observe involvement of the prostanoids and EDHF during pregnancy as we found an increased role for vasodilating prostaglandins and a decreased role for EDHF. In experimental preeclampsia, these pregnancy-specific adaptations of the different endothelial-derived factors were absent. The experimental preeclamptic rats showed similar
vasodilating responses as the non-pregnant rats. This emphasizes again a reversal of the vascular adaptations to pregnancy during experimental preeclampsia. To elucidate this in more detail, in futures studies it would be useful to assess endothelial-dependent vasodilation at different time points during gestation.

Overall, in Chapter 2, we have shown that in the endotoxin model for preeclampsia changes in the RAAS and endothelial function occur. These changes, increased ang II sensitivity and endothelial dysfunction, are also seen in human preeclampsia, suggesting that this model for preeclampsia resembles the human situation in these characteristics. We did not study the mechanisms by which increased ang II sensitivity and endothelial dysfunction were induced. However, it may be suggested that the inflammatory response, induced by the low-dose endotoxin infusion 14, may be involved since inflammatory responses are associated with endothelial cell activation 19, 20, potentially leading to endothelial dysfunction, and ang II mediated inflammatory responses have been described 21. We were not able to detect increased sFlt1 levels in this model (unpublished data), however, the exact role of other angiogenic factors, i.e. placental growth factor, vascular endothelial growth factor (VEGF), or soluble endoglin, need to be examined. For example, VEGF deficiency might play an important role in the development of endothelial dysfunction since VEGF is important in maintaining endothelial health 22. Since ang II sensitivity and endothelial dysfunction have been shown to be present, this animal model is appropriate to specifically study these impairments postpartum in order to elucidate the role of preeclampsia on the postpartum endothelial function and ang II sensitivity.

Renal function, RAAS, and cardiovascular system after preeclampsia
It has been known for some time now, that formerly preeclamptic women are more prone to develop end-stage renal disease later in life 23-25. In Chapter 3 we described potential mechanisms involved in this increased renal risk. We reviewed several studies that have investigated renal function, renal hemodynamics, and the RAAS in women after a pregnancy complicated by preeclampsia. These studies suggest (subtle) persistent impairments. However, since most studies were performed early after pregnancy, it is unknown whether these (sub)clinical impairments are still present, or even worsen, during follow-up at middle age. In addition, the mechanisms involved in the increased long-term risk for cardiovascular and renal disease need to be elucidated in order to make the step towards prevention.

Therefore, we first longitudinally assessed renal function and renal function decline in women with a history of a hypertensive disorder during pregnancy (Chapter 4). Renal function data from women with a history of a (hypertensive) pregnancy collected in the PREVEND (Prevention of REnal and Vascular ENdstage Disease) study, a general population based prospective observational cohort study, were used. In addition, renal function parameters 10-years postpartum were compared in a sub-cohort of women following a healthy pregnancy, following pregnancy induced hypertension (PIH) and following preeclampsia and/or HELLP-syndrome (Chapter 4) to study whether severity of the hypertensive disorder was associated with more prominent effects on renal function. Next, we tested the hypothesis that following preeclampsia, (renal) hemodynamic changes (including
endothelial function) and changes in the RAAS occur. Such changes may be involved in the increased renal risk following an early-onset preeclamptic pregnancy (Chapter 5-7; the RETAP (REsponse To Angiotensin II in formerly Preeclamptic women) study).

To elucidate the mechanisms involved in the increased long-term renal risk following preeclampsia, it is of importance to discriminate between the role of pre-pregnancy risk factors and preeclampsia-induced factors, to distinguish whether preeclampsia itself or common pre-pregnancy risk factors are involved in the postpartum susceptibility to renal disease after preeclampsia. The optimal study design would obviously require pre-pregnancy assessment, but this is extremely difficult to realize in clinical practice. As an alternative, studying formerly preeclamptic women in the absence of cardiovascular risk factors could also shed light on the role of preeclampsia per se. In the RETAP study, we therefore carefully selected formerly early-onset preeclamptic women who were otherwise healthy, i.e. normotensive with a healthy body weight and without cardiovascular or renal diseases or current drug use (Chapter 5, 6, and 7). To study postpartum effects of preeclampsia in a fully clean model, animal studies are more practical; therefore, the endotoxin model for preeclampsia studied in Chapter 2 was also used to study the effects of experimental preeclampsia on the postpartum RAAS (Chapter 6). Since in this model increased ang II sensitivity and endothelial dysfunction was found, it is a suitable model to study these particular impairments postpartum following experimental preeclampsia. In addition, no hypertension or proteinuria was found postpartum in our rats following experimental preeclampsia, showing that indeed healthy rats were studied.

In the next section, we will first elaborate on longitudinal renal function data collected during the PREVEND study in women following a hypertensive disorder during pregnancy (Chapter 4). Thereafter, the mechanisms suggested to be involved in the increased renal risk following preeclampsia that were studied in the RETAP study will be discussed (Chapter 5-7).

Renal function decline following a hypertensive disorder during pregnancy
Since formerly preeclamptic women exhibit an increased renal risk postpartum on the long-run, it is of interest to longitudinally assess renal function for an extended period of time. So far, only one study intended to investigate long-term renal function following preeclampsia. This cross-sectional small study suggested that age-dependent renal function decline was not affected by a history of preeclampsia 26. In Chapter 4 we presented unique longitudinal data from the PREVEND study. We are the first to show an accelerated renal function decline (assessed by estimated glomerular filtration rate (eGFR)) in women after a self-reported history of a hypertensive disorder during pregnancy. In addition, these women had slightly, but significantly lower eGFR as compared to controls, within the normal range. Both the steeper decline in renal function and the lower eGFR fit in the observation that these former hypertensive women were more susceptible to develop chronic kidney disease (CKD). However, as shown previously, the absolute risk for renal disease following preeclampsia is relatively low 23-25. Also, the observed abnormalities in renal function after preeclampsia were subtle. Although our study highlights the importance of longitudinal follow-up, it remains questionable whether it is of additive value to assess renal function (i.e. eGFR) during follow-up in all women...
following a hypertensive disorder of pregnancy. Importantly, formerly hypertensive women exhibited higher blood pressure values, lower renal function, and were using more anti-hypertensive drugs as compared to healthy controls, indicating that women with a history of hypertensive disorder of pregnancy carry a high-risk profile for future cardiovascular and renal disease. Whether this is a cause or a consequence of their hypertensive disorder during pregnancy and long-term increased health risk remains to be investigated in future studies.

As part of the women from the above mentioned PREVEND study delivered in one of the two hospitals in Groningen, we retrieved medical records from these women in order to obtain verified information on the severity of the hypertensive disorder during pregnancy (PIH, the HELLP syndrome, and preeclampsia). This allowed us to compare renal function data from women with PIH, HELLP or preeclampsia with healthy controls 10-years post-partum in a case-control set-up study (Chapter 4). The renal function data showed that women with former preeclampsia or HELLP syndrome had the highest risk for renal function impairment and CKD 10-years postpartum. It must be stated here that the prevalence of hypertensive disorders in the PREVEND cohort was higher than reported in other studies, possibly due to oversampling of subjects with albuminuria, by design of the PREVEND study. Consequently, our findings require confirmation in other cohorts.

If our data can be confirmed, this could provide a basis for recommendations to screen for renal abnormalities (i.e. plasma creatinine, eGFR, and/or albuminuria) at middle age (i.e. from postmenopausal onwards) in women with a history of preeclampsia or HELLP syndrome. In addition, in line with our national guideline for cardiovascular risk management (CVRM), it should be strongly considered to establish a risk profile in all women with a history of (early-onset) preeclampsia/HELLP syndrome. However, before recommendations of standardized renal follow-up in women following a hypertensive disorder during pregnancy can be made, the exact time course at which, for example, renal function decline becomes evident, needs to be identified in long-term longitudinal studies. Furthermore, more mechanistic insight is necessary in order to interfere in this decline by the use of therapeutic interventions.

From preeclampsia to renal disease; a mechanistic role for an altered renal hemodynamic profile?
Several studies suggest persistent impairments in renal hemodynamics after preeclampsia, which might subsequently contribute to the increased renal risk in later life. However, this was mainly found in formerly preeclamptic women that were hypertensive at the time of study, and thus might be related to the hypertension per se. Therefore, these studies cannot discriminate between the role of comorbidities and the role of the former preeclampsia in inducing renal function impairments. Moreover, these prior studies did not standardize for sodium status or stage of menstrual cycle, both known to exert substantial effects of renal hemodynamics. In Chapter 5, we therefore assessed renal hemodynamics in healthy formerly pregnant women and healthy formerly early-onset preeclamptic women, during standardized sodium conditions (i.e. on low and high sodium intake), and during standardized stage of the menstrual cycle. We observed subtle long-term changes in renal hemodynamics, i.e. increased filtration fraction, in formerly preeclamptic women on both sodium intakes. Since healthy women were studied, this may suggest a role for preeclampsia itself.
on postpartum renal hemodynamics. However, pre-pregnancy changes cannot be ruled out in the human situation as mentioned earlier.

The increase in filtration fraction found relatively short (1-10 years) after preeclampsia, might underlie the increased long-term renal risk in these women. Indeed, a high filtration fraction has been independently associated with progressive renal damage in CKD 32, 33. Other potential mechanisms of renal function loss could be the higher blood pressure, and/or presence of additional cardiovascular risk factors, as these are generally associated with an increased risk for long-term renal function loss. As described previously and studied in Chapter 4, in formerly preeclamptic women the rate of renal function decline with aging is more pronounced than in control women. This could potentially lead to overt CKD on long-term, as supported by the observed higher prevalence of CKD in the formerly preeclamptic women in our case-control study. Ideally, one would therefore assess postpartum renal hemodynamics longitudinally, in order to elucidate the time-course of these changes in renal hemodynamics. In addition, although the changes observed 1-10 years postpartum were relatively subtle, these changes can become of clinical significance with further progress at a more advanced age.

Several mechanisms could potentially be involved in the increased filtration fraction found in formerly preeclamptic women. An increase in vasoconstriction of the efferent arteriole relative to the afferent arteriole is one of the renal hemodynamic changes leading to an increased filtration fraction 34. The RAAS is a known pathway that could induce this particular pattern 34. However, no differences in renal ang II responses or RAAS parameters were seen in the formerly preeclamptic women studied in Chapter 5. To assess the mechanisms underlying changes in filtration fraction with certainty and to verify the role of the vasotonus in the arterioles, micropuncture studies 35 in animals that suffered from experimental preeclampsia may gain more insight. Studying renal hemodynamics in animals also allows standardizing conditions, i.e. sodium and RAAS status, body weight, and pre-pregnancy renal function. With regards to the structural impairments, animal studies are also ideal to assess whether glomerular lesions are present following experimental preeclampsia and whether they affect renal hemodynamics. In addition, mechanisms involved in inducing these glomerular lesions can be assessed, i.e. inflammation, imbalance in angiogenic factors or local RAAS activity.

**From preeclampsia to renal disease; a mechanistic role for the renin-angiotensin aldosterone system?**

As observed in Chapter 2 and earlier mentioned studies, during preeclampsia ang II sensitivity is increased and therefore it can be suggested thatincreased ang II sensitivity persists postpartum. Postpartum increased ang II sensitivity might then contribute to the increased renal risk. This can for example be directly via renal vascular tone regulation, or indirectly via increasing systemic blood pressure. So far, three studies investigated ang II sensitivity in women with a history of PIH or preeclampsia 36-38. In general, all three suggest an increased ang II sensitivity postpartum. However, co-morbidity, i.e. hypertension or overweight, was present and studies were not standardized for sodium status or menstrual phase, both factors known to play a role in the activity of the RAAS. Therefore, one cannot conclude that is was preeclampsia itself that induced this postpartum
increased ang II sensitivity.

In order to investigate whether postpartum RAAS alterations are present after preeclampsia, blood pressure response upon ang II infusion was studied (Chapter 6). Although subtle, our data suggested an increased blood pressure response upon ang II infusion postpartum in women after an early-onset preeclamptic pregnancy. To further substantiate between the effect of co-morbidity and a history of preeclampsia on the increased ang II sensitivity postpartum, blood pressure and kidney response upon ang II infusion were studied in an experimental rat model for preeclampsia (Chapter 6). In line with the human study, also rats did not show hypertension or proteinuria following a healthy pregnancy or experimental preeclampsia. And again, increased responsiveness upon ang II was found in formerly experimental preeclamptic rats. Together, our human and experimental data suggest persistent ang II sensitivity following (experimental) preeclampsia which subsequently might contribute to the increased renal risk. In addition, since healthy women and pre-pregnancy identical rats were studied, it can be hypothesized that preeclampsia itself induces this increased ang II sensitivity postpartum.

To assess the mechanisms behind the increased ang II sensitivity postpartum, the function and role of the aligned receptors for ang II is of interest. Since in our experimental work we have shown an increased AT1-R response during preeclampsia (Chapter 2) while a diminished AT2-R response was seen after preeclampsia (Chapter 6), it may not merely be a persistence of ang II sensitivity following preeclampsia, there also appears to be a transition in the balance between these receptors. In addition, the postpartum presence of AT1-AA (which we found in women following preeclampsia (Chapter 6)) might play a direct role in the increased ang II sensitivity or indirectly influences this receptor balance.

From preeclampsia to renal disease; a mechanistic role for altered arterial stiffness?
Increased arterial stiffness is an independent precursor of cardiovascular diseases 39, 40 and could be a candidate mechanism for the increased renal and cardiovascular risk in formerly preeclamptic women. Several studies have reported increased arterial stiffness in women with a history of preeclampsia 41-43. However, these studies show inconsistencies and in the most recent studies no differences in arterial stiffness were reported. However, none of the studies were performed under standardized sodium conditions. Since a high sodium intake increases arterial stiffness 44, while a low sodium intake reduces arterial stiffness 45, 46 in Chapter 7, arterial stiffness was assessed in healthy formerly preeclamptic women under low and high sodium intake. Although baseline blood pressure and blood pressure responses to the change in sodium intake were comparable between the groups, formerly preeclamptic women did not adapt arterial stiffness during the change in sodium intake. This non-adaptation of arterial stiffness has not been described previously, neither in normotensive subjects 47 nor in hypertensive subjects 46. In line with previous studies, although now under standardized conditions, i.e. a high sodium intake (corresponding to the normal Western diet), no differences were observed between formerly healthy women and formerly preeclamptic women. However, after challenging women with a low sodium diet, differences between the groups became apparent.
The mechanism behind this non-adaptation of arterial stiffness in formerly preeclamptic women needs to be elucidated. Several mechanisms have been suggested to be involved in arterial stiffness regulation. Aging increases arterial stiffness probably via changes in the extracellular matrix composition. Also hypertension, diabetes mellitus, atherosclerosis, and renal failure increases arterial stiffness. Since in Chapter 7 only women without comorbidity were studied and women were in the same age range, these mechanisms are not likely to have influenced our results. Both the RAAS and volume status are important determinants of arterial stiffness. Our data presented in Chapter 7 suggest that in our study, the RAAS was not mechanistically involved in the non-adaptation of arterial stiffness since no differences in systemic RAAS parameters were found between groups. Although low sodium reduced volume status in both groups to a similar extent, only formerly healthy pregnant women were capable in reducing arterial stiffness. We feel that in our study, sodium status may have been the factor determining arterial stiffness. Indeed, sodium status is suggested to regulate arterial stiffness and we found sodium status to differently affect arterial stiffness. Future studies thus should aim on exploring the mechanism behind this regulation. For example, serum sodium response was slightly different between our groups studied and this might have influenced the non-adaptation of arterial stiffness in formerly preeclamptic women.

With increased arterial stiffness, signs of endothelial dysfunction might be expected. The gold standard to assess endothelial function is by measuring the flow-mediated dilatation (FMD). Since previous studies found an impaired FMD in formerly preeclamptic women, endothelial dysfunction can be suggested as a mechanism behind the non-adaptation of arterial stiffness in formerly preeclamptic women. As in human preeclampsia it has been suggested that endothelial dysfunction is associated with decreased NO bioavailability. We hypothesized that postpartum increased arterial stiffness is partly caused by decreased NO availability or activity. However, assessment of nitrate and cyclic GMP, surrogates for NO production and activity, could not confirm this in our formerly preeclamptic women (Chapter 7). Whether other endothelial-dependent (i.e. prostanoids or EDHF) or endothelium-independent (i.e. smooth muscle cells) factors are involved deserves further research. In line with the suggestion of endothelial dysfunction following preeclampsia, animal work in our group, has shown subtle signs of disturbed endothelial-dependent relaxation in formerly preeclamptic rats (unpublished results). In the absence of increased blood pressure postpartum, the contribution of the vasoactive factors to relaxation of the thoracic aorta differed between formerly healthy pregnant rats and formerly preeclamptic rats, i.e. more NO and less EDHF for endothelial-dependent relaxation in formerly preeclamptic rats.
Final conclusion
Our studies in formerly preeclamptic women and (formerly) experimental preeclamptic rats in this thesis demonstrated long-term consequences of preeclampsia. Our studies point toward a role for preeclampsia per se on the postpartum impairments in women with a history of preeclampsia, thereby increasing the susceptibility of these women to develop cardiovascular and renal disease later in life. Our studies demonstrated that postpartum changes in renal hemodynamics, persistent increased ang II sensitivity, endothelial dysfunction, and arterial stiffness all provide leads for further research into the exact mechanisms behind this increased susceptibility (Figure 1). Using a history of preeclampsia as an early marker to identify young women at risk for premature cardiovascular and renal disease opens a window of opportunities for preventive treatment in this specific group of women, even in the absence of known cardiovascular risk factors.
Figure 1. Simplified version adapted for this thesis of proposed and studied mechanisms involved in the long-term renal health risk following preeclampsia.

So far, it is unknown whether women who develop preeclampsia during pregnancy, exhibit prior to pregnancy an altered cardiovascular health status, i.e., pre-existing cardiovascular and renal disease risk factors. These pre-existing factors might increase a woman’s risk to develop preeclampsia during pregnancy. Preeclampsia is clinically characterized by hypertension and proteinuria, with alterations in ang II sensitivity, endothelial function, renal function, and arterial stiffness involved. Our thesis has demonstrated that these specific alterations persist postpartum and we hypothesize that these persistent alterations increase the susceptibility of these women to develop renal disease late postpartum.

In addition, traditional cardiovascular risk factors, possibly already present pre-pregnancy, might add to this increased long-term renal risk.

FUTURE PERSPECTIVES

Experimental preeclampsia; what can we learn from it and what should be the next step?

Our animal work has shown pregnancy specific adaptations of the vascular bed with preeclampsia leading to maladaptation. Changes in the contribution of endothelial derived vasoactive factors induced by pregnancy were seen, while during preeclampsia regulation of endothelial-derived vasodilatory factors was altered, suggesting endothelial dysfunction. Also, a pregnancy-specific decrease in ang II sensitivity was observed, which was absent during experimental preeclampsia. However, how experimental preeclampsia affects ang II sensitivity and the endothelial cells has still not completely been elucidated. Therefore, future studies should focus on the intriguing interaction between (experimental) preeclampsia and the vascular bed. By unraveling the exact mechanisms and pathways behind the changes in the vascular bed to pregnancy, via more experimental animal work, future studies can then be conducted focusing on protecting the vascular bed from these changes, and hence from preeclampsia.

Since preeclampsia is a multifactorial disease, with, as we have shown, the inflammatory system, endothelial dysfunction, renal function, and the RAAS involved, combining these pathways in a future animal model, for instance low-dose endotoxin infusion and RAAS over activity combined, might provide more insight into its multifactorial origin. Combining these pathways in a future animal model might hypothetically exaggerate preeclampsia-like features, i.e. hypertension and endothelial dysfunction, via inducing a common final pathway following these distinct but coinciding pathways. Hypothetically, when symptoms exaggerate, it can be stated that the pathways are either independently involved and/or reinforce each other.

Long-term consequences of preeclampsia; from a mechanistical point of view

Since we have shown persistent impairments in renal function, RAAS activity, and vascular function in women with a history of preeclampsia that hypothetically induce their susceptibility to renal disease later in life, more studies are warranted that elucidate the mechanisms involved in more detail.

From a renal perspective, the ultimate goal is to protect formerly preeclamptic women from long-term renal disease. Therefore, to gain more mechanistic insight into renal function decline and altered renal hemodynamics, micropuncture studies in animals following preeclampsia focusing in detail on the role of the glomerular pressure and glomerular lesions in the increased filtration fraction should be performed. It addition, longitudinal studies should be performed in order to investigate which women, i.e. women with a specific cardiovascular/renal risk profile or lifestyle, suffer the highest risk for renal function decline on the long-run and to test whether for example RAAS-blockade, protects against the suggested switch from hyperfiltration to hypofiltration that eventually leads to renal function decline.

With regards to the increased ang II sensitivity, the potential of RAAS blockade in reducing the long-term risk should be assessed in for example experimental preeclampsia. In addition, longitudinal ang II sensitivity studies in (experimental) preeclampsia during gestation (ex-vivo)
and postpartum \((in-vivo \text{ and } ex-vivo)\) are of interest to perform in order to test whether phenotypic changes in ang II receptors occur, to verify the time course of these possible changes, and to assess the mechanisms behind the differentially involved AT1-R and AT2-R in the ang II sensitivity during and after preeclampsia.

Although no sodium-sensitivity of blood pressure was observed in formerly preeclamptic women, low sodium diet distinguished formerly preeclamptic women from healthy women with respect to arterial stiffness. Therefore, the effect of sodium status on the endothelial function following (experimental) preeclampsia is of interest to study. To further substantiate whether the increased arterial stiffness under low sodium intake is caused by impairments in the endothelial-dependent or endothelial-independent vasodilation, flow mediated dilation (FMD) and glyceryl trinitrate-induced vasodilation assessments can be performed respectively. Also, since oxidative stress is associated with endothelial dysfunction and inflammation, it is of interest to study oxidative stress markers, especially in combination with a low and a high sodium diet. To further verify the role of oxidative stress, studying the effect of anti-oxidants on arterial stiffness can be suggested. For example, arterial stiffness and FMD can be assessed under different sodium conditions, in the presence and absence of anti-oxidant treatment.

In addition to longitudinal studies, women with a history of preeclampsia more than 10 or 20 years postpartum can be studied to assess whether the differences we found become more pronounced over-time. Since the menopause is of influence on women's cardiovascular risk, menopausal women specifically following preeclampsia are of interest to study. In addition, since pregnancy is suggested to be a stress-test for life, future studies should also include a group of women that has never been pregnant.

The ideal future perspective: evidence-based structured follow-up and preventive treatment in women with a history of preeclampsia

A history of preeclampsia serves as a risk marker for future premature cardiovascular and renal disease. Preeclampsia history provides an opportunity for early identification of young women at increased risk, even in the absence of classical cardiovascular risk factors. Postpartum follow-up of these women can therefore detect early subclinical damage and gives the opportunity for preventive treatment and interventions at a younger age than usual. This thesis gives insight in renal function after preeclampsia and gives insight into mechanisms involved. To further elucidate the exact renal risk, more prospective case-control studies preferably at a high age, or retrospective studies in large non-selected population based cohorts, i.e. the LifeLines study in which pre-pregnancy data are available, are warranted.

Next to elucidating the effectiveness of lifestyle interventions in these women, it is of importance to verify the most optimal timing to start these interventions, i.e. directly postpartum or several years after the preeclamptic event. Longitudinal follow-up studies are therefore necessary to clarify the time course of cardiovascular and renal impairments to subsequently be able to detect the time point at which an adverse cardiovascular profile can be identified. The American Heart Association (AHA) and the American Stroke Association (ASA), both guidelines on prevention
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of cardiovascular diseases, have recently included a history of preeclampsia as a women-specific risk factor for cardiovascular diseases. Both the AHA and ASA have implemented follow-up and treatment of formerly preeclamptic women in their guidelines 60, 61. In addition, the Netherlands implemented a guideline on cardiovascular risk management after reproductive disorders in women 62. However, the recommendations on screening and treatment are based on a low level of evidence. Therefore, more insight into the mechanisms behind this increased risk is necessary, since there is currently no real evidence how and whom to screen and which drugs and interventions will be of real benefit.

Preventive treatment can include both lifestyle interventions and pharmacotherapy. Since overweight and the metabolic syndrome are linked with both preeclampsia and cardiovascular diseases, a healthy diet and exercising should always be a general advice. Considering the effect of high sodium on renal hemodynamics, blood pressure, and the role of an increased filtration fraction in long-term renal risk, advice on sodium restriction should be incorporated into the dietary advices. Since ang II seems mechanistically involved in these women’s increased risk, the effectiveness of pharmacotherapy directed towards RAAS-blockade deserves further investigation on effectiveness. Albuminuria and increased blood pressure, both endangering cardiovascular and renal health, can effectively be treated by RAAS-blockade. Whether this is beneficial and in which subcategory at what age this should be started, needs to be elucidated. In line with the suggested persistent endothelial dysfunction, increasing the bioavailability of nitric oxide or supplementation of anti-oxidants can be suggested as a preventive treatment.
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SUMMARY

Preeclampsia, a pregnancy specific disorder, complicates up to 8% of the pregnancies worldwide. In the Netherlands, this is approximately 2%. The exact pathophysiology is still unknown, it is, however, generally accepted that preeclampsia is a two-stage disease with the placenta being organ of origin. The first stage of preeclampsia is characterized by poor placentation and during the second stage the placenta secretes several factors, which subsequently induce the maternal response; clinically characterized by hypertension and proteinuria.

Apart from termination of pregnancy there is no treatment option to cure preeclampsia. For a long time, it has been thought that preeclampsia is a completely reversible syndrome. However, it has been shown that women with a history of preeclampsia have an increased risk to develop cardiovascular and renal disease later in life. Although several mechanisms have been suggested to be involved in this increased risk, the exact mechanisms need to be elucidated. Whether common constitutional risk factors for both preeclampsia and cardiovascular disease are involved or whether preeclampsia itself induced the increased this risk is currently still unknown.

To gain more insight into the mechanisms behind the increased postpartum risk for cardiovascular and renal disease, we first evaluated angiotensin II sensitivity and endothelial function in an existing rat model for preeclampsia (Chapter 2). Then, Chapter 3-7 describes long-term renal function after preeclampsia and after experimental preeclampsia, together with studies investigating suggested mechanisms behind the postpartum increased risk for cardiovascular and renal disease in women with a history of preeclampsia.

An animal model for preeclampsia; focus on angiotensin II sensitivity and endothelial function

Pregnancy is characterized by major maternal hemodynamic changes. Blood volume increases and systemic vascular resistance decreases. The renin-angiotensin aldosterone system is an important system involved in this volume and tonus regulation. Healthy pregnant women show decreased sensitivity for the actions of the vasoconstrictor angiotensin II (ang II). In contrast, preeclampsia is characterized by increased sensitivity for ang II, contributing to hypertension and proteinuria. In Chapter 2 we showed that in the low-dose endotoxin rat model for preeclampsia both endothelial dysfunction and alterations in the renin-angiotensin system are present. Healthy pregnant and non-pregnant rats were compared with pregnant rats infused with a low-dose endotoxin. Isolated aortic rings were used to perform isotonic contraction measurements. Acetylcholine dose-response curves were used to study endothelial dysfunction and ang II dose-response curves were used to evaluate ang II sensitivity and the role of the ang II type I and type II receptor (AT1-R and AT2-R respectively). We observed an increased role for the vasodilator prostaglandin and ang II sensitivity was decreased in healthy pregnant rats. This was associated with decreased responses of the AT1-R and AT2-R. However, these pregnancy adaptations were absent in rats with experimental preeclampsia. Therefore, the lack of vascular adaptations to pregnancy may play a role in the development of hypertension in this model. Accordingly, the low-dose endotoxin rat model is therefore a suitable
Renal function, RAAS, and cardiovascular system after preeclampsia

Chapter 3 reviews the current understanding of the association between preeclampsia and the subsequently increased risk of developing chronic kidney disease (CKD). This review first elaborates on the increased renal risk in formerly preeclamptic women and provides an overview of the literature focusing on microalbuminuria and renal hemodynamics in these women. Next, we commented on angiogenic factors and the renin-angiotensin aldosterone system as suggested mechanisms by which the risk of CKD is increased in formerly preeclamptic women. Levels of soluble fms-like tyrosine kinase-1 (sFlt1), the antiangiogenic factor involved in the pathogenesis of preeclampsia, is suggested to be elevated postpartum and via impairing the vascular health might contribute to the development of reno-vascular diseases in later life. In addition, the presence of auto-antibodies to the AT1-R in formerly preeclamptic women and persistent increased ang II sensitivity postpartum is discussed. Unfortunately, it remains still unknown whether the association between preeclampsia and the increased risk for renal diseases can be explained by deleterious effect of preeclampsia itself on the kidney or by underlying risk factors that make women more susceptible to both preeclampsia and renal disease.

Using data from the Prevention of RENal and Vascular ENd-stage Disease (PREVEND) study allowed us to study long-term renal function and renal function decline over time in women with a history of hypertensive disorder during pregnancy (Chapter 4). Women with a history of a self-reported hypertensive disorder during pregnancy and women without a history of a self-reported hypertensive disorder during pregnancy were identified. Data on estimated glomerular filtration rate, albuminuria, and the prevalence of CKD over a time-period of 14-years were collected. Next, detailed information on pregnancy history were collected from the women that delivered in the two local hospitals and a case-control study was performed. In this case-control study, renal function parameters 10-years postpartum were analyzed and compared between healthy controls, pregnancy-induced hypertension and formerly preeclamptic women in order to analyze whether the severity of the disease was related to the long-term renal function loss. We found that a history of a hypertensive disorder during pregnancy was associated with an increased decline in renal function over time. In addition, 10-years postpartum, estimated glomerular filtration rate was significantly reduced in formerly preeclamptic women as compared to healthy controls. Moreover, almost 20% of the formerly preeclamptic women fulfilled the definition of having CKD 10-years postpartum as compared to 8% of the control women. To gain more insight into the exact mechanism behind this increased risk for CKD, more studies focusing on renal hemodynamics in formerly preeclamptic women are warranted. In addition, we suggest regular clinical follow-up of women with a history of a hypertensive disorder during pregnancy to create a window of opportunities for preventive model to study potential treatment options interfering with the endothelium and ang II and to unravel the mechanisms behind these changes. Moreover, this model can be used to assess ang II sensitivity following experimental preeclampsia to investigate whether increased ang II persists postpartum as a mechanism behind the increased risk for cardiovascular and renal disease in formerly preeclamptic women.
treatment. This follow-up should be based on evidence-based mechanisms found to be involved in women's increased susceptibility to renal disease, i.e. assessing renal function parameters (albuminuria, eGFR and/or creatinine).

Persistent impairments in the renal hemodynamics postpartum could be involved in the increased risk for end stage renal disease in women with a history of preeclampsia. In Chapter 5 renal hemodynamics were studied in healthy formerly pregnant women and healthy formerly early-onset preeclamptic women 1-10 years postpartum. To measure glomerular filtration rate and effective renal plasma flow, the clearance of constantly infused radio-labeled tracers, $^{125}$I-iothalamate and $^{131}$I-Hippuran was assessed under standardized low sodium and high sodium conditions. In addition, ang II infusion was performed to investigate whether renal ang II sensitivity was increased, as an additional pathogenic mechanism behind the increased renal risk. We found that both the intake of high sodium and a history of preeclampsia increased filtration fraction. The renal response to ang II was not different between the groups. Thus, we demonstrated that preeclampsia has persistent effects on renal hemodynamics, even in the absence of co-morbidity. An elevated filtration fraction has been suggested to be involved in the development of hypertension and renal damage. Therefore, our results fit in the assumption that persistent disturbances in renal hemodynamics after preeclampsia, could be involved in the mechanism of the long-term increased risk for renal disease, independent of co-morbidity.

Persistent increased systemic ang II sensitivity postpartum is another suggested mechanism behind the increased risk for cardiovascular and renal disease in women with a history of preeclampsia. Chapter 6 described a translational study investigating ang II sensitivity as the pressor response to ang II infusion, in healthy formerly pregnant women and healthy formerly early-onset preeclamptic women 1-10 years postpartum. Baseline blood pressure and blood pressure response upon ang II infusion were measured. Simultaneously, ang II sensitivity was studied both in-vivo and ex-vivo in never pregnant, formerly healthy pregnant, and formerly experimental preeclamptic rats. In both women and rats, baseline blood pressure was not affected after (experimental) preeclampsia. However, the pressor response to ang II was increased in formerly preeclamptic women and formerly experimental preeclamptic rats. Moreover, in response to ang II, only formerly preeclamptic rats had a significant increase in proteinuria. Since we only included healthy formerly preeclamptic women (without comorbidity) and all rats were healthy pre-pregnancy, our results support the hypothesis that preeclampsia itself plays an important role in the altered ang II sensitivity post-partum. This persistently increased ang II sensitivity might contribute to the increased cardiovascular and renal risk of formerly preeclamptic women in later life.

Arterial stiffness, described to be increased during preeclampsia, has also been suggested to be a mechanism behind the increased cardiovascular and renal risk in women with a history of preeclampsia. Chapter 7 assessed arterial stiffness by measuring pulse wave analysis and pulse wave velocity under low and high sodium diet in formerly healthy pregnant women and formerly early-onset preeclamptic women in the absence of co-morbidity. We showed that formerly healthy pregnant women are able to modulate their arterial stiffness in response to a reduction of sodium intake. However, this modulation was absent in formerly preeclamptic women, independent of
blood pressure. To further elucidate the mechanisms, NO metabolites were measured as a reflection of endothelial (dys)function, and volume status response was analyzed. Differences in volume regulation or endothelial markers could not explain this non-modulation, so the mechanism behind this non-modulation remains to be elucidated.