Preeclampsia and the Brain

Epidemiological and Magnetic Resonance Studies

MARIA NELANDER
Preeclampsia is a pregnancy specific syndrome that causes substantial maternal and fetal morbidity and mortality. One major contributor to maternal deaths is eclampsia, i.e. when seizures arise in the context of preeclampsia. The pathophysiology of eclampsia is still incompletely chartered and the long-term cerebral consequences of preeclampsia are also largely unknown.

This thesis consists of a register based cohort study (n=3232, study I), and a cross-sectional neuroimaging study of pregnant women with and without preeclampsia (n=78, studies II-IV).

In paper I, we compared the incidence of dementia and cardiovascular disease (CVD) between women ≥65 years with a self-reported history of hypertensive pregnancy, and women with a normotensive pregnancy. No difference was found regarding dementia, but an increased risk of CVD persisted among these elderly women.

In paper II, we used phosphorus magnetic resonance spectroscopy to measure cerebral magnesium levels (Mg$^{2+}$). We found lower levels of Mg$^{2+}$ in women with preeclampsia than in women with normal pregnancy and non-pregnant women. Further, which was novel, we showed that lower cerebral Mg$^{2+}$ levels correlated with visual disturbances. The findings are interesting, since magnesium sulfate is the most effective treatment and prophylaxis for eclampsia, but with a largely unknown mechanism of action.

In paper III, we measured cerebral organic osmolytes with proton magnetic resonance spectroscopy and found lower levels of osmolytes in pregnancy. Cerebral osmolytes were positively correlated with a decreased plasma osmolality, indicating that there is a joint biological mechanism. The only osmolyte that differed between women with preeclampsia and healthy pregnant women was glutamate. Glutamate is an excitatory neurotransmitter, which also functions as an osmolyte. Thus, lower cerebral glutamate levels could have implications on the pathophysiology of seizures.

In paper IV, cerebral perfusion and edema were assessed with magnetic resonance imaging using intravoxel incoherent motion technique. A reduced perfusion fraction was found in a part of the basal ganglia in women with preeclampsia. No difference in edema was detected.

Our findings indicate Mg$^{2+}$ metabolism, plasma hypoosmolality and possibly cerebral hypoperfusion to be involved in the pathophysiology of cerebral affection in preeclampsia.

Keywords: preeclampsia, eclampsia, seizure, MRI, MRS, IVIM, dementia, cardiovascular disease, magnesium, cerebral organic osmolytes, perfusion

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In memoriam Lisbeth Nelander
1938-1997

To Sven, Edith, Svante and Harriet
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV  **Nelander M**, Hannsberger D, Sundström-Poromaa I, Bergman L, Weis J, Åkerud H, Wikström J, Wikström AK. Assessment of cerebral edema and perfusion in preeclampsia with intravoxel incoherent motion MRI. *In revision*

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Abbreviations

AD    Alzheimer’s disease
BBB   Blood-brain barrier
BMI   Body mass index
CBF   Cerebral blood flow
CPP   Cerebral perfusion pressure
CVD   Cardiovascular disease
DBP   Diastolic blood pressure
DWI   Diffusion-weighted imaging
HELLP Hemolysis, elevated liver enzymes, low platelets
IVIM  Intravoxel incoherent motion
MAP   Mean arterial pressure
MRI   Magnetic resonance imaging
MRS   Magnetic resonance spectroscopy
PRES  Posterior reversible encephalopathy syndrome
ROI   Region of interest
RF    Radio frequency
SBP   Systolic blood pressure
T     Tesla
TCD   Transcranial Doppler ultrasound
VOI   Voxel of interest
Introduction

Preeclampsia

Preeclampsia is a pregnancy-specific syndrome that complicates 3–5 % of all pregnancies. It is a major cause of both maternal and neonatal morbidity and mortality worldwide, with the largest impact in developing countries. Of the estimated half a million maternal deaths each year, 10–15% are attributed to preeclampsia or other hypertensive disorders. Most of these deaths are caused by cerebral complications.

Definition

Preeclampsia is defined by its clinical presentation and the most common definition is a new onset of hypertension (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg) and proteinuria (≥300 mg/24 hours or a spot urine protein/creatinine ratio ≥30 mg/mmol). In 2014 the International Society for the Study of Hypertension in Pregnancy (ISSHP) suggested a new definition. According to this definition, the diagnosis can be made if there is a new onset of hypertension in combination with one or more of the following:

1. Proteinuria (≥300 mg/24 hours or a spot urine protein/creatinine ratio ≥30mg/mmol or ≥1g/L (2+) on a dipstick test.

2. Other maternal organ dysfunction:
   - Renal insufficiency (serum creatinine ≥ 90 µmol/L)
   - Liver involvement (elevated serum transaminases – at least twice the value of the normal upper limit, with or without right upper quadrant or epigastric abdominal pain)
   - Neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headache when accompanied by hyperreflexia and persistent visual scotomata)
   - Hematological complications (thrombocytopenia – platelet count below 150,000/dL, disseminated intravascular coagulation (DIC), hemolysis)

3. Uteroplacental dysfunction
   - Fetal growth restriction
These guidelines are for clinical use, and the recommendation is still to keep proteinuria as a mandatory criterion in research settings.\textsuperscript{6} This applies to our study population in papers II–IV. According to the ISSHP, preeclampsia is defined as severe if SBP\textgreater{} 160 mmHg and/or DBP\textgreater{} 110 mmHg or in the presence of hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome.\textsuperscript{7}

Epidemiology and risk factors

Known risk factors for preeclampsia are nulliparity,\textsuperscript{8} high body mass index (BMI),\textsuperscript{9} multiple pregnancy, diabetes, antiphospholipid antibodies,\textsuperscript{10} a first-degree relative with a history of preeclampsia,\textsuperscript{11} previous preeclampsia,\textsuperscript{12} chronic hypertension,\textsuperscript{13} and renal disease.\textsuperscript{14} Maternal age also has an impact with increased risk in both younger and older pregnant women.\textsuperscript{15}

Paradoxically, smoking seems to have a protective effect\textsuperscript{16} which is hypothesized to be due to reduced levels of antiangiogenic factors because of the carbon monoxide produced during smoking.\textsuperscript{17} A recent study questions this and hypothesizes that the reduced incidence of preeclampsia in smoking patients is due to a bias on the basis of increased early pregnancy loss.\textsuperscript{18}

Pathophysiology

Despite a century of intense research, a full understanding of the etiology and pathophysiology of preeclampsia still eludes us. Over the years, several models have been proposed, and a two-stage,\textsuperscript{19} a three-stage,\textsuperscript{20} as well as a four-stage model are now available.\textsuperscript{21}
In the latter model (see Figure 1), a distinction is made between placental and maternal preeclampsia. Stage 0 of the placental pathway takes place prior to conception and is based on the concept of immunological adaptation to paternal antigens through exposure to semen. Stage 1, which occurs during implantation, is characterized by poor maternal immunoregulation in response to paternal antigens on trophoblasts. During stage 2, the well-studied defective remodeling of spiral arteries in placenta takes place. In normal pregnancy, these arteries are transformed into wide low resistance vessels, providing the placenta and the growing fetus with oxygen and nutrients. When this fails to take place, an environment with hypoxia and oxidative stress develops. The placental production of anti-angiogenic factors such as soluble fms-like tyrosine kinase1 (sFlt1) and soluble endoglin (sEng) increases, whereas the pro-angiogenic placental growth factor (PlGF) decreases. This imbalance precedes the clinical presentation of preeclampsia by several weeks and could explain the generalized endothelial dysfunction seen in preeclampsia. The elevated levels of sFlt1 remain up to 18 months after preeclampsia and are thought to contribute to the increased risk of CVD in these women.

Preeclampsia is also regarded as an exaggerated maternal inflammatory response, beyond the systemic inflammation seen in normal pregnancy. Maternal background factors such as hypertension, obesity, and diabetes, all pro-inflammatory conditions, are thought to contribute to maternal preeclampsia.
Clinical presentation

The symptoms of preeclampsia are in many cases mild or non-existent. Typically, the woman is diagnosed during a routine visit. As endothelial dysfunction is a hallmark of preeclampsia, the clinical manifestations depend on which organs are affected.\(^\text{26}\) Altered levels of vasoactive mediators, an increase in sympathetic tone, and alterations in the renin-angiotensin system all contribute to hypertension. Glomerular capillary endotheliosis is the classic renal lesion in preeclampsia. There is loss of fenestrations in the endothelium, which leads to a reduced filtration rate.\(^\text{27}\)

Liver involvement with raised serum transferases is not unusual, but only 0.2–0.8% of all pregnancies are complicated by HELLP.\(^\text{28}\) Most patients with HELLP also have preeclampsia, which then by definition is severe, but 25% are normotensive. In HELLP, maternal morbidity and mortality are substantial and partly attributable to liver complications, such as necrosis and rupture, but mostly to the coagulation alterations with DIC as a feared endpoint.

Headache and visual symptoms such as blurred vision, scotomas and temporary blindness can occur in preeclampsia.\(^\text{26}\) Sometimes these symptoms are a herald of seizures, i.e. eclampsia.

Eclampsia

Eclampsia is defined as new-onset seizures in the setting of preeclampsia in women with no known or subsequently diagnosed seizure disorder. The incidence in high-income countries has declined and is now 4–5/10,000 births.\(^\text{29}\) In low-income countries, it is more difficult to obtain accurate numbers, but the incidence is reported to be 16–69/10,000 births.\(^\text{30}\) In high-income countries, mortality due to eclampsia has decreased and reported numbers vary between 0 and 1.8%. Unfortunately, the mortality rates in low income countries remain as high as 15%.\(^\text{31}\)

The term eclampsia comes from the Greek *eclampsis*, which can be translated to lightning. Many women have prodromal symptoms, but the seizure can still occur as a lightning bolt from the blue in as many as 17% of the cases.\(^\text{32}\) The most commonly reported prodromal symptoms are headache and visual disturbances.\(^\text{32}\)

Posterior reversible encephalopathy syndrome

In women with eclampsia, MRI can demonstrate cortical or subcortical edema, predominantly in the parietal and occipital lobes.\(^\text{33}\) Further studies have found the same pattern in some women with severe preeclampsia.\(^\text{34}\) This radiological finding in combination with the clinical presentation of nausea, vomiting,
headache, blurred vision, altered mental state, and seizures is known as posterior reversible encephalopathy syndrome (PRES).\textsuperscript{35, 36} PRES is not a unique feature of eclampsia and severe preeclampsia, but has also been described in sepsis,\textsuperscript{37} with immunosuppressive treatment\textsuperscript{38} or chemotherapy,\textsuperscript{39} and in autoimmune conditions, e.g. systemic lupus erythematosus.\textsuperscript{40} In a cohort of 96 patients with PRES, 25% had preeclampsia or eclampsia, 23% were on immunosuppressive treatment, 20% had been subjected to organ transplantation, 19% had an autoimmune disorder, 17% were treated with chemotherapy, and 5% patients suffered from sepsis.\textsuperscript{41}

PRES is the current acronym for this clinico-radiological entity; in fact it was first presented as reversible posterior leukoencephalopathy syndrome (RPLS) by Hinchey et al. in 1996.\textsuperscript{35} Casey et al. demonstrated in 2000 that the edema was evenly distributed between gray and white matter and proposed PRES as a more fitting description.\textsuperscript{42} It is argued that also PRES is a misnomer, since the localization is not always posterior and that the reversibility can be questioned.\textsuperscript{33, 43}

Whether eclampsia is a form of PRES or whether these two conditions have a common pathophysiological pathway is not clear. Brewer et al. reported on MRI findings compatible with PRES in 98% of eclampsia patients, whereas others report numbers between 59–77%.\textsuperscript{36, 44-46} In comparison to patients with other PRES-related etiologies, patients with preeclampsia/eclampsia have less edema, milder symptoms, and better long-term outcomes.\textsuperscript{43, 47}

The pathophysiology behind PRES is still not fully elucidated and two opposing theories exist. In the first, cerebral autoregulation capacity is thought to be exceeded by a sudden or severe rise in arterial blood pressure, and the following hyperperfusion results in the vasogenic edema seen in preeclampsia and eclampsia.\textsuperscript{48} The fact that many women develop eclampsia despite blood pressures far below the limit of autoregulation is, however, hard to explain with this theory. Also, no association has been found between blood pressure and the presence of MRI abnormalities.\textsuperscript{34}

In the second theory, hypoperfusion due to vasoconstriction, with ensuing ischemia and edema, is proposed to be the underlying cause of PRES.\textsuperscript{49} This would partly be driven by a loss of blood brain-barrier (BBB) integrity. The endothelium in the brain differs from the endothelium in other organs. Endothelial cells in the brain are connected with tight junctions, they lack fenestrations, and pinocytosis is more strictly regulated.\textsuperscript{50} In animal models, rats with severe preeclampsia were found to have endothelial dysfunction and increased BBB permeability.\textsuperscript{51} A dysfunctional BBB is also suggested to act on its own as a pathophysiological pathway in PRES and there is also radiological evidence to support this notion.\textsuperscript{52, 53} Cerebral circulation in women with preeclampsia and eclampsia show signs of both hypo- and hyperperfusion, thus, there is still no consensus.\textsuperscript{54-59}
MgSO₄

Though the pathogenesis is not fully understood, magnesium sulfate, MgSO₄, is now a well-established treatment and prophylaxis for eclampsia.⁶⁰ Proposed mechanisms of action of MgSO₄ include vasodilatation, restoration of blood-brain-barrier (BBB) integrity, and an increased threshold for seizures.⁶¹ Extracellular Mg²⁺ has been shown to voltage-dependently block N-methyl-D-aspartate (NMDA) receptors, one of the excitatory neurotransmitter glutamate’s receptors (Figure 2).⁶¹, ⁶² With reduced Mg²⁺ levels, less NMDA receptors are blocked and more can be opened at a relatively low membrane potential, causing hyperexcitability of neurons, and thus lowering the threshold for seizures. Animal models suggest that treatment with MgSO₄ results in reduced neuroinflammation and thereby decreased seizure susceptibility.⁵¹, ⁶³

![Figure 2. The N-methyl-D-aspartate (NMDA) receptor. URL: http://what-when-how.com/neuroscience/neurotransmitters-the-neuron-part-6/](image-url)

Although MgSO₄ is proven superior to other anticonvulsant drugs, the reduction in relative risk for eclampsia is merely 58% and the number needed to treat (NNT) is 91.⁶⁴ Respiratory depression and arrest are potentially serious side effects of MgSO₄ but most women only experience a sense of warmth, flushing, and nausea that is not harmful, but extremely unpleasant.⁶⁵ The administration of MgSO₄ thus requires a more vigilant surveillance and the increased presence of staff. Since our prediction of eclampsia is poor, the decision if and when to treat a woman with preeclampsia with MgSO₄ is difficult to make.
Long-term consequences of preeclampsia and eclampsia

Preeclampsia and eclampsia were for a long time considered as solely pregnancy-related complications with no long-term sequelae. Today there is consensus that women with pregnancy hypertensive disease are at increased risk for hypertension, cardiovascular disease (CVD) and stroke later in life.66-68 Whether preeclampsia should be regarded as a “failed stress test” in which women who develop the disease are already predisposed to future CVD, or whether preeclampsia leads to enduring vascular damage is not clear. In a large population-based prospective study, Magnussen et al demonstrated that women who subsequently had preeclamptic pregnancies had unfavorable pre-pregnancy serum levels of triglycerides and cholesterol, as well as higher blood pressure, compared to those who did not develop preeclampsia.69 On the other hand, changes in maternal circulating proteome associated with CVD long after delivery was noted in an experimental model of preeclampsia in mice.70 During normal pregnancy, the plasma volume increases 50% and the cardiovascular system adapts accordingly. Echocardiographic studies have revealed increased size of all cardiac chambers and eccentric hypertrophy.71 These changes were more pronounced in women with preeclampsia.72 In a follow-up two years postpartum, women with preeclampsia had a higher incidence of persisting left ventricular dysfunction, which in turn was associated with an increased risk of postpartum hypertension.73

However, studies focusing on pre-eclampsia and CVD risk in elderly women (>65 years) are lacking. Since the risk of CVD increases after menopause, the effect of pre-eclampsia may vary with age of follow-up.74 Evidence has also emerged of residual impact in the maternal brain. Both self-reported and objectively demonstrated cognitive impairment are reported.75-77 Long-term follow-up with MRI have revealed increased number of white matter lesions (WML),78, 79 known to correlate with cognitive decline and dementia.80, 81 The distribution of WML has not been shown to correspond to areas most often affected in PRES, a finding which disagrees with the notion of a direct causal pathway.82 A recent study did, however, show lower gray matter volume in posterior localizations in women with a history of preeclampsia and current hypertension.83 Others have also reported reduced cortical volume or total brain volume in women with a history of preeclampsia.84, 85 In the study by Mielke et al., women with a history of pregnancy hypertensive disease performed worse on a cognitive test measuring processing speed and had greater brain atrophy compared to women with previous normal pregnancies. These recordings were made decades after the affected pregnancy, and differences remained statistically significant even after adjustment for traditional cardiovascular risk factors.85

A few reports are published examining the risk of dementia after a hypertensive pregnancy. One study focused on Alzheimer’s disease (AD), but failed to find an increased risk.86 Fields et al. included 40 patients with prior
preeclampsia and 40 controls. The women were assessed by neuropsychologists and a neurologist, and significantly more women with a history of preeclampsia were diagnosed with dementia or multiple domain mild cognitive impairment. Finally, a cohort study of women giving birth 1973–1975, identified through the Swedish Medical Birth Registry (n = 284,598) reported an adjusted HR of 6.27 (95% CI 1.65–27.44) for vascular dementia after hypertension and proteinuria in pregnancy.

Dementia is increasing world-wide, and to identify persons at risk will represent an important preventive strategy.

Cerebral perfusion and autoregulation

The brain constitutes 2% of the body weight but receives 12% of the cardiac output. Cerebral metabolism is almost exclusively aerobic, rendering the brain vulnerable to hypoperfusion. Hyperperfusion on the other hand can lead to edema, which is poorly tolerated by the brain as it is enclosed in the rigid skull. Thus, the brain has great need for a strict autoregulation. Cerebral blood flow (CBF) is kept at 50 ml/min per 100 g brain tissue when the cerebral perfusion pressure (CPP), defined as the difference between arterial pressure at the Circle of Willis and the intracranial pressure, remains within 50–160 mmHg.

Figure 3. Taken from Pires et al. 2013 American Journal of Physiology Heart and Circulatory Physiology.
Cerebral autoregulation is exerted through the neurovascular unit, which is comprised of neuronal, vascular, and glial components. CBF is mainly preserved through changes in vessel calibers in large arteries as well as arterioles, thereby altering the cerebral vascular resistance (CVR). When blood pressure exceeds the limits of autoregulation, CBF will be directly dependent on mean arterial pressure resulting in hyperperfusion that can lead to endothelial damage, edema, and risk of brain injury.

Cerebral circulation in pregnancy and preeclampsia

Pregnancy infers considerable hemodynamic changes, among these a 40–50% increase in plasma volume that puts a considerable strain on the body. Adjustments include increased cardiac output, decreased peripheral resistance and hyperpermeability, all of which could increase the potential for edema formation and a rise in intracranial pressure. In animal studies, pregnancy was found to cause remodeling of cerebral arterioles and increased capillary density. In pregnant rats with induced acute hypertension with no preeclamptic features, this resulted in reduced CVR, increased CBF, and increased BBB permeability. Pregnancy also calls for adaptation of the endothelium to an increase in circulating vasoconstrictors. This has again been demonstrated in animal models, where in non-pregnant rats, plasma from pregnant was shown to cause vasoconstriction, whereas this reaction was absent in late-pregnant rats.

In humans, there are some conflicting results on how pregnancy and preeclampsia affect the cerebral circulation. This is in part due to the limitations of the non-invasive examinations methods at hand. In a longitudinal MRI study by Zeeman et al., decreased CBF was observed in late pregnancy compared to the postpartum measurement. In contrast, a study using Doppler of the internal carotid artery, as a surrogate marker of CBF, found an increased CBF. This is in line with findings from transcranial Doppler examination revealing increased CPP in late pregnancy. Another way to evaluate changes in cerebral circulation is to define how well the cerebral autoregulation is functioning. This can be described as the autoregulatory index (ARI). Pregnancy in itself did not negatively affect the dynamic autoregulation, but a lower ARI was found in women with preeclampsia and chronic hypertension. The women with chronic hypertension who later developed superimposed preeclampsia had further reduced ARI compared to those who did not. CBF in preeclampsia has been found to both increase and decrease, as mentioned in the section on PRES. To conclude, our knowledge about the cerebral adaptations during pregnancy and preeclampsia is still scarce.
Osmolality in pregnancy and preeclampsia

As well as a steady perfusion, neurons are also dependent on a stable cell size to function properly. This is challenged when plasma osmolality changes. In cases of hypoosmolality, most often caused by hyponatremia, there is risk of intracellular edema when osmotic forces cause water to enter the cells. Hyponatremia is associated with increased risk of seizures, which could be related to the decrease in extracellular space when cells expand, causing ephaptic transmissions and hyperexcitability. The cells defend themselves with mechanisms called regulatory volume decrease (RVD), primarily by extrusion of electrolytes, but in cases of more longstanding hypoosmolality, by extrusion of cerebral organic osmolytes.

Pregnancy causes fundamental changes in hemodynamics and body fluid hemostasis, and it has been established for decades that plasma osmolality and sodium levels are reduced. A small number of case reports have described severe hyponatremia in preeclampsia. The authors theorize that hyponatremia could be due to nephrotic syndrome or an increased release of vasopressin, but give no clear-cut explanation. Whether the hyponatremia in pregnancy or preeclampsia affects the brain and levels of cerebral organic osmolytes has not been studied before.

Excitability

Several pregnancy changes can influence the excitability of neurons. Among these are the increased levels of sex steroids, estrogen being considered mostly proconvulsant and progesterone anticonvulsant. Estrogen increases the number of N-methyl-D-aspartate (NMDA) receptors and decreases gamma-aminobutyric acid (GABA) synapses, thereby lessening the inhibition, and finally binds to a receptor in the membrane of neurons, thereby increasing their resting potential and decreasing their firing threshold. Estrogen has also been found to inhibit extracellular glutamate uptake, an important mechanism to maintain non-toxic concentrations. Progesterone has the opposite effect by decreasing the number of synapses and also the number of estrogen receptors. Allopregnanolone, a progesterone metabolite, affects GABA and increases its inhibitory effect on cell membranes.

Peripheral inflammation has been shown to increase neuronal excitability through tumor necrosis factor alpha (TNF-α) activated microglia. This was also found in a preeclampsia model in rats. Here, the hyperexcitability was reversed by administration of MgSO4, which decreased the number of microglia in an active state.

As mentioned in the section on osmolality above, conditions with hypoosmolality are associated with an increased risk of seizures. Binder et al. tested the hypothesis that glial aquaporin channels, which transport water across cell membranes, may modulate intrinsic brain excitability. In aquaporin (AQP) 4-
deficient mice, they found less seizure activity after administration of a chemoconvulsant.90 Studies in rats have found a significant upregulation of AQP 4 in pregnancy, a finding postulated to increase edema formation in acute hypertension.112

Methods to study cerebral circulation

Transcranial Doppler ultrasound
In humans the most commonly used method to study cerebral circulation has been transcranial Doppler ultrasound (TCD). The advantages are obvious as it is a non-invasive, easy and accessible examination technique. Flow velocity measurements in the middle cerebral artery in combination with blood pressure measurements are used to calculate CPP, which has been shown to be elevated in preeclampsia.113 Arterial blood flow and flow velocity in the great arteries in the brain can also be measured with MRI, and the flow in both the middle and posterior cerebral arteries has been shown to be elevated in preeclampsia.55

Single-photon emission computed tomography
Single-photon emission computed tomography (SPECT) is a tomographic gamma camera technique, yielding a three-dimensional presentation of the spatial distribution of a radiotracer. The most commonly used radioisotope is technetium-99m. SPECT does not measure absolute CBF but rather perfusion indices.114 Currently, SPECT is used for evaluation of epilepsy surgery and for the differential diagnostics of Parkinson and other neurodegenerative diseases.115

Positron emission tomography
In positron emission tomography (PET) a cyclotron is used to produce a radioactive tracer that is subsequently injected in the patient. One commonly used tracer for assessment of cerebral perfusion is 15O-labeled water. This is considered the gold standard for perfusion measurements and the technique also allows detailed mapping of metabolic processes. Limiting factors are the availability of PET scanners and the radiation dose which precludes the use in children and pregnant women.

Magnetic resonance imaging (MRI)-based techniques
The principles of MRI are described further in a later paragraph.
**Velocity encoded phase-contrast MRI**
Velocity encoded phase-contrast MRI allows for measurements of flow velocities in major arteries. Compared to TCD, the advantages are that all arteries are equally accessible to examination and that MRI also permits flow measurements.

**Dynamic susceptibility contrast-enhanced MRI**
With the aid of a gadolinium-based contrast medium, a magnetic field gradient is created between the capillaries and the surrounding tissue. As the bolus passes through the voxel, the signal intensity drops at a rate depending on the local CBF. Cerebral blood volume and mean transit time can also be calculated by the method. Dynamic susceptibility contrast MRI (DSC) is widespread in clinical practice. In recent years, some concerns have been raised regarding the safety of gadolinium as it has been shown that patients undergoing repeated examinations end up having gadolinium deposits in the brain.\(^{116}\)

**Dynamic contrast-enhanced magnetic resonance imaging**
Also called permeability MRI, dynamic contrast-enhanced magnetic resonance imaging (DCE) uses T1-weighted images to quantify microvascular permeability after administration of a gadolinium-based contrast agent.

**Arterial spin labelling**
Arterial spin labeling (ASL) is a non-invasive MRI technique that utilizes magnetically labeled water molecules as an endogenous tracer. This magnetisation is done at a labeling plane, usually at the neck to include the major arteries that supply blood to the brain. Measurements are made at an imaging plane downstream, reflecting the perfusion of the tissue. The major drawback of ASL is its low signal-to-noise ratio (SNR), with long scan times as a result, increasing the risk of motion artefacts.\(^{117}\)

**Intravoxel incoherent motion**
This is a technique proposed by Le Bihan already in 1988, but which has been made feasible by improved technology relatively recently.\(^{118}\) Intravoxel incoherent motion (IVIM) is based on a diffusion weighted imaging (DWI) sequence, where image contrast is largely affected by differences in the random molecular motion of water protons, so that no exogenous contrast agent is needed.\(^{119}\) IVIM uses several recordings made in a DWI protocol, at different degrees of sensitivity to incoherent motion, i.e. to different b-values. Since diffusion is slow and perfusion faster, it is possible to separate the contributions from each component and thereby calculate the so called perfusion frac-
tion.\textsuperscript{120} This reflects the volume fraction of the water within a voxel that consists of capillaries. The equation for the IVIM signal can be described as follows:

\[
\frac{S(b)}{S_0} = f e^{-b D^*} + (1 - f) e^{-bD}
\]

\(S^{(b)}\) is the MR signal obtained when images are acquired with diffusion-encoding gradient pulses at a given b-value; \(S_0\) when they are acquired without. D stands for the diffusion coefficient, \(D^*\) for the pseudo-diffusion-coefficient, b for the b-value, and f for the perfusion fraction. Results for f obtained with IVIM correlate reasonably well with those for the cerebral blood volume obtained with DSC.\textsuperscript{121}

### Magnetic resonance

#### Principles

MRI is based on the physical phenomenon of magnetic resonance, the fact that the magnetization of certain atomic nuclei, when placed in a strong magnetic field, rotates with a frequency dependent on the strength of the magnetic field. It was primarily used for studies of chemical structure of substances. In the 1970s, the development toward the MRI of today started, and the scientists responsible for this were subsequently awarded the Nobel Prize in Physiology and Medicine in 2003. The MR scanner consists of a main magnet and gradient coils, enabling MR images in multiple planes, and finally a radio frequency (RF) transmitter and receiver coil. The magnetic field causes the magnetization of all nuclei to align in the same direction. A radio frequency pulse at a certain (resonance) frequency will disturb and shift the orientation of the magnetization. When the RF pulse is turned off, the excited nuclei return to their initial equilibrium state. In this process of relaxation, another RF signal is generated that is picked up by the receiver coil. The magnitude of this signal depends on the density of the observed nucleus type, and on two different processes, T1 and T2 relaxation. T1 reflects the rate at which the magnetization along the main magnet field recovers and is also referred to as spin-lattice relaxation or longitudinal relaxation. This process is slower than T2 relaxation, which reflects the decay of transverse magnetization and is known as spin-spin relaxation. MRI can be weighted toward T1 or T2 relaxation depending on what is the purpose of the examination. This is achieved by changing the repetition time between RF pulses or the echo time, the time when the signal is being collected.
Figure 4. A schematic description of how the magnetic fields of protons are randomly distributed in equilibrium (A), how they align in a strong magnetic field (B) provided by the MR scanner, when exposed to a radio frequency wave (RF) there is a flip (C) and finally, how the signal from the relaxation to equilibrium is recorded and is used to create the imaging (D).

URL: http://journals.openedition.org/primatologie/508

Compared to computed tomography the main advantages with MRI are that it involves no ionizing radiation, a much higher soft-tissue contrast, and that the use of different imaging sequences gives improved tissue characterization.

Safety

MRI is overall a safe imaging method and involves no ionizing radiation. However, pacemakers and other implanted electronic devices can be affected by the magnetic field. It is also important to take into consideration that ferromagnetic objects can become projectiles in the vicinity of the machine and can harm the patient, staff, and the MR camera. For some patients, claustrophobia can be an aggravating circumstance.

As with all new techniques, precaution has been observed for use in pregnancy. There are no reports of teratogenic harm, in either humans or animals,122 or of short term injury, such as affected fetal heart rate,123 and the American College of Radiology recommends that MRI can be used in all trimesters of pregnancy.124 They conclude that the majority of reports come from research at 1.5T or less, although no harmful effects of exposure to the developing fetus have so far been reported for 3T.
MR spectroscopy

Magnetic resonance spectroscopy (MRS) utilizes the fact that the magnetization of a certain nucleus resonates at slightly different frequencies depending on its molecular surrounding. This difference is called chemical shift. Through Fourier transformation, a spectrum can be calculated which depicts different metabolites depending on which nucleus is observed. The most frequently used nucleus, as in conventional MRI, is the proton, as it is abundant in the human body. In this spectrum information on markers of membrane metabolism, cell proliferation, and cellular energetics are obtained. Other nuclei observed include $^{13}$C and $^{31}$P, the latter giving information on bioenergetics and intracellular magnesium.

The use of MRS in pregnancy has largely focused on the fetal brain and there are few reports on the maternal brain in preeclampsia. These are all published more than 10 years ago, a decade which has seen major progress in MRS technology. In one study, lower levels of cerebral magnesium was reported, though with no information on any correlation with cerebral symptoms. Another study found a lower n-acetyl aspartate/choline ratio in preeclampsia compared to normal pregnancy, interpreting this as indications of cerebral ischemia in preeclampsia. To conclude, MRS provides a non-invasive method to study levels of several significant metabolites in the brain. The possibility to use this during pregnancy and preeclampsia has so far not been fully utilized.
Aims

The overall aim of this thesis is to study how the maternal brain is affected by preeclampsia in the short and long term.

I  
To study whether pregnancy hypertensive disease is associated with subsequent increased risk of dementia, and to study whether the increased risks of cardiovascular disease and stroke after pregnancy hypertensive disease persist in an elderly population.

II  
To study whether cerebral magnesium levels differ between women with preeclampsia, normal pregnant, and non-pregnant women using phosphorus magnetic resonance spectroscopy, and further, to correlate the magnesium levels to cerebral symptoms.

III  
To compare cerebral organic osmolyte levels in women with preeclampsia, normal pregnant and in non-pregnant women using proton magnetic resonance spectroscopy.

IV  
To estimate whether women with preeclampsia have an affected cerebral perfusion or signs of edema in comparison with normal pregnant and non-pregnant women, using intravoxel incoherent motion MRI.
Material and Methods

The study population and study design

Study I

The cohort for this study (n = 3232) was collected from the Swedish Twin Registry. It comprised women older than 65 years who had participated in a telephone-based interview including questions regarding hypertension and proteinuria during pregnancy. They had all performed a cognitive test, TELE, and we only included women with intact cognition at this baseline interview. Details of the selection process can be found in Figure 1. The main outcomes, dementia, CVD and stroke, were retrieved through the National Patient Register and the Cause of Death Register. Dementia diagnoses included AD, vascular dementia, and dementia of unspecified causes. CVD diagnoses were hypertension, ischemic heart disease, atrial flutter, heart failure, and atherosclerosis. Cerebral hemorrhage or infarction, transitory ischemic attack, and non-specified cerebrovascular disease constituted diagnoses for the outcome of stroke. For a comprehensive list of diagnoses, see supplementary Table 1 in paper I. Covariates in the study were collected at the time of the interview and included age (continuous) at interview, current BMI (continuous), education (9 years of school /more than 9 years of school), and current smoking habits (daily smoking yes/no).
Studies II-IV

The study was designed as a cross-sectional study with inclusion during the years 2013–2016. General exclusion criteria were chronic hypertension, diabetes mellitus, pre-existing renal disease, or contraindications for MRI, e.g. claustrophobia or pacemaker. Only singleton pregnancies were included.

Three groups were included, women with preeclampsia, normal pregnancy and non-pregnant women. The first group consisted of 30 women with preeclampsia. Preeclampsia was defined as de novo hypertension after 20 weeks of gestation in combination with proteinuria. Hypertension was defined as SBP of $\geq 140$ mmHg and/or DBP of $\geq 90$ mmHg measured on two subsequent occasions at least 6 hours apart and proteinuria $\geq 2+$ on a dipstick or $\geq 300$ mg/24h in a urine collection. Preeclampsia was defined as severe when blood pressure was $\geq 160$ mmHg systolic and/or $\geq 110$ mmHg diastolic; or if HELLP-syndrome was present.7

Further, we included 32 women with normal pregnancies and 16 non-pregnant women. A normal pregnancy was defined as a normotensive pregnancy
resulting in term delivery (gestational week ≥37) of an infant with normal birth weight (± 2 standard deviations of the mean birth weight for gestational age and sex). We aimed to include controls where the gestational length matched the cases within 2 weeks. The non-pregnant controls included healthy women, either parous or nulliparous. We excluded women with prior pregnancy hypertensive disease in both the normal pregnant and non-pregnant groups.

All participants underwent blood pressure measurement (BP), blood and urine sampling and MRI and MRS examination within a 12-hour period after enrolment in the study. The plasma samples were collected in Vacutainer tubes, centrifuged for 10 minutes at 1,500 g, and the plasma was immediately frozen at −70°C for later analysis. A 1.5 Tesla MR system (Achieva, Philips Healthcare, The Netherlands) was used for studies II–IV. MR examination took approximately 1 hour. Directly prior to examination in the MR scanner, an interview was conducted with questions regarding cerebral symptoms.

Data on medical history, early pregnancy BMI, and birth weight and information on the course of the rest of the pregnancy were obtained from the participants’ medical journals.

Methods

Study II

Plasma was analyzed for Mg^{2+} levels. A transmit-receive quadrature head coil was used for phosphorous spectroscopy (31P-MRS) while the whole-body RF-coil served for morphological imaging. Morphological images were first obtained for the purpose of MRS planning. Single-voxel 31P-MRS was then performed using image-selected in vivo spectroscopy (ISIS) localization sequence with a spectral bandwidth of 1500 Hz, repetition time 3500 ms, 1024 points, and 256 acquisitions. The voxel was positioned over the parieto-occipital region. The net measurement time was 15 min 3 sec.

Magnetic resonance user interface (MRUI) software package was used for spectrum processing. The spectra were fitted using AMARES method in the time domain. Spectral intensities of the following metabolites were fitted by Lorentzians: phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), PCr, γ-, α-, and β-adenosine triphosphate (ATP) (Figure 2). The cytosolic concentration of free magnesium (Mg^{2+}) was calculated from the chemical shift between PCr and β-ATP using the equation of Iotti et al. Since the position of the β-ATP triplet can be difficult to determine in an individual spectrum due to a low signal-to-noise ratio (SNR), spectra from each group of the volunteers (preeclampsia, normal pregnancy, and non-pregnant) were also averaged (Figure 6) and mean Mg^{2+} levels of each group were
computed from these spectra. The physicist analyzing the MRS levels was not aware of which study group the woman belonged to.

Figure 6. $^{31}$P-MR spectrum of the brain. (a) Mean spectrum of preeclampsia patients; (b) Fitted spectrum. Phosphomonoesters (PME); inorganic phosphate (Pi); phosphodiesters (PDE); phosphocreatine (PCr); $\gamma$, $\alpha$, and $\beta$-adenosine triphosphate (ATP).

Study III
Plasma samples for analysis of osmolality, sodium and glutamate were obtained. Single-voxel spectra were acquired using the point-resolved spectroscopy (PRESS) sequence (spectral bandwidth 1000 Hz, 1024 points, TR/TE 5000/30 ms, 16 phase cycle steps). Volume of interest (voxel) with a size of 20x20x20 mm$^3$ was positioned in the posterior midline, at the junction between the parietal and occipital lobes (Figure 7).

Study IV
Diffusion-weighted images were obtained by a spin-echo sequence with echoplanar read-out (SE-EPI), repetition time: 4150 ms, and echo time: 62 ms. Images were acquired in the axial plane with a slice thickness of 5 mm, in-plane resolution of 1.2x1.5 mm, and an inter-slice gap of 1 mm. Diffusion gradients were applied in three directions ($b = 0, 50, 100, 150, 200, 400, 600, \text{ and } 800 \text{s/mm}^2$). IVIM parameters were estimated using bi-exponential fitting using Olea Sphere 3.0 (Olea Medical Solutions, France). Regions of interest (ROI) were manually drawn in a slice at the level of the lateral ventricles in the following regions: the bilateral frontal white matter (including genu corpus callosum), bilateral parieto-occipital white matter (including splenium corpus
callosum), thalamus, caudate, and lentiform nuclei. These points were chosen to represent blood supply areas of both the carotid and vertebrobasilar arteries and to include both white and gray matter.

![Figure 7. T2- (left) and T1-weighted (right) turbo spin echo images with the typical voxel position in the posterior midline at the parieto-occipital fissure.](image)

**Statistical analysis**

**Study I**

Risks of dementia, CVD and stroke after pregnancy hypertensive disease were estimated using Cox proportional hazards regression models, and age at interview was set as the time-dependent variable. Women who reported no pregnancy hypertensive disease were the reference. Adjustments were made for BMI, education, and smoking at time of interview. To account for relatedness (twins), the analyses were clustered on twin-pair identity. The regression parameters in the Cox model were estimated by the maximum partial likelihood estimates under an independent working assumption and a robust sandwich covariance matrix estimate was used to account for the intracluster dependence. Analyses were performed using SAS version 9.3 (SAS Institute, Inc).

**Study II**

Frequency histograms and the Shapiro–Wilk’s test were used to test cerebral and plasma Mg$^{2+}$ levels in each study group for normal distribution. Thereafter, we used analysis of variance (ANOVA) for overall comparisons of means and Tukey’s test for multiple pair-wise comparisons. Chi-square and Fisher’s exact tests were used for comparisons between categorical variables. Spearman’s correlation test was used to estimate correlations between cerebral
Mg$_2^+$ levels and maternal age, BMI, and gestational length at examination in the normal pregnant population, as well as between cerebral and plasma Mg$_2^+$ levels, and cerebral Mg$_2^+$ levels and cerebral symptoms in women with preeclampsia. All significance tests were two-tailed. P < 0.05 was considered to denote a statistically significant difference. All analyses were performed using IBM SPSS Statistics 23 (IBM SPSS, Inc., Chicago, IL).

Study III
Clinical characteristics, median levels of cerebral $^1$H-metabolites, plasma osmolality, and plasma levels of glutamate and sodium were compared between the women with preeclampsia, women with normal pregnancies and non-pregnant women by the Kruskal–Wallis test. Pair-wise comparisons between the study groups were made with the Mann–Whitney U test. Adjustment for maternal age and BMI was made with ANCOVA after testing for adequate normal distribution of the variables. Correlations between the different cerebral osmolytes and between cerebral osmolytes and plasma variables were assessed with Spearman’s correlation test. All significance tests were two-tailed. P < 0.05 was considered to denote a statistically significant difference. Analyses were performed using IBM SPSS Statistics 24 (IBM SPSS, Inc., Chicago, IL, USA).

Study IV
Comparisons at the group level (preeclampsia, normal pregnancy and non-pregnant women) were tested with the Kruskal–Wallis followed by pairwise Mann-Whitney U tests. The chi square test ($\chi^2$) was used to compare proportions. Differences between gray and white matter were assessed with the Wilcoxon signed-rank’s test. Correlations were assessed with Spearman’s correlation test. All significance tests were two-tailed, and a p-value < 0.05 was considered to denote a statistically significant difference. Analyses were performed using IBM SPSS Statistics 24 (IBM SPSS, Inc., Chicago, IL, USA). No correction for multiple comparisons was performed. The rationale for this was to avoid type II errors and also the fact that the measurements in the different brain areas cannot be regarded to be independent of each other.
Results

Study I

Background characteristics are presented in Table 1. After exclusion of women who did not remember if they had had high blood pressure during pregnancy, 419 of 3065 (1.4%) reported that they had had at least one pregnancy with pregnancy hypertensive disease. Women who reported that they had a history of pregnancy hypertensive disease had slightly higher BMI and education, but were less often smokers compared with those without pregnancy hypertensive disease. Women who reported a history of pregnancy hypertensive disease more often had a diagnosis of CVD, but not of stroke, in the Patient Register before the interview.

Table 1 Characteristics of the study population at the time of the interview (i.e. start of follow up).

<table>
<thead>
<tr>
<th></th>
<th>NO (n = 2646)</th>
<th>YES (n = 419)</th>
<th>DON'T KNOW (n = 167)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Any (n = 419)</td>
<td>With proteinuria (n = 269)</td>
</tr>
<tr>
<td>Age at inclusion</td>
<td>71.8 ± 5.6</td>
<td>71.0 ± 5.1</td>
<td>71.0 ± 5.0</td>
</tr>
<tr>
<td>Age at end of follow-up</td>
<td>82.2 ± 5.6</td>
<td>81.5 ± 5.3</td>
<td>81.5 ± 5.2</td>
</tr>
<tr>
<td>BMI1</td>
<td>24.9 ± 3.8</td>
<td>26.2 ± 4.2</td>
<td>26.0 ± 4.2</td>
</tr>
<tr>
<td>Education &gt; 9 years</td>
<td>1197 (45.3)</td>
<td>201 (48.1)</td>
<td>137 (50.9)</td>
</tr>
<tr>
<td>Smoking</td>
<td>254 (9.6)</td>
<td>28 (6.7)</td>
<td>20 (7.4)</td>
</tr>
<tr>
<td>Previous disease2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVD3</td>
<td>200 (7.6)</td>
<td>47 (11.2)</td>
<td>28 (10.4)</td>
</tr>
<tr>
<td>Stroke</td>
<td>63 (2.4)</td>
<td>11 (2.6)</td>
<td>8 (3.0)</td>
</tr>
</tbody>
</table>

Data are presented as mean± SD or numbers (%); 1 Body mass index. Data are missing in 99 women. 2 Prior to interview according the Patient Registry. 3 Cardiovascular disease
We found no significant association between dementia and pregnancy hypertensive disease, either adjusted or unadjusted. Women who reported that they had a pregnancy hypertensive disease, had slightly higher risks of both CVD and stroke compared with women who reported no hypertensive disease. (Table 2)

Table 2.

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>Crude</th>
<th>95% CI</th>
<th>P-value</th>
<th>Adjusted1</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CVD</strong></td>
<td>1.29</td>
<td>1.02–1.61</td>
<td>0.03</td>
<td>1.29</td>
<td>1.02–1.61</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Stroke</strong></td>
<td>1.35</td>
<td>0.99–1.79</td>
<td>0.05</td>
<td>1.36</td>
<td>1.00–1.81</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1Adjust by BMI, education and smoking.

Studies II–V

Background characteristics of study participants are presented in Table 3. There were significant differences between study groups for age, BMI, parity, and BP.

Table 3.

<table>
<thead>
<tr>
<th>Preeclampsia (n = 29) *</th>
<th>Normal pregnant (n = 30) †</th>
<th>Non-pregnant (n = 16) ‡</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27 (7)</td>
<td>33 (6)</td>
<td>27 (12)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26 (6)</td>
<td>24 (5)</td>
<td>22 (5)</td>
</tr>
<tr>
<td>Prior births, n (%)</td>
<td>5 (17)</td>
<td>20 (67)</td>
<td>7 (44)</td>
</tr>
<tr>
<td>At examination:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, days</td>
<td>246 (55)</td>
<td>246 (74)</td>
<td>0.93</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>150 (20)</td>
<td>110 (10)</td>
<td>110 (9)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95 (13)</td>
<td>70 (13)</td>
<td>70 (5)</td>
</tr>
</tbody>
</table>

a p-value according to Kruskal–Wallis, comparison of all groups.
* In study II n = 28
† In study II n = 28, study IV n = 29
‡ In study II n = 11, in study IV n = 14
Data are presented as median (inter-quartile range)
BMI, Body mass index, measured in the first trimester in the two pregnant study groups.

Study II

Figure 8 shows a scatter plot of cerebral Mg²⁺ levels for the three study groups. The mean Mg²⁺ level was 0.12 ± 0.02 mM in the preeclampsia group, 0.14 ±
0.03 mM in the normal pregnant group and 0.14 ± 0.03 mM in the non-pregnant group. This yielded a significant difference between groups (ANOVA; \( p = 0.04 \)), where women with preeclampsia had a significantly lower cerebral level of \( \text{Mg}^{2+} \) compared to women with normal pregnancy (Tukey; \( p = 0.04 \)). There was no significant difference in cerebral \( \text{Mg}^{2+} \) content between the preeclampsia and non-pregnant group (\( p = 0.22 \)), nor between normal pregnant and non-pregnant women (\( p = 0.98 \)).

Figure 8. Cerebral \( \text{Mg}^{2+} \) levels for each study group. Mean levels are shown with lines.

We found a significant correlation between cerebral \( \text{Mg}^{2+} \) levels and presence of visual disturbances. However, \( \text{Mg}^{2+} \) levels did not correlate with the presence of headache.

Non-pregnant women had a higher plasma \( \text{Mg}^{2+} \) level than normal pregnant women (0.76 mM ± 0.06 vs. 0.68 mM ± 0.06, \( p = 0.005 \)) and a borderline higher level than the preeclampsia group (0.70 mM ± 0.08, \( p = 0.054 \)). Women with preeclampsia and the normal pregnant group did not differ in plasma \( \text{Mg}^{2+} \) levels (\( p = 0.479 \)). In the preeclampsia group, the cerebral and plasma \( \text{Mg}^{2+} \) levels did not correlate.

Study III

The reliability of concentration estimates is defined by Cramér-Rao Lower Bound (CRLB, defined as SD of the spectral line fit expressed in %). CRLB < 20% is considered as a good criterion for acceptable reliability.\(^{135}\) We note that our spectra were of good quality, and CRLBs were less than 10% for glutamate, creatine, choline, and myo-inositol. CRLB of taurine was between 20% and 40% (average 33%).

Cerebral levels of \(^1\text{H}-\)metabolites in each study group are depicted in Figures 9A-F. We found significant differences on group level for glutamate,
myo-inositol, choline and creatine ($p = 0.001$ or less). Normal pregnancy was characterized by lower cerebral levels of myo-inositol, choline, and creatine ($p = 0.001$ or less), whereas cerebral levels of n-acetylaspartate, taurine, and glutamate did not differ from the non-pregnant women. These results remained after adjustment for maternal age and BMI. Women with preeclampsia had lower cerebral glutamate levels, both in comparison with women with normal pregnant and non-pregnant controls ($p = 0.009$ and $0.001$ respectively). Otherwise, the two pregnant groups did not differ.

Figures 9A-F. Boxplots of $^1$H-MRS metabolites in women with preeclampsia ($n = 29$), normal pregnancies ($n = 30$) and in non-pregnant women ($n = 16$).

Women with preeclampsia had higher levels of plasma glutamate in comparison with women with normal pregnancies, and non-pregnant women, 0.22, 0.17, and 0.15 mmol, respectively (both $p < 0.001$). This difference persisted after adjustment for maternal age and BMI. No difference in plasma glutamate levels was noted between women with normal pregnancy and non-pregnant...
women. Compared to the non-pregnant group, the two pregnant groups had lower osmolality and plasma sodium levels \((p < 0.001)\). Compared to normal pregnancy, women with preeclampsia had higher osmolality, but lower levels of sodium \((p = 0.02\) and \(p = 0.04\), respectively). There were no differences in levels of osmolytes between those who were on antihypertensive treatment and those who did not receive such medication.

There were significant correlations between cerebral levels of the osmolytes glutamate, myo-inositol, choline and creatine. The cerebral levels of taurine correlated with levels of choline, but not to the other cerebral osmolytes. There was a significant negative correlation between cerebral and plasma glutamate levels. Plasma osmolality correlated with cerebral levels of myo-inositol, choline and creatine, but not with cerebral levels of glutamate and taurine. The plasma levels of sodium correlated with plasma osmolality, plasma glutamate, and all cerebral metabolites except taurine.

Study IV

We found no difference in perfusion-related IVIM parameters across the five brain ROI’s (parietal-occipital white matter, frontal white matter, thalamus, caudate nucleus, and lentiform nucleus) except for the caudate nucleus. Women with preeclampsia had lower cerebral blood volume measure \((f)\) in the caudate nucleus in comparison with both normal pregnant and non-pregnant women \((p = 0.01\) and \(p = 0.03\), respectively) (Figure 10, left). Additionally, the blood flow measure \((fD^*)\) was lower in the caudate nucleus in preeclampsia compared to normal pregnant, and non-pregnant women (both \(p = 0.02\)) (Figure 10, right). We did not find any evidence of cerebral edema in pregnant women or women with preeclampsia.

![Figure 10. Cerebral blood volume measure \((f)\) in caudate nucleus (left). Cerebral blood flow measure \((fD^*)\) in caudate nucleus (right).](image-url)
Discussion

Without exaggeration, at least 100 women over the world die each day due to cerebral complications of preeclampsia and eclampsia. The vast majority of these die in low resource settings. Our knowledge of how seizures arise in preeclampsia is limited. Despite MgSO₄ being the most effective treatment, we do not know how it exerts its action, the side effects are considerable, availability world-wide low, and the number needed to treat substantial. Research has been hampered in part by the complexity of preeclampsia, which has made a consistent definition hard to achieve. Also, since preeclampsia is almost exclusively a human disease, the animal models that do exist are not entirely representative of human pathophysiology. Studies in humans, which in this case means pregnant women, calls for limitations in examination methods.

We are becoming more and more aware of the long-term implications of preeclampsia, especially regarding CVD. But growing evidence also points to long-term cerebral complications, and preeclampsia is now regarded as a gender-specific risk factor for stroke by the American Heart Association. Impairment in cognition has also been reported, but whether this entails increased risk of dementia is not known.

The papers included in this thesis have aimed at gaining more insight into both the short-term and long-term effects of preeclampsia for the maternal brain.

Methodological considerations

Study I

Study I is based on data from the Swedish Twin Registry, primarily established in the 1950s to give an opportunity to study the impact of smoking and alcohol on the risk of CVD and cancer, while controlling for genetic factors. Our cohort was retrieved from women in the registry, who participated in a telephone based interview which included questions regarding high blood pressure and proteinuria in pregnancy and also a cognitive test, TELE. This procedure gives rise to one of the major limitations of this study, the self-reported incidence of the exposure. Since the reliance on self-reported data often is necessary, especially in settings without comprehensive national
health registers, great effort has been applied in the validation of data acquired in this manner. Stuart et al. has published a systematic review on the validity of self-reported preeclampsia diagnoses. This review included 10 studies where the positive predictive value (PPV) varied between 51–59%, but the negative predictive value (NPV) was found to be 99%. Length of recall varied greatly, from 48 h to 30 years. This did not seem to affect the reliability of results, since the sensitivity in the study with the shortest interval was 33% among controls compared with 36% when the time interval was >30 years. When potentially modifying factors were studied, only one study found a significant result, higher maternal education and multiparity being associated with a more accurately self-reported preeclampsia diagnosis.

In contrast to the results from the systematic review, a validation of a Norwegian epidemiological survey, the Tromsø study, reported PPV of 80% and NPV of 57% for a self-reported preeclampsia diagnosis. When stratifying for age, the proportion of false negatives was highest among the eldest, which, in contrast to the conclusions from Stuart et al., enforces the risk for recall bias when self-reported data is obtained remote from exposure, as is the case for our cohort.

In an attempt to lessen recall bias, we only included women with no cognitive impairment on TELE. This, very likely, affected the number of dementia diagnoses in our cohort, since cognitive decline is known to precede dementia by years, if not decades. Consequently, the incidence of dementia in our cohort was 7% against the 12% expected in that age group. Another explanation for this could be that we lack information on diagnoses made in primary care.

We used data from the Swedish Twin Registry since it contained information on pregnancy complications as well as possible confounders such as education level, smoking and BMI. There could be concerns on how generalizable our results are. Lower birth weight and shorter gestational length are associated with twins and are also known risk factors for CVD. Epidemiological studies have not shown a higher incidence of CVD in twins, but since both our exposed and non-exposed women were twins, this should not have affected our results anyway.

The strengths of our study are the large cohort and that we were able to follow them to an advanced age when the incidence for both dementia and CVD increases steeply.

Studies II-IV:
These are all based on the same cohort collected 2013–2016 in a cross-sectional study. The generalizability of results from this study design depend on the representativeness of the sample and the sample size. We were limited by our exclusion criteria, especially regarding a multiple gestation, the availability of the MR camera, and a stable clinical condition in preeclampsia patients enabling transportation to the MR facility and the one hour MR investigation.
Of eligible preeclampsia patients, only a handful declined participation, most often invoking claustrophobia as the cause.

Studies II-III:
When reporting data from MRS, an important factor is the signal-to-noise ratio (SNR). This is dependent on field strength, homogeneity of the magnetic field, and quality of the receiver coils. Other factors influencing the quality of the spectrum are the size of the ROI where data acquisition is made and the acquisition time. Artefacts can arise from movement, and unlike MRI this may not be apparent when visually reviewing the spectrum.

Our spectra have been measured in ROIs carefully outlined by the same physicist who was blinded for the study group status of all study participants. The ROIs were positioned over the parietal and occipital lobes of the brain, regions often affected in PRES. There is, however, a regional variation in metabolite levels, such as between gray and white matter. Our MRS results can therefore only be generalized for differences in the parietal and occipital lobes of the brain.

We have been limited to 1.5 T out of consideration for the growing fetus. It should be noted that spectra acquired at a higher field strength (e.g. 3 T) have improved SNR and spectral resolution. We did, however, have spectra of excellent quality for all cerebral osmoles, except for taurine.

For study II, the levels of cytosolic Mg\textsuperscript{2+} was calculated using the equation from Iotti et al.\textsuperscript{133} Mg\textsuperscript{2+} can be calculated in different ways; Gupta et al. used the chemical shift difference of the $\alpha$- and $\beta$-phosphoryl resonances of ATP.\textsuperscript{151} This has been viewed as an over-simplification by others, considering that the chemical shift of $\beta$-ATP is affected by the number of phosphorylated compounds present in the cell cytosol, the ionic strength, temperature, and pH of the medium. The equation of Iotti et al. takes all of this into consideration, rendering the results more robust. In the previous study on Mg\textsuperscript{2+}-levels in preeclampsia, the calculation was based on the equation from Gupta.\textsuperscript{126}

Study IV
To assess the cerebral circulation we have used the MRI technique IVIM, which is not new, but has been made feasible relatively recently. It is mostly used in oncology research to grade tumors and determine treatment efficiency. IVIM has been evaluated for the assessment of the cerebral circulation, although there are some concerns regarding the robustness of the method. In a study in which a coefficient of variance for different perfusion measurements measured on different sites and on different scanners was calculated, the IVIM parameters had the largest variance. In another critical appraisal of the method, measurements in gray matter were more robust than in white, due to the higher perfusion fraction in this type of tissue. The strengths of the present study lie in our well-described cohort and our utilization of a technique to measure perfusion that corresponds to the capillary level in areas of
the brain mostly affected in preeclampsia/eclampsia. Limitations are the relatively small numbers of participants with severe preeclampsia and the participant’s use of antihypertensive drugs. Although antihypertensive drugs have been shown not to affect CBF or autoregulation, data suggest a decrease in CPP with Labetalol treatment.\textsuperscript{155,156} Whether this has any bearing on perfusion fraction when measured with IVIM has not been studied.

**General discussion**

**Long-term cerebral effects**

In study I, we investigated a potential association between pregnancy hypertensive disease and future risk of dementia. The notion that there is a link was based on reports about impaired cognition after preeclampsia and the presence of white matter lesions (WML) in the brain several years after preeclampsia and eclampsia.\textsuperscript{75-79} Also, pregnancy hypertensive disease has emerged as a gender-specific risk factor for future CVD, stroke, and hypertension, conditions well-known to increase risk of cognitive decline and dementia.\textsuperscript{66, 67, 157, 158}

In our study, we could not show an increased risk of dementia after hypertensive pregnancy. There are few reports addressing this proposed association. A large retrospective cohort study from Utah, USA, which included 60,580 women with a history of hypertensive pregnancy in 1939–2012, found an increased all-cause mortality in these women compared to women with a history of normotensive pregnancies. A major contributor was an increased risk of AD, with a hazard ratio of 3.44, 95% (CI 1.00–11.82).\textsuperscript{159} Contrary to this report, no difference in reported history of pregnancy hypertensive disease was found between women with AD and healthy controls in another study.\textsuperscript{86} Since pregnancy hypertensive disease is associated with later hypertension and CVD, one hypothesis could be that pregnancy hypertensive disease would increase the risk of vascular dementia as opposed to AD. AD is the most common form of dementia, accounting for 60–70% of all dementia cases, whereas vascular dementia accounts for approximately 15%.\textsuperscript{160} In a recent study with data from the Swedish Birth Register the authors reported on increased risk of vascular dementia after hypertension and proteinuria in pregnancy.\textsuperscript{88} The confidence interval reported was however wide and the authors themselves questioned the reliability of the result. The reliability of the dementia subgroup diagnosis can also be questioned since the positive predictive value of a vascular dementia diagnosis was found to be 57% in one report.\textsuperscript{161} Future work in the area is warranted that focuses on associations between pregnancy hypertensive disease and subtypes of dementia, including vascular dementia. Demographic trends indicate a growth in the elderly population and increasing
prevalence of dementia that will infer a great socioeconomic burden. To identify persons at risk will represent an important preventive strategy.

Concerning our second aim, we could confirm that the increased risks of CVD and stroke after pregnancy hypertensive disease persist in an elderly population. The point estimate is lower than what is reported for women below 65 years, 1.3 versus 2.4, but since the absolute risk for women of CVD increases sharply after menopause, even a small increase in risk in this age, has a bigger impact on the total number.

**Implications for seizure susceptibility**

In study II, we found lower cerebral Mg\(^{2+}\) levels in women with preeclampsia than in normal pregnant women. Furthermore, a novel finding was the correlation between visual disturbances in women with preeclampsia and lower cerebral Mg\(^{2+}\)-levels. In study III, we found lower levels of cerebral organic osmolytes in pregnant women than in non-pregnant women. Glutamate, the excitatory neurotransmitter, was reduced in women with preeclampsia compared to women with a normal pregnancy.

These findings are all intriguing considering the unchartered pathophysiology of seizures in preeclampsia, i.e. eclampsia. Today intravenous MgSO\(_4\) is the most effective treatment and prophylaxis, proven after large and laborious trials. The exact mechanisms of action are not fully understood but some proposed pathways are outlined in the section MgSO\(_4\) in the introduction. Our data for cytosolic Mg\(^{2+}\) was 0.14 mM in normal pregnancy and 0.12 mM in preeclampsia. We do not know whether this has any clinical significance. In an earlier study of intravenous MgSO\(_4\) treatment of subarachnoid hemorrhage, the authors could, however, demonstrate an improved outcome in study participants when cerebral intracellular Mg\(^{2+}\) increased by 0.018 mM. Based on these results, our measured difference could have clinical implications.

A reduction in cerebral organic osmolytes in conditions of hyponatremia is well established, both in animal studies and in humans. These findings has been linked to the increased susceptibility for seizures seen in hypoosmotic conditions. In vitro studies have found that hypoosmolarity triggers a release of glutamate from nerve endings which could increase excitatory postsynaptic potentials.

Pregnancy is a state of hyponatremia and hypoosmolality brought about by an altered osmotic threshold for thirst and antidiuretic hormone release. Antidiuretic hormone, or vasopressin, has been shown to be increased in preeclampsia and also that the rise precedes the clinical diagnosis. The plasma sodium level in pregnancy is decreased, but still within normal limits. If the lower sodium levels in pregnancy have an effect on cerebral organic osmolyte levels has never been studied in humans previously. Only one small animal study from 1989 has been published to date, and in this study the authors demonstrated lower levels of taurine in pregnant rats. We found lower levels of myo-inositol, creatine, and choline in both pregnant study...
groups than in the non-pregnant group and lower levels of glutamate in women with preeclampsia than in normal pregnant women. The extrusion of glutamate from the cells could mean higher levels of the neurotransmitter in the synaptic cleft, where it can exercise its excitatory effect. In combination with the lower levels of Mg\(^{2+}\) that we also report, where fewer glutamate receptors would presumed to be blocked, this could implicate a lower threshold for seizures.

**Cerebral perfusion**

In study IV, we used a novel technique to investigate how the cerebral circulation is affected in preeclampsia. This issue has been extensively explored over the years with the use of different techniques, yielding diverging results. An understanding of cerebral perfusion and its adaptation to pregnancy and preeclampsia is regarded as a prerequisite for insight into the pathophysiology of eclampsia and PRES. However, reports on changes in cerebral circulation in both pregnancy and preeclampsia are contradictory. Pregnancy has been found to both decrease and increase CBF.\(^97,98\) In preeclampsia, findings have pointed to both hyper- and hypoperfusion.\(^54,55,57,58\)

A common examination technique has been TCD, since it is easily accessible. From the measures of blood flow velocities and BP, an estimation of CPP can be made.\(^173\) Results from studies published thus far, show evidence of impaired autoregulation in preeclampsia and increased CPP.\(^174,175\) However in one study, CPP was higher in only women with severe preeclampsia, whereas women with mild to moderate disease had lower CPP than women with normal pregnancies.\(^54\)

Since TCD can only be used for major arteries, the TCD-derived findings may not reflect how the peripheral circulation is affected by preeclampsia. Our aim was to investigate the perfusion on the capillary level. For this purpose we chose to use IVIM, an MRI technique in which contributions from diffusion and perfusion can be separated.\(^118\) The IVIM variables corresponding to cerebral blood flow and volume, were lower in the caudate nucleus in women with preeclampsia than in both normal pregnant and non-pregnant women. The caudate nucleus is a part of the basal ganglia, a brain structure that is more often affected in preeclampsia or eclampsia-induced PRES.\(^176,177\) It is hypothesized that this region is more vulnerable due to a different vascular architecture with more non-anastomotic vessels.\(^177\) However, no difference between women with preeclampsia and controls was noted in another part of the basal ganglia, namely the lentiform nucleus, in turn questioning this potential explanation. Thus, our results must be interpreted with caution.

In PRES, the edema seen is predominantly vasogenic, but cytotoxic edema could be more common than we have believed so far.\(^46\) How and when edema arises is not clear. Using IVIM, we had a unique possibility to measure the diffusion coefficient with the aim of assessing the occurrence of edema not
visible on standard MR images. In our material we could not detect any differences, even though a third of our preeclampsia group had a severe disease, and two-thirds had cerebral symptoms. Whether this is enough information to consider edema formation as a late step in the pathophysiology of PRES and eclampsia is uncertain.
Conclusion

Study I

No association between hypertensive complications during pregnancy and later dementia was found. The risk for CVD was shown to persist in this elderly cohort.

Study II

Preeclampsia was associated with lower cerebral Mg\(^{2+}\) levels, and this correlated with visual disturbances.

Study III

The levels of most cerebral organic osmolytes were lower in women with preeclampsia and normal pregnancy than in non-pregnant women. Glutamate was significantly lower in preeclampsia than in normal pregnancy.

Study IV

Perfusion fraction was comparable between preeclampsia, normal pregnancy and non-pregnant women, except in the caudate nucleus where it was decreased in preeclampsia. No difference in measurements of cerebral edema was found between study groups.
Future perspectives

Plasma biomarkers of cerebral damage are currently explored as a tractable approach to monitor conditions such as traumatic brain injury, epilepsy and neurodegenerative diseases. Also in preeclampsia, two of these biomarkers, S100B and neuron-specific enolase (NSE) have been found to be increased, remaining so up to one year post-partum.\textsuperscript{178-180} Initial results, in which we have measured S100B and NSE in plasma samples from our cohort in study II-IV confirm previous studies, thus strengthening the potential for these biomarkers in preeclampsia. Interestingly, NSE and glutamate are found to correlate, with unknown implications. Building on these early findings, we will extend the investigation to additional plasma protein biomarkers with potential promise in preeclampsia, including tau protein and NfL, mostly studied in AD.\textsuperscript{181, 182}

Much of the focus in experimental eclampsia is on changes and dysfunction in the BBB. An intact BBB seems protective for the pro-excitatory changes brought on by pregnancy.\textsuperscript{183} In a collaboration with the University of Bio-Bio, Chillan, Chile, plasma samples from our cohort will be added to human brain endothelial cells \textit{in vitro} to study changes in BBB permeability. Effects of S100B and NSE on the endothelium will be investigated. Are increased levels of the biomarkers a direct cause of endothelial disruption or a secondary effect to increased BBB permeability?

The findings from studies II and III point toward an increased seizure susceptibility in preeclampsia. To evaluate whether reduced Mg\textsuperscript{2+} and cerebral organic osmolyte levels have a direct effect on seizures, an animal model could be used. Further, it would be of interest to follow up our findings from these studies with a longitudinal approach. When in the course of preeclampsia does Mg\textsuperscript{2+} and organic osmolytes decrease? For how long in the postpartum period would a difference persist in comparison to non-pregnant women?
Sammanfattning på svenska

Havandeskapsförgiftning, eller preeklampsii som det kallas inom läkarvetskapen, drabbar ca 3-5% av alla graviditeter. I ett globalt perspektiv har preeklampsii stor påverkan på sjuklighet och död hos såväl gravida kvinnor som deras barn. Sjukdomen innebär stor risk för förtidig födsel med alla de komplikationer som det kan medföra.

Diagnosen preeklampsii ställs när en kvinna drabbas av nyttillkommet högt blodtryck och äggvita i urinen under andra halvan av sin graviditet. Sjukdomen kan påverka hela Kroppen, inklusive hjärnan. Kvinnan kan i sådant fall drabba av kramper och får då diagnosen eklampsii. Majoriteten av dödsfallen vid preeklampsii beror på komplikationer i hjärnan.

Insjuknande i preeklampsii medför i många fall sjukhusvistelse, ibland under lång tid, behandling med blodtryckssänkande medicinering och ställningstagande till att sätta igång förlossning, vilket idag är den enda botande åtgärden. Avvägningen mellan att inte riskera att kvinnan ska bli sjukare mot att behöva förlösa ett barn prematurt kan vara mycket svår.

Kvinnor som haft en graviditet med preeklampsii löper ökad risk att få högt blodtryck, hjärtinfarkt och stroke senare i livet. Det finns också studier som tyder på att den kognitiva förmågan kan vara påverkad.

Huvudhypotesen till varför preeklampsii uppstår har länge varit att anläggningen av moderkakan av olika orsaker blivit undermålig. Det uppstår då en syrefattig miljö vilket kan frisätta ämnen som leder till den kliniska bilden med högt blodtryck och njurpåverkan. Många barn som föds i graviditeter komplicerade av preeklampsii är också tillväxthämmade. De flesta barn är dock normalstora och ett nytt spår i forskningen kring preeklampsii har de senare åren varit att studera hur en bristande omställning av hjärt-kärlsystemet till graviditeten kan bidra till preeklampsii.

**Delarbete I**
Vi använde ett svenskt register, tvillingregistret, för att undersöka om kvinnor med preeklampsi och/eller högt blodtryck under graviditeten har större risk för demens och hjärtärl-sjuklighet efter 65 års ålder. Kvinnorna deltog i en telefonintervju där de bland mycket annat, besvarade frågor om de haft högt blodtryck och äggvita i urinen under sina graviditeter. Dessutom genomgick de ett kognitivt test. För att inkluderas i studien krävde vi intakt kognition, detta för att kunna lita på uppgifterna om graviditetskomplikationer. Därefter följdes kvinnorna under 12 års tid och information om insjuknande i demens och hjärt- och kärlsjukdomar samlades in från patientregistret samt dödsregistret.

Vi kunde i vår studie inte se något samband mellan en graviditet med preeklampsi eller högt blodtryck och ökad risk för ett senare insjuknande i demens. Däremot kunde vi bekräfta att det sedan tidigare kända sambandet mellan preeklampsi och högt blodtryck under graviditeten och senare ökad förekomst av hjärt- och kärlsjukdomar kvarstod även hos vår äldregrupp kvinnor. De flesta tidigare studier har tittat på betydligt yngre kvinnor som har en lägre absolut risk att drabbas av dessa sjukdomar.

**Delarbete II**
Med hjälp av en magnetkameraundersökning, kallad spektroskopi, kan magnesiumnivåerna i hjärnan mätas. Eftersom magnesium i form av magnesiumsulfat används som förebyggande behandling vid svår preeklampsi, ville vi undersöka om nivåerna skiljer sig mellan kvinnor med preeklampsi, normal graviditet och hos icke-gravida kvinnor. Vi kunde visa att kvinnorna med preeklampsi hade de lägsta nivåerna. När vi vidare tittade på nivåerna hos de kvinnor med preeklampsi som klagat på synstörningar vid undersökningen, fann vi att de hade de allra lägsta nivåerna. En slutsats man skulle kunna dra av våra fynd vore att noga efterfråga ögonsymtom hos kvinnor med preeklampsi och överväga magnesiumsulfatbehandling tidigare vid förekomst av synstörningar.

**Delarbete III**
Graviditet medför stora förändringar i kroppen med bland annat ökad plasmavolym, högre hjärtfrekvens och ökad filtration i njurarna. Man ser också att nivåerna av natrium sjunker i blodet. Vid tillstånd med mer uttalad natriumsänkning t.ex. vid alltför stort intag av vatten vid idrottprestationer, eller vid hjärtsvikt, har man sett att krampbenägenheten ökar. Detta kan bero på risken för ödem, vätska i cellerna, vid akut natriumsänkning. Med hjälp av magnetisk resonansspektroskopi har man kunnat se att cellerna i hjärnan stöter ut en rad ämnen för att försvara sig mot ödembildning. Dessa kallas osmyler och bland dem finns glutamat, som också fungerar som excitatorisk signalsubstans
i hjärnan. Om mängden glutamat i utrymmet mellan cellerna ökar, kan stimulering av fler celler ske, vilket kan öka krambenägenheten.

Vi har i detta delarbete också använt magnetisk resonansspektroskopi och kan visa att gravida kvinnor, både de med preeklampsi och de utan, har lägre nivåer av osmolyter. Vidare fann vi lägre nivåer av glutamat inne i cellerna hos kvinnor med preeklampsi. Detta skulle kunna ha betydelse för hur kramper uppstår.

**Delarbete IV**

Här ville vi studera hur blodgenomströmningen i hjärnan ändras under preeklampsi. Vid svår preeklampsi och eklampsi kan man se ödem på magnetkamerabilder. Det finns olika idéer om hur det uppstår, där man föreslår såväl ökad som minskad genomblödning. Olika undersökningstekniker och studier har gett stöd för båda teorierna. De bästa metoderna för att undersöka blodgenomströmning i hjärnan går inte att använda under graviditet, av hänsyn för fötret. Vi har använt en relativt ny metod, en variant på magnetkameraundersökning kallad IVIM. Med denna metod kan man uppskatta hur mycket blod som är i rörelse på kapillär nivå, d.v.s. i de allra minsta blodkärllon. Dessutom ger metoden en uppskattning av graden av ödem i vävnaden.

Vi fann i stort sett likartade resultat hos våra tre studiegrupper, kvinnor med preeklampsi, normal graviditet och icke-gravida kvinnor. I en del av hjärnan som styr frivilliga rörelser, de s.k. basala ganglierna såg vi dock minskad blodgenomströmning hos kvinnor med preeklampsi. Vi kunde inte påvisa någon skillnad i förekomst av ödem.
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Pregnancy hypertensive disease and risk of dementia and cardiovascular disease in women aged 65 years or older: a cohort study

M Nelander,1 S Cnattingius,2 H Åkerud,1 J Wikström,3 N L Pedersen,4 A-K Wikström1,2

ABSTRACT

Objective: The primary aim was to study pregnancy hypertensive disease and subsequent risk of dementia. The second aim was to study if the increased risks of cardiovascular disease (CVD) and stroke after pregnancy hypertensive disease persist in an elderly population.

Design: Cohort study.

Setting: Sweden.

Population or sample: 3232 women 65 years or older (mean 71 years) at inclusion.

Methods: Cox proportional hazards regression analyses were used to calculate risks of dementia, CVD and/or stroke for women exposed to pregnancy hypertensive disease. Exposure data were collected from an interview at inclusion during the years 1998–2002. Outcome data were collected from the National Patient Register and Cause of Death Register from the year of inclusion until the end of 2010. Age at inclusion was set as a time-dependent variable, and adjustments were made for body mass index, education and smoking.

Main outcomes measures: Dementia, CVD, stroke.

Results: During the years of follow-up, 7.6% of the women exposed to pregnancy hypertensive disease received a diagnosis of dementia, compared with 7.4% among unexposed women (HR 1.19; 95% CI 0.79 to 1.73). The corresponding rates for CVD were 22.9% for exposed women and 19.0% for unexposed women (HR 1.29; 95% CI 1.02 to 1.61), and for stroke 13.4% for exposed women and 10.7% for unexposed women (HR 1.36; 95% CI 1.00 to 1.81).

Conclusions: There was no increased risk of dementia after self-reported pregnancy hypertensive disease in our cohort. We found that the previously reported increased risk of CVD and stroke after pregnancy hypertensive disease persists in an older population.

INTRODUCTION

Pre-eclampsia used to be regarded as an isolated event during pregnancy and delivery, but we now know that women with a pregnancy complicated by pre-eclampsia have an increased risk of cardiovascular disease (CVD), such as ischaemic heart disease and stroke, later in life. Pre-eclampsia and CVD share many risk factors, including obesity and diabetes. Pregnancy has been described as a stress test for the cardiovascular system, where the demands of pregnancy unmask underlying phenotypic susceptibility to CVD. There is also the possibility that pre-eclampsia is an independent risk factor due to endothelial changes during the pregnancy, changes that have been shown to persist at least years after the pregnancy. Most previous studies of the association between pre-eclampsia and later CVD are limited to follow-up of women who have not yet reached menopause, and studies focusing on pre-eclampsia and CVD risk in elderly women (>65 years) are lacking. Since the risk of CVD generally increases with age, the effect of pre-eclampsia may vary with age of follow-up. The prevalence of dementia is increasing worldwide. It has been estimated that the number of people living with dementia will almost double in the coming 20 years.

Strengths and limitations of this study

This is the first study addressing a potential association between hypertensive complications during pregnancy and dementia later in life.

The strengths of this study lie in the well-characterised cohort and our ability to follow-up women during a life period with a suspected high prevalence of dementia.

The major limitation of our study was that the information on pregnancy hypertensive disease was self-reported and collected many years after pregnancy.
prevalence increases with age and is very rare in women below 65 years. Dementia is a heterogeneous disorder with Alzheimer’s disease and vascular dementia being the two most common types.

When followed up several years after the index pregnancy, women with eclampsia and pre-eclampsia have significantly more brain white matter lesions on MRI than controls with normotensive pregnancies. These lesions can have different causes and be asymptomatic, but are often due to microangiopathy and have been found to correlate with impaired cognition and dementia, especially in periventricular location. There are also recent reports of detectable cognitive impairment a few months after severe pre-eclampsia, and self-reported impairment of cognitive function up to 7 years after eclampsia. On the basis of these findings together with the known association between pre-eclampsia and different cardiovascular complications, we hypothesised that pregnancy hypertensive disease is associated with increased dementia risk.

In this study, we included women 65 years or older. The primary aim was to explore if women with a history of pregnancy hypertensive disease have an increased risk of dementia. Second, we wanted to study if pregnancy hypertensive disease is associated with increased risks of CVD and stroke in this elderly population.

MATERIAL AND METHOD

Study population
The cohort was retrieved from the nationwide Swedish Twin Register. Between 1998 and 2002, all twins in the Register born in 1958 or earlier were invited to a telephone interview, which was a part of the SALT (Screening Across the Lifespan Twin) study. The interview included questions about pregnancy complications, such as whether or not they had suffered from high blood pressure during pregnancy and, if so, also from proteinuria. Further, it included general questions, such as current weight and height, years of education and smoking habits. For those who were 65 years or older at the time of the interview, the interview included a short cognitive screening test called TELE. The cognitive test included the Mental Status Questionnaire, combined with other cognitive items, such as three-word recall, counting backwards and questions about the need for help with daily life due to memory impairment. In our study population, we included women who had given birth, answered questions regarding hypertensive complications during pregnancy, were 65 years or older at the time of the interview and performed the cognitive screening TELE (figure 1). Since exposure data of our study were collected at the time of interview, we excluded women who were not cognitively intact according to the TELE test. We also excluded one woman who had a dementia diagnosis within 6 months after the interview, although she was screened cognitively intact at the interview. The final study population included 3232 women, aged

Figure 1 Flow chart of the study population (SALT, Screening Across the Lifespan Twin).

65 years or more with available self-reported data on pregnancy hypertensive disorder and intact memory at the start of follow-up (figure 1).

Exposure data
The exposure was a history of pregnancy hypertensive disease and data were retrieved from the interview, which included two questions regarding hypertension and pregnancy. The first question was if the woman had high blood pressure during any pregnancy (yes/no/do not know). The second question was, if she had high blood pressure, did she also have proteinuria (yes/no/do not know). First, we divided the women into five groups: (1) no hypertension; (2) pre-eclampsia (yes for both hypertension and proteinuria); (3) gestational hypertension (yes for hypertension and no for proteinuria); (4) high blood pressure, unclear proteinuria (yes for hypertension and do not know for proteinuria) and (5) do not know (do not know for hypertension). In further analyses, we combined the second, third and fourth groups to ‘any hypertensive complication’.

Outcome data
The primary outcome was a diagnosis of dementia and the secondary outcomes were diagnosis of CVD and

stroke after the time of the interview. Outcome data were retrieved from the National Patient Register and the Cause of Death Register. Linkage between the Swedish Twin Register and the National Patient Register and Cause of Death Register was made possible through each individual’s unique personal registration number. The National Patient Register includes information on dates of hospital admissions and diagnoses, which are classified according to International Classification of Diseases (ICD) codes. From 1987, the registry includes all in-patient care in Sweden and from 2001 also hospital outpatient visits. ICD diagnoses for each diagnosis are presented in online supplementary table S1.

**Covariates**

Covariates in the study were collected at the time of the interview and included age (continuous) at interview; current body mass index (BMI) (continuous), education (9 years of school/ >9 years of school) and current smoking habits (daily smoking, yes/no).

**Statistics**

Risks of dementia, CVD and stroke after pregnancy hypertensive disease were estimated using Cox proportional hazards regression models and age at interview was set as a time-dependent variable. Women who reported no pregnancy hypertensive disease were the reference. Adjustments were made for BMI, education and smoking at the time of interview. To account for relatedness (twins), the analyses were clustered on twin-pair identity. The regression parameters in the Cox model were estimated by the maximum partial likelihood estimates under an independent working assumption and a robust sandwich covariance matrix estimate was used to account for the intraclass dependence.\(^\text{19}\)

**RESULTS**

Table 1 includes the basic characteristics of the study population at the time of interview (ie, start of follow-up). Women who did not remember if they had high blood pressure during pregnancy were somewhat older at the interview than the other exposure groups. After exclusion of women who did not remember if they had high blood pressure during pregnancy, 419 of 3065 (14%) women reported that they had at least one pregnancy with pregnancy hypertensive disease. Women who reported that they had a history of pregnancy hypertensive disease had a slightly higher BMI and education, but were less often smokers compared with those without pregnancy hypertensive disease. Women who reported a history of pregnancy hypertensive disease more often had a diagnosis of CVD, but not of stroke, in the Patient Register before the interview.

Table 2 presents rates of dementia, CVD and stroke after the time of the interview by exposure to pregnancy hypertensive disease. Rates of dementia seemed similar between women who reported a history of pregnancy hypertensive disease and those who did not (7.6% vs 7.4%). Rates of CVD and stroke seemed slightly higher in women who reported a history pregnancy hypertensive disease than those who did not (22.9% vs 19.0% and 13.4% vs 10.7%, respectively).

Table 3 presents the risk of dementia after any hypertensive complication during pregnancy, before and after adjustments for confounders. We found no significant association between dementia and pregnancy hypertensive disease. Figure 2A illustrates the cumulative incidence of dementia for women with a history of pre-eclampsia, gestational hypertension or no hypertension. The three groups exhibit very similar graphs. If anything, the cumulative incidence of dementia increased with gestational hypertension towards the end

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**Table 1** Characteristics of the study population at the time of the interview (ie, start of follow-up)

<table>
<thead>
<tr>
<th>Pregnancy hypertensive disease</th>
<th>No (N=2646)</th>
<th>Any (N=419)</th>
<th>With proteinuria (N=269)</th>
<th>Without proteinuria (N=113)</th>
<th>Unclear proteinuria (N=37)</th>
<th>Do not know (N=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at inclusion</td>
<td>71.8±5.6</td>
<td>71.0±5.1</td>
<td>71.2±5.0</td>
<td>71.0±5.4</td>
<td>69.9±5.4</td>
<td>73.9±6.1</td>
</tr>
<tr>
<td>Age at end of follow-up</td>
<td>82.2±5.6</td>
<td>81.5±5.3</td>
<td>81.5±5.2</td>
<td>81.7±5.5</td>
<td>80.5±5.7</td>
<td>84.1±6.0</td>
</tr>
<tr>
<td>BMI*</td>
<td>24.9±3.8</td>
<td>26.2±4.2</td>
<td>26.2±4.2</td>
<td>26.4±5.3</td>
<td>25.6±3.7</td>
<td>24.8±3.5</td>
</tr>
<tr>
<td>Education &gt;9 years</td>
<td>1197 (45.3)</td>
<td>201 (48.1)</td>
<td>137 (59.0)</td>
<td>46 (40.7)</td>
<td>18 (50.0)</td>
<td>71 (42.8)</td>
</tr>
<tr>
<td>Smoking</td>
<td>254 (9.6)</td>
<td>28 (6.7)</td>
<td>20 (7.4)</td>
<td>6 (5.3)</td>
<td>2 (5.4)</td>
<td>22 (13.2)</td>
</tr>
<tr>
<td>Previous disease†</td>
<td>200 (7.6)</td>
<td>47 (11.2)</td>
<td>28 (10.4)</td>
<td>17 (15.0)</td>
<td>2 (5.4)</td>
<td>13 (7.8)</td>
</tr>
<tr>
<td>CVD</td>
<td>63 (2.4)</td>
<td>11 (2.6)</td>
<td>8 (3.0)</td>
<td>1 (0.9)</td>
<td>2 (5.4)</td>
<td>11 (6.6)</td>
</tr>
</tbody>
</table>

Data are presented as means±SD or numbers (%).

*BMI. Data are missing in 99 women.

†Prior to interview according to the Patient Registry.

BMI, body mass index; CVD, cardiovascular disease.
of the observation period, possibly reflecting that the overall incidence is quite low at the start of follow-up.

Table 4 presents risks of CVD and stroke after any hypertensive complication during pregnancy. Women who reported that they had a pregnancy hypertensive disease had slightly higher risks of CVD and stroke compared with women who reported no hypertensive disease.

Figure 2B,C illustrate the cumulative incidences of CVD and stroke by history of pre-eclampsia, gestational hypertension or no hypertension. The cumulative incidence of CVD and stroke increased with age in all three exposure groups. For CVD, the increase was slightly higher in women with a history of pre-eclampsia, as well as in women with gestational hypertension compared with non-hypertensive women (figure 2B). Women who experienced pre-eclampsia seemed to be more affected during the first 7 years of follow-up, but thereafter women with pre-eclampsia and gestational hypertension were affected. For stroke, pre-eclampsia also conferred an increased risk during follow-up (figure 2C).

DISCUSSION
Main findings
To the best of our knowledge, this is the first study addressing a potential association between hypertensive complications during pregnancy and dementia later in life. We could not detect a significant difference in the incidence of dementia between women with or without a history of pregnancy hypertensive disease. We could also show that the well-established increased risk for CVD in women with previous pre-eclampsia also applies to women of advanced age, but the risk increase seems somewhat lower compared with women in middle age.1

Strengths and limitations
The strengths of this study lie in the well-characterised cohort and our ability to follow the cohort until an age where the incidences of dementia and CVD are rapidly increasing. The major limitation of our study was that the information on pregnancy hypertensive disease was self-reported and collected many years after pregnancy. We also lacked further information regarding other pregnancy complications, such as giving birth to a preterm or growth retarded infant, that are associated with both our exposure and adverse long-term health of the mother.20 21

Since the need to rely on self-reported data often arises, especially if there is a long period between exposure and outcome, efforts have been made to try to validate these data. Klemmensen et al22 reported a positive predictive value of 59% for self-reported diagnosis of pre-eclampsia in a telephone interview 6–18 months after delivery. In contrast, Falkegård et al23 reported a positive predictive value of 80% for pregnancy hypertensive disease, although their population ranged from 25 to 88 years. To increase the validity of the self-reported data, we only included women who were cognitively intact according to a test performed at the same time as exposure data were collected. The prevalence of dementia in women aged around 80 is supposed to be approximately 12%.24 The incidence of dementia in our cohort was 7.4% and 7.6% in women unexposed and exposed to hypertensive complication in pregnancy, respectively. The major reason for the relatively low incidence in our cohort is probably the requirement of intact cognition at start of follow-up (mean age around 71 years at start). Owing to the study design, our results cannot be generalised to women with early impaired cognition. The association between pregnancy hypertensive disease and risk of CVD is more pronounced in women in middle-ages than among older women.23 This pattern could also be present concerning pregnancy hypertensive disease and risk of dementia. However, owing to the study design, our results cannot be generalised to women with early impaired cognition.
The incidence of any pregnancy hypertensive disease was 14% in our cohort, which is slightly higher than the usual reported incidence of 5–10%.26 Our finding is, however, in line with the results of Klemmensen et al.,22 who also reported a higher incidence of self-reported gestational hypertension compared with data from registries.

In a post hoc analysis, using data from the current study regarding the number of women exposed and the observed incidence of dementia, we have estimated that we had the power to detect an 80% difference in risk of dementia between women exposed and unexposed for pregnancy hypertensive disease, with a 80% power and 0.05 significance level.

Our cohort was retrieved from a twin registry with information on pregnancy complications and lifelong follow-up. Compared with singletons, twins have a lower birth weight and shorter gestational length. In singletons, low birth weight and preterm birth are known risk factors for obesity and future CVD.27 28 However, earlier studies have not shown that twins have a higher incidence of CVD than singletons29 30 and both the exposed and non-exposed women in our cohort were twins. Thus, having a study population of twins should not have influenced our results.

Interpretation

We found no increased risk of dementia after self-reported hypertensive pregnancy. Since pregnancy hypertensive disease is associated with later hypertension and CVD, one hypothesis could be that pregnancy hypertensive disease would increase the risk of vascular dementia as opposed to Alzheimer’s disease. Alzheimer’s disease is the most common form of dementia, accounting for 60–70% of all dementia cases, whereas vascular dementia accounts for approximately 15%.31 The difficulty lies in the reliability of the differential diagnosis. The Swedish Dementia Register reports that 30% of all dementia diagnosis are unspecified.32 Furthermore, the positive predictive value of a vascular dementia diagnosis is 57%.33 To obtain accurate results would therefore require a clinical validation of each patient’s dementia diagnosis, which was not possible with our study design. On the other hand, there is support for a vascular aetiology in Alzheimer’s disease,34 35 in which case we would expect an overall increase in dementia diagnoses.

In our study, we could confirm the established link between pre-eclampsia and CVD also in an elderly population.13 6 Our finding concerning CVD is in accordance with the study of Arnadottir et al.,25 reporting that pregnancy hypertensive disease was associated with a relative risk of 1.5 of death from CVD in women 65 years or older. We had a somewhat lower point estimate (1.3) compared with Arnadottir, which may be explained by an older study population. Our results extend the results of Arnadottir, since they also apply to morbidity from CVD and are adjusted for smoking and BMI, two important confounders for CVD.37 38 The study by Arnadottir further found that the relative risk for ischaemic heart disease death in women exposed to pregnancy hypertensive disease is higher in women below 65 years than in...
women 65 years or older (2.4 and 1.5, respectively). It could be that age itself is such an important risk factor that the risk induced by prior hypertensive pregnancy is attenuated. Since the absolute risk of CVD in women increases sharply after menopause, even a small increase in risk at this age has a bigger impact on the total number.

We also found a higher risk of stroke in women with pregnancy hypertensive disease, though with borderline significance. This is in line with previous work of Wilson et al. Our finding only refers to risk of stroke in women aged at least 65 years. As stated above concerning CVD, risk of stroke might also be relatively higher in younger than older women after exposure to pregnancy hypertensive disease.

The main hypothesis of the study was that women exposed to pregnancy hypertensive disease would have an increased risk of dementia later in life. Neuroimaging 5 years after eclampsia and pre-eclampsia has shown that women exposed to these pregnancy complications have an increased amount of white matter lesions compared with unexposed women. Pathological findings corresponding to white matter lesions are myelin loss and mild gliosis. Clinically, associations have been shown between white matter lesions and the incidences of stroke, dementia and cognitive decline. The white matter lesions seen after pre-eclampsia might be interpreted as either permanent brain effects of the disease or, perhaps more plausible, as merely another manifestation of the propensity of women with pre-eclampsia to develop vascular disease.

CONCLUSION
In this national study, we could not find an association between hypertensive complications during pregnancy and later dementia. Our cohort was retrieved from a twin registry and data on exposure were self-reported at an average age of 70 years. To account for this, we excluded a proportion of women not cognitive of intact at this time point, which can affect the generalisability of our study. Future work in the area is warranted to focus on associations between pregnancy hypertensive disease and subtypes of dementia, including vascular dementia. Demographic trends show an ageing population and increasing prevalence of dementia that will infer a great socioeconomic burden. To identify persons at risk will represent an important preventive strategy.

Contributors A-KW, HÅ and SC had the original idea for the study. MN, SC, HÅ, JW, NLP and A-KW all contributed to the design of the study. MN wrote the first draft of the manuscript. MN, SC, HÅ, JW, NLP and A-KW all made substantial contribution to the interpretation of results and manuscript revision.

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Competing interests None declared.

Ethics approval The study was approved by one of the Regional Ethical Review Boards in Stockholm, Sweden (reference number 2013/496; date of approval 19 April 2013).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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REFERENCES


Cerebral magnesium levels in preeclampsia; a phosphorus magnetic resonance spectroscopy study

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Disclosure: The authors declare no conflict of interests.

Key words: Preeclampsia, Eclampsia, Magnesium, Magnetic Resonance, ³¹P- magnetic Resonance Spectroscopy

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Abstract

**Background:** Magnesium sulfate (MgSO₄) is used as a prophylaxis for eclamptic seizures. The exact mechanism of action is not fully established. We used phosphorus magnetic resonance spectroscopy (³¹P-MRS) to investigate if cerebral magnesium (Mg²⁺) levels differ between women with preeclampsia, normal pregnant and non-pregnant women.

**Methods:** This cross-sectional study comprised 28 women with preeclampsia, 30 women with normal pregnancies in corresponding gestational week (range: 23-41 weeks) and 11 non-pregnant healthy controls. All women underwent ³¹P-MRS from the parieto-occipital region of the brain and were interviewed about cerebral symptoms. Differences between groups were assessed by ANOVA and Tukey’s post hoc test. Correlations between Mg²⁺ levels and specific neurological symptoms were estimated with Spearman’s rank test.

**Results:** Mean maternal cerebral Mg²⁺ levels were lower in women with preeclampsia (0.12 mM ±0.02) compared to normal pregnant controls (0.14 mM±0.03) ($p = 0.04$). Non-pregnant and normal pregnant women did not differ in Mg²⁺ levels. Among women with preeclampsia, lower Mg²⁺ levels correlated with presence of visual disturbances ($p = 0.04$). Plasma levels of Mg²⁺ did not differ between preeclampsia and normal pregnancy.

**Conclusions:** Women with preeclampsia have reduced cerebral Mg²⁺ levels, which could explain the potent anti-seizure prophylactic properties of MgSO₄. Within the preeclampsia group, women with visual disturbances have lower levels of Mg²⁺ than those without such symptoms.
Introduction

Magnesium sulfate (MgSO₄), has been shown to be the most efficient treatment for eclamptic seizures. While the mechanism of action still remains unclear, proposals include vasodilation, decreased permeability of the blood-brain-barrier (BBB), decreased neuroinflammation and anticonvulsant mechanisms by way of acting as an N-methyl-D-aspartate receptor antagonist. MgSO₄ is also used as a prophylaxis in preeclampsia when seizures are deemed imminent. There is however no clear consensus worldwide concerning indication for treatment. Eclampsia is not always preceded by severe preeclampsia and although there are known prodromal symptoms such as severe headache and visual disturbances, many women are asymptomatic immediately prior to their seizure.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have dramatically increased our ability to non-invasively study morphological as well as metabolic and functional changes in different tissues. Importantly for the aim of the present study, intracellular Mg²⁺ levels can be quantified using phosphorous MR spectroscopy (³¹P-MRS). Up to date, only one ³¹P-MRS study focusing on the brain in preeclampsia has been published so far, but the included study population was very small and the technique vaguely described. However, the authors found lower cerebral Mg²⁺ levels in normal pregnancy compared to non-pregnant controls and even lower levels in study patients with preeclampsia. No information was available regarding cerebral symptoms.

In this cross-sectional study we aimed to estimate cerebral Mg²⁺ levels in women with preeclampsia, women with normal pregnancies and non-pregnant women. We hypothesized that lower Mg²⁺ levels would correlate with cerebral symptoms in women with preeclampsia.
Material and methods

Study design and population

The study participants in this cross-sectional study were recruited in Uppsala, Sweden during 2013-2016. Uppsala University Hospital is a tertiary referral centre with approximately 4000 deliveries per year. General exclusion criteria were chronic hypertension, diabetes mellitus, pre-existing renal disease or contraindications for MRI, e.g. claustrophobia or pacemaker. Only singleton pregnancies were included.

Cases were thirty women with preeclampsia who were recruited from the obstetric ward or outpatient clinic. Preeclampsia was defined as de novo hypertension after 20 weeks of gestation in combination with proteinuria. Hypertension was defined as systolic blood pressure (BP) of $\geq 140\text{mmHg}$ and/or diastolic BP of $\geq 90\text{mmHg}$ measured on two subsequent occasions with at least 6 hours apart and proteinuria as $\geq 2^+$ on a dipstick or $\geq 300\text{mg/24h}$ in a urine collection. Preeclampsia was defined as severe, when blood pressure was $\geq 160\text{mmHg}$ systolic and/or $\geq 110\text{mmHg}$ diastolic; or if HELLP-syndrome was present. This is according to the recommendations from the International Society for studies on Hypertension in Pregnancy (ISSHP). Only women with singleton pregnancies and a gestational length between 22+0 and 41+6 were eligible. They also had to be clinically stable enough to be transported to the MR facility. None of the women were treated with MgSO$_4$. Every woman diagnosed with preeclampsia, either admitted or monitored as an outpatient that came to the main researcher’s (M.N.) knowledge, was approached regarding study participation. Only a small fraction of respondents abstained participation in the study, most often due to fear of claustrophobia. The included women were subsequently followed through their medical record and data on infant birth weight, mode of delivery and gestational age at delivery was noted. Small for gestational age was defined as a birth weight below 2 standard deviations...
from the sex-specific national reference curve.\textsuperscript{8} Gestational age was estimated with an early second trimester ultrasound.

We recruited two control groups: normal pregnant women in corresponding gestational age as the preeclampsia group at inclusion and a non-pregnant group. The normal pregnant group included 32 women and they were recruited through information posters at antenatal outpatient clinics in Uppsala and at university facilities. A normal pregnancy was defined as a normotensive pregnancy resulting in term delivery (gestational week $\geq$37) of an infant with normal birth weight ($\pm$ 2 standard deviations of the mean birth weight for gestational age and sex).\textsuperscript{8} This group was also followed through their medical record and women who subsequently developed preeclampsia were excluded. The non-pregnant control group included eleven women, both parous and nulliparous. These participants were recruited through Facebook and local networks. In addition to the general exclusion criteria, women with prior history of preeclampsia or gestational hypertension were not included in the control groups.

All participants underwent blood pressure measurement (BP), blood and urine sampling and MRS examination within a 12-hour period after enrollment in the study. The BP recording closest to the MRS examination was used (0.25-4 h). Systolic and diastolic BPs were measured in supine position in the right arm after a 15 min rest. A manual sphygmomanometer (Unmedico CE) with appropriate cuff size depending on arm circumference was used. The plasma samples were collected in Vacutainer$^\text{®}$ tubes with Lithium Heparin (Becton, Dickinson, Franklin Lakes, NJ, USA). The samples were centrifuged for 10 min at 1500g and the plasma was immediately frozen at -70$^\circ$ C for later analysis. Plasma Mg$^{2+}$ levels were analyzed on a BS380 instrument (Mindray, Shenzhen, China) with reagent (3P68) from Abbott Laboratories (Abbott Park, IL, USA)\textsuperscript{9}. Fresh midstream urine samples were collected and analyzed with a Combur 9 test (Roche) or
Clinitek Status Analyzer (Siemens). Directly prior to the MRS, an interview was conducted with questions regarding cerebral symptoms. This covered detailed questions regarding headache and visual disturbances, including scotomas, blurred vision and diplopia occurring in the last three days.

MRS

Data acquisition

Cases and controls were scanned on a 1.5 Tesla MR system (Achieva, Philips Healthcare, The Netherlands). The whole-body RF-coil served for imaging and transmit-receiver quadrature head coil (diameter 29 cm, length 25 cm) was used for phosphorous spectroscopy ($^{31}$P-MRS). Morphological images were first obtained for purpose of MRS planning. This was performed using T$_2$-weighted multi-shot turbo spin-echo sequences in para-sagittal, coronal and transverse planes (acquisition pixel size $1.5 \times 1.9$ mm$^2$, slice thickness, 6 mm). Single-voxel $^{31}$P-MRS was performed using image-selected in vivo spectroscopy (ISIS) localization scheme with a spectral bandwidth 1500 Hz, repetition time 3500 ms, 1024 points, and 256 acquisitions. Iterative first-order shimming improved magnetic field inhomogeneity inside the volume of interest (voxel). Typical voxel size was $35 \times 75 \times 50$ mm$^3$ with a position in the parieto-occipital region (Figure 1). Quality of the spectra was improved by proton ($^1$H) broad band decoupling and nuclear Overhauser enhancement (NOE). The whole body coil was used for this purpose. Proton decoupling consisted of standard WALTZ-4 cycle. The mixing time of NOE broad band irradiation was 2400 ms. The net measurement time was 15 min 3 sec.
Spectrum processing

Magnetic resonance user interface (MRUI) software package\textsuperscript{10} was used for spectrum processing. The spectra were fitted using AMARES method in time domain.\textsuperscript{11} No apodization of the free induction decay to improve the signal-to-noise ratio was used during fitting. Nevertheless, for presentation purpose (Figure. 2), a Lorentzian apodization corresponding to 2 Hz line broadening was applied. Phosphocreatine (PCr) peak was placed to 0 ppm. Multiplication (weighting) of the first twenty FID points with a quarter-sine wave was used to remove the broad background signal that typically underlines \textsuperscript{31}P brain spectra. Thus the broad component of the signal became part of the residue. Spectral intensities of following metabolites were fitted by Lorentzians: phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), PCr, \( \gamma \)-, \( \alpha \)-, and \( \beta \)-adenosine triphosphate (ATP) (Figure 2). Cytosolic concentration of free magnesium (Mg\textsuperscript{2+}) was calculated from the chemical shift between PCr and \( \beta \)-ATP using the equation of Iotti et al.\textsuperscript{5} Since position of \( \beta \)-ATP triplet can be difficult to determine in individual spectra due to low signal-to-noise ratio (SNR), spectra from each group of the volunteers (preeclampsia, normal pregnant and non-pregnant) were also averaged (Figure 2) and mean Mg\textsuperscript{2+} levels of each group were computed from these spectra.

\textbf{Figure 1.} Voxel position in the medial bilateral parieto-occipital cortex, sagittal and coronal plane.
The physicist analyzing the MRS levels was not aware of which study group the woman belonged to.

**Figure 2.** $^{31}$P MR spectrum of the brain. (a) Mean spectrum of preeclampsia patients; (b) Fitted spectrum. Phosphomonoesters (PME); inorganic phosphate (Pi); phosphodiesters (PDE); phosphocreatine (PCr); $\gamma$-, $\alpha$-, and $\beta$-adenosine triphosphate (ATP).

Statistics

Frequency histograms and Shapiro-Wilk’s test was used to test cerebral and plasma Mg$^{2+}$ levels in each study group for normal distribution. Thereafter, we used analysis of variance (ANOVA) for overall comparisons of means and Tukey’s test for multiple pair-wise comparisons. Chi-square and Fisher’s exact tests were used for comparisons between categorical variables. Spearman’s correlation test was assessed when we estimated correlations between cerebral Mg$^{2+}$ levels and maternal age, body mass index (BMI) and gestational length at examination in the normal pregnant population, as well as between cerebral and plasma Mg$^{2+}$ levels and cerebral Mg$^{2+}$ levels and cerebral symptoms in women with preeclampsia. All significance tests were two-tailed. $P<0.05$ was considered to denote a statistically significant difference. All analyses were performed using IBM SPSS Statistics 23 (IBM SPSS, Inc., Chicago, IL).
Ethics

The study was approved by the Regional Ethical Review Board in Uppsala, Sweden, and informed consent was obtained from each woman participating in the study.
Results

Of the 73 included women, two women with preeclampsia did not complete the $^{31}$P-MRS examination due to discomfort. Four normal pregnant controls were excluded since two of them developed preeclampsia after their inclusion in the study, one had a spectrum where intracellular Mg$^{2+}$ could not be calculated and one had a level of Mg$^{2+}$ that was an extreme outlier (>3 interquartile range). The final analysis was based on spectra from 67 women (28 preeclampsia, 28 normal pregnant and 11 non-pregnant).

Table 1 presents the clinical characteristics of the study population. The non-pregnant group was significantly younger than the pregnant participants, but there was no difference in maternal age between the preeclampsia and normal pregnant groups, 29.4 and 32.3 years respectively. Both the normal pregnant and the non-pregnant groups had lower mean BMIs than the preeclampsia group, $p = 0.048$ and $p = 0.001$, respectively. As expected the proportion of primiparas was high in the preeclampsia group, 82%. In contrast, in our normal pregnant group most women were parous.

**Table 1. Clinical characteristics of study population**

<table>
<thead>
<tr>
<th>Characteristics of the women</th>
<th>Preeclampsia (n=28)</th>
<th>Normal pregnant (n=28)</th>
<th>Non-pregnant (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>29.4 ± 5.5</td>
<td>32.3 ± 4.3</td>
<td>28.3 ± 6.5</td>
<td>0.047</td>
</tr>
<tr>
<td>BMI*, mean ± SD</td>
<td>26.5 ± 4.3</td>
<td>24.3 ± 2.7</td>
<td>21.6 ± 2.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Prior births, number (%)</td>
<td>5 (18%)</td>
<td>19 (68%)</td>
<td>5 (46%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational week, mean ± SD</td>
<td>33.6 ± 4.6</td>
<td>33.0 ± 5.1</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic, mean ± SD</td>
<td>146 ± 10</td>
<td>112 ± 8</td>
<td>112 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic, mean ± SD</td>
<td>93 ± 11</td>
<td>68 ± 8</td>
<td>67 ± 5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SD, standard deviation. N.s, non significant

*B*Body mass index. Early pregnancy BMI for the pregnant study groups
In Table 2 the preeclampsia group is further described. The mean gestational week at onset of preeclampsia was 33.2 weeks, with a range from 25 to 41 weeks. Most women had antihypertensive treatment (21/28), predominantly with a mono-therapy of Labetalol, (n= 18). At examination 8 women were found to have severe preeclampsia, a number that increased to 15 at the time of delivery. Mode of delivery was mostly cesarean delivery (16/28) and 7 women gave birth to infants small for gestational age. No woman was treated with MgSO4.

**Table 2. Characterization of the preeclampsia population**

<table>
<thead>
<tr>
<th>Characteristics of the women</th>
<th>Preeclampsia (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of preeclampsia, weeks (mean; range)</td>
<td>33.2; 25.3-41.1</td>
</tr>
<tr>
<td>At examination</td>
<td></td>
</tr>
<tr>
<td>Gestational length, weeks (mean; range)</td>
<td>33.6; 25.6-41.1</td>
</tr>
<tr>
<td>Severe preeclampsia*, n (%)</td>
<td>8 (29%)</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>21 (75%)</td>
</tr>
<tr>
<td>One drug, n (%)</td>
<td>18 (64%)</td>
</tr>
<tr>
<td>Two drugs, n (%)</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>At delivery</td>
<td></td>
</tr>
<tr>
<td>Gestational length, weeks (mean; range)</td>
<td>34.4; 26.0-41.4</td>
</tr>
<tr>
<td>Severe preeclampsia*, n (%)</td>
<td>15 (54%)</td>
</tr>
<tr>
<td>Vaginal/ Caesarean delivery (n/n)</td>
<td>12/16</td>
</tr>
<tr>
<td>Infant small for gestational age†, n (%)</td>
<td>7 (25%)</td>
</tr>
</tbody>
</table>

* According to the guidelines from the International Society for the study of Hypertension in Pregnancy (ISSHP)
† Birthweight deviation >22% below the sex specific reference curve for gestational age

Figure 3 shows a scatter plot of cerebral Mg$^{2+}$ levels for the three study groups. The mean Mg$^{2+}$ level was 0.12±0.02 mM in the preeclampsia group, 0.14±0.03 mM in the normal pregnant group and 0.14±0.03 mM in the non-pregnant group. This yielded a significant difference between groups (ANOVA; \( p = 0.04 \)) where women with preeclampsia had a significantly lower cerebral level of Mg$^{2+}$ compared to women with normal pregnancy (\( p = 0.04 \)). There was no significant difference in cerebral Mg$^{2+}$ content between the preeclampsia and non-pregnant group (\( p = 0.22 \)), nor between normal pregnant and non-pregnant women (\( p = 0.22 \)).
It should be noted, however, that accuracy of Mg\textsuperscript{2+} concentrations estimated from individual spectra suffer from the lower signal-to-noise ratio (SNR) of β\textsuperscript{-}ATP resonances. Therefore, we also calculated cerebral Mg\textsuperscript{2+} levels from the averaged spectra of each study group. In the preeclampsia group, the cerebral Mg\textsuperscript{2+} level was 0.12 mM, in the normal pregnant group 0.15 mM and in the non-pregnant group 0.17 mM. Since each study group was represented by single (averaged) spectrum, significance of differences between groups was not possible to compute.

Figure 3. Cerebral Mg\textsuperscript{2+} levels for each study group. Mean levels are shown with lines.
Table 3 presents correlations between cerebral Mg$^{2+}$ levels and cerebral symptoms in women with preeclampsia. There was a significant correlation between Mg$^{2+}$ levels and presence of visual disturbances ($p = 0.04$). However, Mg$^{2+}$ levels did not correlate with presence of headache.

**Table 3.** Correlations between cerebral magnesium Mg$^{2+}$ (mM) levels and cerebral symptoms in women with preeclampsia (n=28)

<table>
<thead>
<tr>
<th>Cerebral symptoms</th>
<th>Yes</th>
<th>No</th>
<th>Mg$^{2+}$ levels</th>
<th>Mg$^{2+}$ levels</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median</td>
<td>IQR</td>
<td>n</td>
<td>Median</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>10</td>
<td>0.11</td>
<td>0.10-0.13</td>
<td>18</td>
<td>0.12</td>
</tr>
<tr>
<td>Headache (any)</td>
<td>17</td>
<td>0.12</td>
<td>0.10-0.14</td>
<td>11</td>
<td>0.12</td>
</tr>
<tr>
<td>Severe headache (VAS&gt;5)</td>
<td>9</td>
<td>0.11</td>
<td>0.10-0.15</td>
<td>19</td>
<td>0.12</td>
</tr>
</tbody>
</table>

IQR; interquartile range
n.s, non significant

Supplemental Figure 1 illustrates plasma Mg$^{2+}$ levels in each study group. Non-pregnant women had a higher plasma Mg$^{2+}$ level than normal pregnancy (0.76 mM ±0.06 vs.0.68 mM ± 0.06, $p = 0.005$) and a borderline higher level than the preeclampsia group (0.70 mM ± 0.08, $p = 0.054$). Women with preeclampsia and the normal pregnant group did not differ in plasma Mg$^{2+}$ levels ($p = 0.479$).

Supplemental figure 1 Plasma Magnesium (S-Mg$^{2+}$) levels in each study group. Mean levels are shown with lines.
Cerebral Mg$^{2+}$ levels did not differ between those with antihypertensive treatment and those without (0.12±0.03 mM vs 0.12±0.02 mM, $p = 0.92$). Cerebral Mg$^{2+}$ levels did not correlate with maternal age ($p = 0.50$), BMI ($p = 0.90$) or gestational age at examination ($p = 0.43$). In the preeclampsia group, the cerebral and plasma Mg$^{2+}$ levels did not correlate ($p = 0.60$) (results are not shown in tables).

Discussion

In this MRS study, we found lower cerebral Mg$^{2+}$ levels in women with preeclampsia compared to normal pregnant women. Furthermore we found a novel correlation between visual disturbances in women with preeclampsia and a lower cerebral Mg$^{2+}$. Our volume of interest (VOI) was placed partly in the medial occipital lobe, the site of the primary visual cortex, which also strengthens the argument that Mg$^{2+}$ plays a central role in the pathophysiology of eclampsia/preeclampsia.

To our knowledge only one prior study has used $^{31}$P-MRS to examine the maternal brain in preeclampsia. The paper was published by Resnick et al in 2004, and their results are consistent with ours regarding a lower cerebral Mg$^{2+}$ level in women with preeclampsia compared to normal pregnant women. There are however differences in absolute levels of intracellular Mg$^{2+}$ between their study and ours. For normal pregnant women Resnick et al reported an average Mg$^{2+}$ level of 0.34 mM in contrast to our 0.14 mM. This is most probably explained by differences in methodologies. They used an equation described by Gupta et al in 1984, whereas we have used the approach further developed by Iotti et al. In the study by Iotti they reported cytosolic Mg$^{2+}$ of 0.182 mM from an averaged spectra of all 36 participants which is lower than previously published. The variation is explained by the difference in models estimating Mg$^{2+}$, where the equation by Iotti et al. considers more species that bind Mg$^{2+}$ than earlier reports. When we estimated Mg$^{2+}$ levels based on the average spectrum from all non-pregnant women, our result is 0.17 mM, i.e. very similar to Iotti et al. When comparing Mg$^{2+}$ levels calculated from individual spectra, we could not replicate Resnick et al’s finding of reduced Mg$^{2+}$ levels in normal pregnant women compared to non-pregnant women. When we compared Mg$^{2+}$ levels calculated from the averaged spectra of the three groups, our results are however consistent with Resnick et al also in this regard. Results from averaged spectra may be more reliable since this increases the signal-to-noise ratio. On
the other hand, no formal significance tests on differences between groups can be performed using this technique.

Besides using different calibration techniques, there are other differences between our study and Resnick et al. Their study was smaller, with a total of 30 patients whereof only 7 with preeclampsia. No patients with medication were included, thereby excluding those with a more severe disease. There is no information regarding cerebral symptoms. There are also differences in MRS methodology. We have used a voxel localization technique (ISIS)⁹ to be able to determine the VOI with high accuracy. In the previous study the size and position of their VOI was not clearly described. We focused on the parieto-occipital region since this has been shown to be the most commonly affected regions in posterior reversible encephalopathy syndrome (PRES).¹³ This syndrome is a combination of edema in predominantly the occipital and parietal lobes on MRI and clinical symptoms of headache, altered mental state, nausea, visual disturbance and seizures. PRES is seen in a number of conditions, including eclampsia and preeclampsia.¹⁴

Our finding that women with preeclampsia have lower cerebral Mg²⁺ levels than normal pregnant women is interesting considering that the most effective treatment and prophylaxis of eclampsia is intravenous MgSO₄. Although the exact mechanisms of action is not fully understood, it is known that Mg²⁺ can act as an antagonist to the glutamate receptor N-methyl-D-aspartate receptor (NMDA) in the brain.² With reduced Mg²⁺ levels, less NMDA receptors are blocked and more can be opened at a relatively low membrane potential causing hyperexcitability of neurons, lowering the threshold for seizures. In an earlier study of subarachnoid hemorrhage, an increase in cerebral intracellular Mg²⁺ by 0.018 mM was observed in subjects treated with intravenous MgSO₄.¹⁴ In our study the average difference in cerebral Mg²⁺ levels between women with preeclampsia and women with normal pregnancy was 0.02mM, a difference that could indicate clinical importance based on the results above. There is however no data regarding the effect on cerebral Mg²⁺ by treatment with MgSO₄ in preeclamptic women. We can only speculate if treatment failure is due to inadequately raised cerebral Mg²⁺ levels.

An important strength of this study is our detailed information regarding cerebral symptoms, such as headache and visual disturbances, immediately preceding the MRI examination. This gives us a unique opportunity to link clinical presentation with cerebral Mg²⁺ levels.
Although larger than previously reported studies, our cohort consists of relatively few women. Our measurements of Mg$^{2+}$ is limited to one VOI, it would be interesting to proceed to examine other regions in the brain and compare results. Due to concerns of possible negative effects on the fetus at higher levels we were limited to 1.5T. Imaging at 3T would improve both the signal-to-noise ratio and the spectral resolution, both of which would increase measurement accuracy.

Since participants in the study needed to be transported to the MRI scanner, those with an unstable condition could not be included, possibly underestimating the differences between groups. A high proportion of women with preeclampsia were on antihypertensive treatment, mostly a monotherapy with Labetalol. Labetalol has been shown not to affect the cerebral blood flow or autoregulation, but possibly lowering the cerebral perfusion pressure.\textsuperscript{15, 16} If this also affects the cerebral Mg$^{2+}$ levels is not known.

In conclusion, we have found that cerebral Mg$^{2+}$ levels are lower in preeclampsia, an observation that agrees with the fact that MgSO$\textsubscript{4}$ is the best available prophylaxis for seizures, i.e. eclampsia. The finding of lower Mg$^{2+}$ levels in the medial parieto-occipital region of women with preeclampsia and visual disturbances could indicate that these symptoms should warrant a closer monitoring and a consideration to use MgSO$\textsubscript{4}$-prophylaxis.

Details of ethics approval:

Approved by the local Ethics board: Reference number: 2012-087, May 23\textsuperscript{rd} 2012

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References


Paper III
Cerebral osmolytes and plasma osmolality in pregnancy and preeclampsia: a proton magnetic resonance spectroscopy study

Running title: Cerebral osmolytes in pregnancy and preeclampsia

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Disclosure: The authors declare no conflict of interests.

Key words: Preeclampsia, eclampsia, proton magnetic resonance spectroscopy, cerebral osmolytes, glutamate

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Abstract

Background: Cerebral complications contribute substantially to mortality in preeclampsia. Pregnancy calls for extensive maternal adaptations, some associated with increased propensity for seizures, but the pathophysiology behind the eclamptic seizures is not fully understood. Plasma osmolality and sodium levels are lowered in pregnancy. This could result in extrusion of cerebral organic osmolytes, including the excitatory neurotransmitter glutamate, but this remains to be determined. The hypothesis of this study was that cerebral levels of organic osmolytes are decreased during pregnancy, and that this decrease is even more pronounced in women with preeclampsia.

Method: We used proton magnetic resonance spectroscopy to compare levels of cerebral organic osmolytes, in women with preeclampsia (n=30), normal pregnancy (n=32) and non-pregnant controls (n=16). Cerebral levels organic osmolytes were further correlated to plasma osmolality, and plasma levels of glutamate and sodium.

Results: Compared to non-pregnant women, women with normal pregnancy and preeclampsia had lower levels of the cerebral osmolytes myo-inositol, choline and creatine (p=0.001 or less), and all these metabolites correlated with each other (p<0.05). Women with normal pregnancies and preeclampsia had similar levels of osmolytes, except for glutamate, which was significantly lower in preeclampsia. Cerebral and plasma glutamate levels were negatively correlated with each other (p<0.008), and cerebral myo-inositol, choline and creatine levels were all positively correlated with both plasma osmolality and sodium levels (p<0.05).

Conclusion: Our results indicate that pregnancy is associated with extrusion of cerebral organic osmolytes. This includes the excitatory neurotransmitter glutamate, which may be involved in the pathophysiology of seizures in preeclampsia.
Introduction

Cerebral complications in preeclampsia/eclampsia contribute substantially to maternal deaths world-wide.\(^1\) Although seizures during pregnancy are described in the literature since Hippocrates and the most effective treatment, magnesium sulfate (MgSO\(_4\)), has been in practice for almost a century, our knowledge of how and when eclampsia arises is surprisingly scarce. Eclampsia and severe preeclampsia are described as a form of posterior reversible encephalopathy syndrome.\(^2\) The predominant theory of the etiology includes breakdown of cerebral autoregulation due to a rapid rise in blood pressure (BP), with ensuing damage to the blood brain barrier and vasogenic edema as a result.\(^3\) However, the fact that a large proportion of women with eclampsia have moderately elevated, or even normal BP, prior to seizures, questions this theory.\(^4\)

Pregnancy causes fundamental changes in hemodynamics and body fluid hemostasis,\(^5,\)\(^6\) including reductions in plasma osmolality and plasma sodium levels. These changes appear directly after conception, reaches a steady state at gestation week 10 and are achieved by pregnancy-induced changes in the osmotic thresholds for thirst and arginine and vasopressin secretion.\(^7,\)\(^8\) A growing body of evidence show that the increase in vasopressin is higher in pregnancies that subsequently develops preeclampsia and it has been proposed as a possible predictive biomarker.\(^9,\)\(^10\)

When osmolality falls there is a risk of edema when osmotic forces causes water to enter the cells. This is prevented by mechanisms called regulatory volume decrease, intricate systems inherent in all animal species. The ability to preserve a stable volume despite changes in osmolality of the surrounding environment, is especially important for brain cells with the potentially life-threatening effects of cerebral edema.\(^11\) Studies using magnetic resonance spectroscopy (MRS) have shown that neurons in a chronic state of hypoosmolality protect themselves from swelling by efflux of organic osmolytes (amino acids, polyalcohols, sugars and methylamines), including glutamate, the most important neurotransmitter.\(^12\) Plasma hypoosmolality is also known to increase susceptibility to seizures.\(^13\)

No prior study has used MRS to investigate levels of cerebral organic osmolytes in women with preeclampsia and normal pregnancy. To our knowledge, there are only two publications with brain proton MRS in preeclampsia or eclampsia.\(^14,\)\(^15\) These studies are limited by small sample sizes and the latter focused on post-eclampsia status, rather than the changes leading up to eclampsia.

Using proton MRS (\(^1\)H-MRS) where the spectrum obtained contains most organic osmolytes of interest, such as N-acetyl aspartate, glutamate, myo-inositol, choline, creatine and taurine,
our aim was to compare levels of cerebral organic osmolytes between women with preeclampsia, normal pregnancy, and non-pregnant women and assess if organic osmolytes correlate to plasma osmolality and plasma sodium levels. Our hypotheses were that levels of cerebral organic osmolytes are decreased during pregnancy, that this decrease is more pronounced in preeclampsia and that levels of cerebral organic osmolytes correlate to plasma osmolality and plasma sodium levels.

**Material and Methods**

*Study design and population*

The study participants in this cross-sectional study were recruited in Uppsala, Sweden during 2013-2016. Uppsala University Hospital is a tertiary referral center with approximately 4000 deliveries per year. The study population is in part described previously. Only women with singleton pregnancies and a gestational age between 22+0 and 41+6 according to an early second trimester ultrasound dating were eligible. General exclusion criteria in the present study were chronic hypertension, diabetes mellitus, pre-existing renal disease, or standard contraindications for MRI, e.g. claustrophobia metallic implants or pacemaker.

We recruited three groups of women: women with preeclampsia (n=30), normal pregnant women (n= 32), and non-pregnant women (n=16). Women with preeclampsia were recruited from the obstetric ward or outpatient obstetric clinic. Preeclampsia was defined as de novo hypertension after 20 weeks of gestation in combination with proteinuria, according to the recommendations of the International Society for studies on Hypertension in Pregnancy (ISSHP). Hypertension was defined as systolic blood pressure (BP) of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg measured on two subsequent occasions, at least 6 hours apart, and proteinuria defined as ≥ 2+ on a dipstick or ≥ 300 mg/24 h in a urine collection. Preeclampsia was defined as severe when systolic BP was ≥160 mmHg and/or diastolic BP ≥110 mmHg; or if HELLP-syndrome was present. Women with preeclampsia had to be sufficiently clinically stable to be transported to the MR facility. None of the women were treated with MgSO₄ or had developed eclampsia, neither before inclusion nor after. Every woman diagnosed with preeclampsia, either admitted or monitored in outpatient care, which came to the principal investigator’s (M.N.) knowledge, was approached regarding study participation. Only a small fraction of respondents refrained participation in the study, most often due to claustrophobia. The normal pregnant group was recruited through information posters at antenatal outpatient clinics in Uppsala and at Uppsala University. Women in the normal pregnant group were
matched for gestational age at examination to women in the preeclampsia group. After examination, women in the normal pregnant group were monitored and those who developed preeclampsia, delivered preterm (<37 weeks) or did not deliver an infant of normal birth weight (± 2 standard deviations of the mean birth weight for gestational age and sex) were excluded. The non-pregnant group was recruited through Facebook and local networks and included both nulliparous and parous women. In addition to the general exclusion criteria, women with prior history of preeclampsia or gestational hypertension were not included either in the normal pregnant or non-pregnant study groups.

All participants underwent BP measurement, blood and urine sampling and MRS examination within a 4 hour period. Systolic and diastolic BPs were measured in supine position in the right arm after a 15 min rest. A manual sphygmomanometer (Boso, Germany or WelchAllyn, USA) with appropriate cuff size depending on arm circumference was used. Fresh midstream urine samples were collected and analyzed with a Combur 9 test (Roche) or Clinitek Status Analyzer (Siemens). Blood samples were centrifuged 10 minutes at 1500 g and then immediately stored as aliquots in -70°C until analysis.

*MRS*

MR examinations were performed using a 1.5 T scanner (Achieva, Philips Healthcare, Best, The Netherlands). The whole body coil was used for excitation and an eight-element receiver head coil served for signal reception. T1- and T2-weighted turbo spin echo (TSE) images were obtained in the axial, sagittal, and coronal orientations (spatial resolution 1x1x4 mm³, interslice gap 0 mm). Single-voxel spectra were acquired using the point-resolved spectroscopy (PRESS) sequence (spectral bandwidth 1000 Hz, 1024 points, TR/TE 5000/30 ms, 16 phase cycle steps). Volume of interest (voxel) with a size of 20×20×20 mm³ was positioned in the posterior midline, at the junction between the parietal and occipital lobes (figure 1). The position was chosen to include areas mostly affected when vasogenic edema is found in eclampsia and severe preeclampsia. Magnetic field homogeneity was improved by iterative first-order shimming. Sixteen non-water-suppressed and 128 water-suppressed scans were performed in consecutive acquisition in 12 minutes. Intensities of spectral lines were estimated by fitting using the LCModel. Levels of the following 1H-metabolites were measured: n-acetyl-aspartate (NAA), choline, creatine, glutamate, myo-inositol and taurine. Quantitation error of each metabolite concentration estimate was assessed from the standard deviation (Cramér-Rao Lower Bound - CRLB) expressed in percent of the estimated
concentration. CRLB below 20% is commonly used as a criterion for estimates of acceptable reliability.

**Figure 1.** T₂- (left) and T₁- weighted (right) turbo spin echo images with the typical voxel position in the posterior midline at the parieto-occipital fissure.

*Plasma samples*

Plasma analyzes were routine tests and performed at the accredited laboratory in the department of Clinical Chemistry and Pharmacology, Uppsala University Hospital. Plasma osmolality was assessed using a Fiske Osmometer, model 210. Plasma sodium was analyzed on an Architect c16000 with a reagent from Abbott Laboratories. Plasma glutamate was analyzed with a glutamate assay kit from Sigma-Aldrich (MAK004).

*Statistics*

Clinical characteristics, median levels of cerebral ¹H-metabolites, plasma osmolality and plasma levels of glutamate and sodium were compared between the women with preeclampsia, women with normal pregnancies and non-pregnant women by Kruskal-Wallis test. Pair-wise comparisons between the study groups were made with Mann-Whitney U test. Adjustment for maternal age and BMI was made with ANCOVA after testing for adequate normal distribution for the variables.

Correlations between the different cerebral osmolytes and between cerebral osmolytes and plasma variables were assessed with Spearman’s correlation test.

All significance tests were two-tailed. P<0.05 was considered to denote a statistically significant difference. Analyses were performed using IBM SPSS Statistics 24 (IBM SPSS, Inc., Chicago, IL).
**Ethics**

The study was approved by the Regional Ethical Review Board in Uppsala, Sweden, and informed consent was obtained from each woman participating in the study.

**Results**

Of the 78 women included in the study, three were excluded; one woman with preeclampsia could not complete the $^1$H-MRS due to discomfort, and two women in the normal pregnancy group later developed preeclampsia. Thus, the final analysis was based on the spectra of 75 women (29 women with preeclampsia, 30 with normal pregnancies, and 16 non-pregnant women). Further, the levels of taurine could not be calculated in eight women with normal pregnancies and in one non-pregnant woman. For cerebral glutamate, two outliers were excluded, one in the preeclampsia group and one in the normal pregnant group.

Table 1 depicts the clinical characteristics of the study population. There were significant differences between study groups for age, BMI and parity. Among the women with preeclampsia, ten had developed severe preeclampsia at the time of examination, with a recorded SBP ≥160 and/or DBP ≥110\textsuperscript{18}. Anti-hypertensive treatment, usually monotherapy with labetalol, was used by 23 (79\%) women with preeclampsia. There was no difference in gestational age at the time of the MRI scanning between women with preeclampsia and those with a normal pregnancy.
Table 1. Clinical characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia (n=29)</th>
<th>Normal pregnant (n=30)</th>
<th>Non-pregnant (n=16)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>27 (7)</td>
<td>33 (6)</td>
<td>27 (12)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>26 (6)</td>
<td>24 (5)</td>
<td>22 (5)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Prior births, n (%)</strong></td>
<td>5 (17)</td>
<td>20 (67)</td>
<td>7 (44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>At examination:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age, days</strong></td>
<td>246 (55)</td>
<td>246 (74)</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Blood pressure, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>150 (20)</td>
<td>110 (10)</td>
<td>110 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95 (13)</td>
<td>70 (13)</td>
<td>70 (5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a p-value according to Kruskal-Wallis, comparison of all groups.

Data are presented as median (inter-quartile range)

BMI, Body mass index, measured in the first trimester in the two pregnant study groups

Cerebral levels of \(^1\)H-metabolites in each study group are depicted in figures 2A-F. Normal pregnancy was characterized by lower cerebral levels of myo-inositol, choline, and creatine (p=0.001 or less), whereas cerebral levels of NAA, taurine and glutamate did not differ from the non-pregnant women. These results remained after adjustment for maternal age and BMI. Women with preeclampsia had lower cerebral glutamate levels, both in comparison with women with normal pregnancies and in comparison with the non-pregnant controls (p=0.01 vs 0.001). Cerebral levels of myo-inositol, choline and creatine were lower in women with preeclampsia than in the non-pregnant women (p<0.001), but did not differ from women with healthy pregnancies, figure 2. These results also remained significant after adjustment for age and BMI. CRLB was lower than 20% for all metabolites except taurine.

Plasma levels of organic osmolytes are demonstrated in Table 2. Women with preeclampsia had higher levels of plasma glutamate in comparison with women with normal pregnancies and non-pregnant women (p<0.001, respectively). This difference persisted after adjustment for maternal age and BMI. No difference in plasma glutamate levels was noted between women with normal pregnancy and non-pregnant women. Compared to the non-pregnant group, the two pregnant groups had lower osmolality and plasma sodium levels (p<0.001).
Compared to normal pregnancy, women with preeclampsia had higher osmolality, but lower levels of sodium (p=0.02 and p=0.04, respectively). There were no differences in levels of osmolytes between those who were on antihypertensive treatment and those who did not receive medication.

Figure 2 A-F: Boxplots of $^1$H-MRS metabolites in women with preeclampsia (n = 29), normal pregnancies (n = 30) and in non-pregnant women (n = 16).

Table 2. Plasma levels of glutamate, osmolality and sodium

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia (n=29)</th>
<th>Normal pregnant (n=30)</th>
<th>Non-pregnant (n=16)</th>
<th>p-value$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate (mmol/L)</td>
<td>0.22 (0.07)$^{b,d}$</td>
<td>0.17 (0.08)</td>
<td>0.15 (0.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osmolality (osm/kg)</td>
<td>289 (6.5)$^{a,c}$</td>
<td>285 (7)$^d$</td>
<td>292 (2.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>138 (1.5)$^{a,d}$</td>
<td>139 (4)$^d$</td>
<td>146 (4.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$ p < 0.05 compared to normal pregnant, Mann-Whitney U-test

$^b$ p < 0.001 compared to normal pregnant, Mann-Whitney U-test

$^c$ p <0.05 compared to non-pregnant, Mann-Whitney U-test
The results of the correlation analyses, based on all three study groups, are presented in Table 3. There were significant correlations between cerebral levels of the osmolytes glutamate, myo-inositol, choline and creatine. The cerebral levels of taurine correlated with levels of choline, but not to the other cerebral osmolytes. There was a significant negative correlation between cerebral and plasma glutamate levels. Plasma osmolality correlated with cerebral levels of myo-inositol, choline and creatine, but not with cerebral levels of glutamate and taurine. The plasma levels of sodium correlated with plasma osmolality, plasma glutamate, and all cerebral metabolites except taurine.

**Table 3.** Correlations (Spearman) between the cerebral levels of the organic osmolytes glutamate, myo-inositol, choline, and creatine and the plasma (P) levels of glutamate, osmolality and sodium for all three study groups.

<table>
<thead>
<tr>
<th></th>
<th>Myo-Inositol</th>
<th>Choline</th>
<th>Creatine</th>
<th>Taurine</th>
<th>P-Glutamate</th>
<th>P-Osmolality</th>
<th>P-Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11</td>
<td>-0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
<td>0.22</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>-</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08</td>
<td>-0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Choline</td>
<td>-</td>
<td>-</td>
<td>0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27</td>
<td>-0.18</td>
<td>0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
<td>0.16</td>
<td>0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Taurine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>P-Glutamate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-0.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-Osmolality</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.001  
<sup>b</sup> p < 0.01  
<sup>c</sup> p < 0.05
Discussion

Main Findings:

This is to our awareness the first report of cerebral osmolytes in pregnancy and in women with preeclampsia. Our findings indicate that pregnant women have lower levels of cerebral osmolytes than non-pregnant women. Preeclampsia is further characterized by reduced levels of glutamate, which in turn acts both as an osmolyte and as an excitatory neurotransmitter.

It has been well established for decades that plasma osmolality and plasma sodium in pregnancy are significantly reduced,\(^2\) and in our study we expand this knowledge by also demonstrating that the levels of cerebral organic osmolytes are correlated with plasma osmolality and sodium levels in pregnancy. Ultimately, this finding suggests that altered circulating fluid metabolism is the underlying mechanism to the reduced levels of cerebral osmolytes we report.

Strengths and limitations

Strengths of our study are the usage of an advanced technique familiar in other research areas, but innovative in pregnancy, the relatively large study population and the standardized protocols for collection of BP and plasma samples in close proximity with the \(^1\)H-MRS examination. The region of interest was positioned over both parietal and occipital lobes, areas mostly affected when vasogenic edema is found in eclampsia and severe preeclampsia.\(^2\) However, we have only measured in one voxel, and there might be regional differences in levels of the osmolytes, which we have failed to register. The voxel was positioned so as to avoid cerebrospinal fluid and vessels, but the MRS technique cannot separate the contributions from intracellular and interstitial compartments, which would have been of interest. Imaging at higher field strength (3 Tesla instead of 1.5 Tesla) would improve signal-to-noise ratio and spectral resolution, but there is at present no consensus regarding its safe use during pregnancy.

When presenting data from \(^1\)H-MRS it has been customary to use a metabolite concentration ratio to creatine, a metabolite considered stable. This is to avoid partial volume effects due to contamination of metabolite free cerebrospinal fluid. Since we detect differences in creatine levels as a part of the same biological phenomenon, a ratio against creatine would be misleading in our case. Since we did not perform correction for partial volume effect of cerebrospinal fluid, our metabolite concentrations could be underestimated.

Interpretations:
This is the first study using \(^1\)H-MRS to measure cerebral organic osmolytes in pregnancy and preeclampsia in women. This technique has, however, been previously used to examine the maternal human brain in a few publications.\(^{14, 15}\) In a study of ten women with eclampsia, studied 3-5 days after their seizures, a decreased NAA peak in comparison to healthy age-matched non-pregnant women was reported.\(^{15}\) A more recent publication reported normal NAA levels in women with preeclampsia, which we also did.\(^{14}\) This study by Rutherford et al. found lower concentrations of choline and creatine in pregnancy, parallel to our results. They further found that choline was significantly higher in preeclampsia compared to normal pregnancy, a finding we came close to replicate, since the unadjusted p-value is 0.056 in our data. Their interpretation of this result is that it reflects an ischemia in preeclampsia since higher choline is also found in patients with unilateral severe carotid stenosis.\(^{22}\) In our study we could further show decreased levels of cerebral glutamate and myo-inositol levels, compounds not reported from earlier \(^1\)H-MRS examinations in pregnancy. Glutamate, myo-inositol, choline and creatine are all described as cerebral organic osmolytes\(^{11}\) and has been shown to decrease in conditions with hyponatremia.\(^{12, 23, 24}\) This is called regulatory volume decrease and is part of the mechanisms by which cells defend themselves from edema when extracellular osmolality decreases.\(^{11}\) In cases of acute hyponatremia, water enters the cells by osmotic forces, but when the decline is slower, the cells defend themselves, firstly by extrusion of electrolytes. Since altered ion gradients can affect cellular functions, a more stable and long-lasting solution is the efflux of organic osmolytes.\(^{25}\) Our study is the first in humans and pregnancy, but decreased levels of organic osmolytes has previously been demonstrated in pregnant rats.\(^{26}\)

We demonstrated lower cerebral levels of glutamate, myo-inositol, choline and creatine in both women with preeclampsia and women with normal pregnancies. All four metabolites correlated with each other, indicating that their decline was part of the same biological mechanism (Table 3). For myo-inositol, choline and creatine there was a clear correlation with plasma osmolality and sodium. This strengthens the argument that the observed decrease in these compounds is indeed a response to the lower plasma osmolality associated with pregnancy. Cerebral levels of glutamate was, however, not correlated with either plasma osmolality or sodium. If this is due to a separate biological mechanism or simply caused by a lack of statistical power, remains to be explained.
Interestingly, glutamate i.e. the most common excitatory neurotransmitter in the brain, was lower in women with preeclampsia than in women with normal pregnancies. This is an intriguing finding considering the incompletely chartered pathophysiology of seizures in preeclampsia. There has been speculation that extrusion of glutamate from cells raise extracellular levels of the neurotransmitter and thereby causing hyperexcitability. Plasma levels of glutamate also differed between women with preeclampsia and normal pregnancy, with the highest concentrations seen in preeclampsia, in line with findings from previous studies. There is evidence that plasma glutamate levels negatively correlates with worse outcomes in stroke and traumatic brain injury. Glutamate can also be released from aggregating platelets and polymorphonuclear leukocytes, which may influence permeability of endothelial cells, including brain endothelium, and pregnancy alters expression of certain glutamate receptors on peripheral blood mononuclear cells. An increased endothelial permeability has been proposed to play a crucial role in the pathophysiology of posterior reversible encephalopathy syndrome, a clinical-radiological entity seen in eclampsia and severe preeclampsia.

Although our results with lower cerebral glutamate, myo-inositol, choline and creatine are consistent with what has previously been shown in hyponatremia our detected differences in cerebral organic osmolytes are small and the clinical relevance is not clear. In the previous studies, myo-inositol was 40-49% lower as compared to 20% in our population. All women in our study had plasma osmolality and sodium levels within normal range, although the pregnant women had lower levels.

It remains to be established if and how these reductions in cerebral organic osmolytes contribute to the hyperexcitability observed in pregnancy, especially preeclampsia, and if these changes can explain how eclamptic seizures can arise without any prodromal symptoms or hypertension.

**Conclusions:**

With the use of $^1$H-MRS, we have found lower levels of cerebral organic osmolytes in pregnancy and preeclampsia compared to non-pregnant women. This correlates to plasma osmolality and the finding that even the relatively small decrease in plasma osmolality seen in pregnancy is associated with a measureable decrease in cerebral osmolytes is intriguing. This adds to our knowledge of cerebral adaptations in pregnancy, but if and how, lowering of cerebral organic osmolytes contributes to hyperexcitability and seizures remains to be established.
**Disclosure:** The authors report no conflict of interests.

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References:


Assessment of cerebral perfusion and edema in preeclampsia with intravoxel incoherent motion MRI

Running title: Cerebral perfusion and edema in preeclampsia

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Abstract

Background

Cerebral complications are the main reasons for morbidity and mortality in preeclampsia and eclampsia. Still we do not know if the pathophysiology entails hypo- or hyperperfusion of the brain, or how and when edema emerges, due to the difficulty to examine the cerebral circulation.

Material and methods

We have used a non-invasive diffusion weighted magnetic resonance imaging (MRI) technique, intravoxel incoherent motion, to study cerebral perfusion on the capillary level and cerebral edema in women with preeclampsia (n=30), normal pregnancy (n=32) and non-pregnant women (n=16). Estimates of cerebral blood volume, blood flow and edema were measured in five different regions. These points were chosen to represent blood supply areas of both the carotid and vertebrobasilar arteries, and to include both white and grey matter.

Results

Except for the caudate nucleus, we did not detect any differences in cerebral perfusion measures on a group level. In the caudate nucleus we found lower cerebral blood volume and lower blood flow in preeclampsia compared to both normal pregnancy (p=0.01 and p=0.03, respectively) and non-pregnant women (both p=0.02). No differences in edema were detected between study groups.

Conclusion

The cerebral perfusion measures were comparable between the study groups, except for a portion of the basal ganglia where hypoperfusion was detected in preeclampsia compared to normal pregnancy and non-pregnant women.

Key words: Cerebral circulation, Edema, Eclampsia, Intravoxel incoherent motion, Magnetic Resonance Imaging, Perfusion, Preeclampsia.
**Abbreviations:**

BP Blood pressure  
DWI Diffusion weighted imaging  
IVIM Intravoxel incoherent motion MRI  
MRI Magnetic resonance imaging  
PRES Posterior reversible encephalopathy syndrome

**Key message:**

Cerebral perfusion and edema measures obtained by IVIM MRI were comparable between women with preeclampsia, normal pregnancy and non-pregnant women, except in a portion of the basal ganglia where hypoperfusion was seen in preeclampsia compared to both study groups.
Introduction

Cerebral complications are responsible for the great majority of maternal deaths due to preeclampsia (1) but our knowledge is still scarce regarding the pathophysiology. A majority of women with eclampsia and some women with preeclampsia, present with signs of the posterior reversible encephalopathy syndrome, PRES (2, 3). This is a neuroradiological entity defined by subcortical edema on magnetic resonance imaging (MRI) or computed tomography (CT), predominantly found in the parietal and occipital lobes of the brain, and associated with clinical symptoms of headache, nausea, visual disturbances and seizures (4). PRES can arise in as diverse conditions as hypertensive disorders, autoimmune disease, chemotherapy and sepsis, among others (5, 6). If the edema is due to hypoperfusion and resulting ischemia or hyperperfusion due to a defect autoregulation is not fully clarified (7).

The limitations in our ability to non-invasively study the cerebral circulation in pregnancy and preeclampsia attributes to this gap in knowledge. The most commonly used method is transcranial Doppler ultrasound (TCD), where perfusion assessment is based on the velocity in a large artery, most often the middle cerebral artery. The validity of this technique has been questioned since a number of assumptions have to be made (8). Since TCD can only be used for major arteries, this may also not reflect how the peripheral circulation in the brain is affected by preeclampsia.

MRI enables the acquisition of both morphological and physiological information. Tissue perfusion may be estimated with several different MRI techniques. Most commonly, perfusion MRI utilizes the administration of a gadolinium contrast agent, though this is not possible to use in pregnant women due to concerns of accumulation in the fetus. Intravoxel incoherent motion (IVIM) is a non-invasive MRI technique for assessment of perfusion fraction and edema. IVIM enables estimation of blood perfusion at the capillary level, without contrast agent administration. The technique is based on the separation of true diffusion from perfusion related “pseudodiffusion” using a diffusion weighted imaging (DWI) sequence with multiple b-values (9). It also yields a diffusion measure (D), indicative of edema. Our group has used IVIM to describe alterations in placental perfusion in preeclampsia, indicating different pathophysiology for early and late onset preeclampsia (10). There are also documented applications in the brain, e.g. in stroke and brain tumors (11). To our knowledge, no prior study has used IVIM to investigate the cerebral circulation in preeclampsia.
The aim of this study was to estimate if women with preeclampsia have an affected cerebral perfusion or signs of edema in comparison with normal pregnant and non-pregnant women, using IVIM MRI.

**Material and methods**

**Study design and population**

The study participants for this cross-sectional study were recruited at the Department of Obstetrics and Gynecology, Uppsala University Hospital, Sweden during 2013-2016. Uppsala University Hospital is a tertiary referral center with approximately 4 000 deliveries per year. General exclusion criteria for cases and controls were chronic hypertension, diabetes mellitus (both pre-pregnancy and gestational), pre-existing renal disease or contraindications for MRI, e.g. claustrophobia or pacemaker. Only singleton pregnancies with a gestational age between 22+0 and 41+6 were included. In addition to the general exclusion criteria, women with a prior history of preeclampsia or gestational hypertension were not included either in the normal pregnant or non-pregnant study groups.

We recruited three groups of women: women with preeclampsia (n=30), normal pregnant women (n=32), and non-pregnant women (n=16). Women with preeclampsia were recruited from the obstetric ward or the obstetric outpatient clinic. Preeclampsia was defined as *de novo* hypertension after 20 weeks of gestation in combination with proteinuria. Hypertension was defined as systolic blood pressure (BP) of ≥140 mmHg and/or diastolic BP of ≥90 mmHg measured on two subsequent occasions with at least six hours apart and proteinuria as ≥2+ on a dipstick or ≥300 mg/24 hours in a urine collection. Preeclampsia was defined as severe, when BP was ≥160 mmHg systolic and/or ≥110 mmHg diastolic; or if HELLP-syndrome was present (12). Women with preeclampsia had to be sufficiently clinically stable to be transported to the MR facility. None of the women were treated with MgSO₄ or had developed eclampsia, neither before inclusion nor after. Every woman diagnosed with preeclampsia, either admitted or monitored in outpatient care, who came to the principal investigator’s (M.N.) knowledge, was approached regarding study participation. Only a small fraction of respondents declined participation in the study, most often due to fear of claustrophobia. The normal pregnant group was recruited through information posters at antenatal outpatient clinics in Uppsala and at
Uppsala University. Women in the normal pregnant group were matched for gestational age at examination to women in the preeclampsia group. After examination, women in the normal pregnant group were monitored and those who developed preeclampsia, delivered preterm (<37 weeks) or did not deliver an infant of normal birth weight (±2 standard deviations of the mean birth weight for gestational age and sex (13)) were excluded. The non-pregnant group was recruited through Facebook and local networks and included both nulliparous and parous women.

All participants underwent blood pressure measurement (BP), urine sampling and MRI examination within 12 hours after enrollment in the study. The BP recording closest to the MRI examination was used (range 0.25-4 h). Systolic and diastolic BPs were measured in supine position in the right arm after a 15-minute rest. A manual sphygmomanometer (Boso, Germany or WelchAllyn, USA) with appropriate cuff size depending on arm circumference was used. Fresh midstream urine samples were collected and analyzed with a Combur 9 test (Roche) or Clinitek Status Analyzer (Siemens). Directly prior to the MRI, all subjects were interviewed about cerebral symptoms. The interview included detailed questions on headache and visual disturbances, including scotomas, blurred vision and diplopia occurring in the last three days.

MRI

All study participants were scanned on a 1.5 Tesla MR system (Achieva, Philips Healthcare, Best, The Netherlands) using an eight channel receiver head-coil. Diffusion weighted images were measured by a spin-echo sequence with echo-planar read-out. Diffusion gradients were applied in three directions with multiple diffusion sensitizing values (b = 0, 50, 100, 150, 200, 400, 600, and 800 s/mm²). Images were acquired in the axial plane with a slice thickness of 4 mm and an inter-slice gap of 0.4 mm. The IVIM parameters f, D*, and D were at a later point estimated using bi-exponential fitting using Olea Sphere 3.0 (Olea Medical Solutions, France). These measures can be translated to standard perfusion parameters as presented in Table 1 (11, 14). Regions of interest were manually drawn in a slice at the level of the lateral ventricles in the following regions: the bilateral frontal white matter (including genu corpus callosum), bilateral parieto-occipital white matter (including splenium corpus callosum), and the unilateral thalamus, caudate and lentiform nuclei. These points were chosen to represent blood supply areas of both the carotid and vertebrobasilar arteries and to include both white and grey matter. All IVIM-parameters were estimated by one observer, blinded to which study group the woman belonged to.
Table 1. Translation of IVIM parameters to standard perfusion parameters and edema.

<table>
<thead>
<tr>
<th>IVIM parameter</th>
<th>Unit</th>
<th>Corresponding perfusion/edema parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion fraction f</td>
<td>Unitless</td>
<td>Cerebral blood volume</td>
</tr>
<tr>
<td>True diffusion coeff</td>
<td>$10^{-3}$ mm$^2$/s</td>
<td>Extracellular edema</td>
</tr>
<tr>
<td>Pseudo-diffusion coeff $D^*$</td>
<td>$10^{-3}$ mm$^2$/s</td>
<td>Mean transit time$^{-1}$</td>
</tr>
<tr>
<td>fD$^*$</td>
<td>$10^{-3}$ mm$^2$/s</td>
<td>Cerebral blood flow</td>
</tr>
</tbody>
</table>

Statistics

Comparisons at the group level (preeclampsia, normal pregnant and non-pregnant women) were tested with Kruskal-Wallis followed by pairwise Mann-Whitney U-tests. Chi square test ($\chi^2$) was used to compare proportions. Differences between grey and white matter were assessed with Wilcoxon signed rank’s test. Correlations were assessed with Spearman’s correlation test. All significance tests were two-tailed and a p-value $<$0.05 was considered to denote a statistically significant difference. Analyses were performed using IBM SPSS Statistics 24 (IBM SPSS, Inc., Chicago, IL). No correction for multiple comparisons was performed. The rationale for this was to avoid type II errors and also the fact that the measurements in the different brain areas cannot be regarded to be independent from each other.

Ethics

Approved by the Regional Ethical Review Board in Uppsala, Sweden: Reference number: 2012-087, May 23rd 2012. Informed consent was obtained from each woman participating in the study.
Results

We included a total of 78 women. One woman in the preeclampsia group discontinued the MRI due to discomfort. Two women in the normal pregnant control group developed preeclampsia after their participation in the study and were excluded. Due to technical reasons, no perfusion fraction results were obtained in one normal pregnant woman and two non-pregnant women. Thus, the final analysis was based on readings from 72 women (29 with preeclampsia, 29 normal pregnant and 14 non-pregnant).

Table 2 presents the clinical characteristics of the study population. Women with normal pregnancies were older than those with preeclampsia or non-pregnant women. Body mass index (BMI) in early pregnancy was higher in women with preeclampsia compared to both normal pregnant and non-pregnant women (p=0.03 and <0.001 respectively). As expected, the proportion of primiparas was high in the preeclampsia group (83%). In contrast, in our normal pregnant group only 35% of women were primiparas.

Table 2. Clinical characteristics of the women

<table>
<thead>
<tr>
<th>Characteristics of the women</th>
<th>Preeclampsia (n=29)</th>
<th>Normal pregnant (n=29)</th>
<th>Non-pregnant (n=14)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>27 (23-43)</td>
<td>33 (23-45)</td>
<td>27 (21-40)</td>
<td>0.025</td>
</tr>
<tr>
<td>Early pregnancy BMI (kg/m²)</td>
<td>26 (20-44)</td>
<td>24 (20-29)</td>
<td>22 (18-26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Primiparas</td>
<td>24 (83%)</td>
<td>10 (25%)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>At inclusion:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>35.1 (24.1-41.1)</td>
<td>35.4 (23.6-39.1)</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>150 (130-170)</td>
<td>110 (100-135)</td>
<td>110 (100-120)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95 (60-110)</td>
<td>70 (55-80)</td>
<td>70 (60-75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>113 (88-128)</td>
<td>83 (70-98)</td>
<td>83 (73-90)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Numbers are presented as median (range) or number (%)
BMI; body mass index.

* p-value according to Kruskal-Wallis, comparison of all groups.
The clinical characteristics of the women with preeclampsia are presented in Table 3. Women with severe preeclampsia were examined at an earlier time-point in pregnancy and had higher systolic and diastolic blood pressures than women with moderate preeclampsia. However, women with severe and moderate preeclampsia did not differ in terms of antihypertensive treatment or cerebral symptoms.

Table 3. Characterization of the preeclampsia group (n= 29)

<table>
<thead>
<tr>
<th></th>
<th>Severe preeclampsia (n= 10)</th>
<th>Moderate preeclampsia (n= 19)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of preeclampsia, weeks</td>
<td>31.0 (22.3 - 36.1)</td>
<td>36.1 (25.3 - 41.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Gestational age at delivery, weeks</td>
<td>32.6 (24.6-37.3)</td>
<td>37.3 (26.0-41.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labetalol only</td>
<td>7 (70)</td>
<td>13 (68)</td>
<td>0.20</td>
</tr>
<tr>
<td>Labetalol and Nifedepin</td>
<td>2 (20)</td>
<td>1 (5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Cerebral symptoms a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>6 (60)</td>
<td>12 (63)</td>
<td>0.87</td>
</tr>
<tr>
<td>Visual disturbance</td>
<td>3 (30)</td>
<td>8 (42)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Numbers are presented as median (range) or number (%)

a For the last three days before the MRI examination

* p-value according to Mann-Whitney U-test.

To validate our method, we pooled the results from all regions with grey and white matter respectively from the study population. As expected, higher levels of perfusion-related IVIM parameters (f, D* and fD*) were found in grey compared to white matter. (All p<0.001. Supplementary table 1).

Perfusion-related IVIM parameters across the five brain regions-of-interest (parietal-occipital white matter, frontal white matter, thalamus, caudate nucleus and lentiform nucleus) are presented in table 4.
Table 4. Median values of IVIM measures for cerebral blood volume (f), edema (D), mean transit time\(^{-1}\) (D*) and cerebral blood flow (fD*) by cerebral regions and study group.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia (n=29)</th>
<th>Normal pregnant (n=29)</th>
<th>Non-pregnant (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parieto-occipital WM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.93</td>
</tr>
<tr>
<td>D (10^{-3} mm(^2)/s)</td>
<td>0.77 (0.04)</td>
<td>0.77 (0.05)</td>
<td>0.77 (0.04)</td>
<td>0.80</td>
</tr>
<tr>
<td>D* (10^{-3} mm(^2)/s)</td>
<td>32.16 (2.83)</td>
<td>32.19 (2.23)</td>
<td>32.33 (1.37)</td>
<td>0.86</td>
</tr>
<tr>
<td>fD* (10^{-3} mm(^2)/s)</td>
<td>1.06 (0.28)</td>
<td>1.04 (0.28)</td>
<td>1.04 (0.31)</td>
<td>0.94</td>
</tr>
<tr>
<td>Frontal WM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0)</td>
<td>0.26</td>
</tr>
<tr>
<td>D (10^{-3} mm(^2)/s)</td>
<td>0.78 (0.04)</td>
<td>0.78 (0.05)</td>
<td>0.77 (0.04)</td>
<td>0.26</td>
</tr>
<tr>
<td>D* (10^{-3} mm(^2)/s)</td>
<td>32.7 (3.33)</td>
<td>31.26 (2.88)</td>
<td>32.31 (1.65)</td>
<td>0.18</td>
</tr>
<tr>
<td>fD* (10^{-3} mm(^2)/s)</td>
<td>1.00 (0.31)</td>
<td>1.04 (0.30)</td>
<td>0.98 (0.12)</td>
<td>0.77</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.04 (0.02)</td>
<td>0.04 (0.01)</td>
<td>0.035 (0.01)</td>
<td>0.71</td>
</tr>
<tr>
<td>D (10^{-3} mm(^2)/s)</td>
<td>0.71 (0.02)</td>
<td>0.71 (0.02)</td>
<td>0.71 (0.03)</td>
<td>0.85</td>
</tr>
<tr>
<td>D* (10^{-3} mm(^2)/s)</td>
<td>36.4 (3.32)</td>
<td>36.93 (3.29)</td>
<td>36.18 (1.81)</td>
<td>1.00</td>
</tr>
<tr>
<td>fD* (10^{-3} mm(^2)/s)</td>
<td>1.24 (0.45)</td>
<td>1.39 (0.35)</td>
<td>1.27 (0.35)</td>
<td>0.56</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.03 (0.02)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>D (10^{-3} mm(^2)/s)</td>
<td>0.73 (0.04)</td>
<td>0.73 (0.02)</td>
<td>0.73 (0.02)</td>
<td>0.86</td>
</tr>
<tr>
<td>D* (10^{-3} mm(^2)/s)</td>
<td>36.69 (6.38)</td>
<td>35.21 (5.37)</td>
<td>35.48 (2.60)</td>
<td>0.69</td>
</tr>
<tr>
<td>fD* (10^{-3} mm(^2)/s)</td>
<td>1.01 (0.48)</td>
<td>1.17 (0.51)</td>
<td>1.23 (0.34)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>0.05 (0.02)</td>
<td>0.05 (0.02)</td>
<td>0.05 (0.01)</td>
<td>0.46</td>
</tr>
<tr>
<td>D (10^{-3} mm(^2)/s)</td>
<td>0.71 (0.04)</td>
<td>0.69 (0.03)</td>
<td>0.70 (0.05)</td>
<td>0.23</td>
</tr>
<tr>
<td>D* (10^{-3} mm(^2)/s)</td>
<td>37.53 (5.22)</td>
<td>36.47 (3.67)</td>
<td>37.43 (5.61)</td>
<td>0.37</td>
</tr>
<tr>
<td>fD* (10^{-3} mm(^2)/s)</td>
<td>1.72 (0.53)</td>
<td>1.72 (0.72)</td>
<td>1.64 (0.49)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Numbers are presented as median (interquartile) range.
WM; White matter
* p-value according to Kruskal Wallis, comparison of all groups.

The cerebral perfusion measures (f and fD*) differed between study groups in caudate nucleus (both p= 0.02). Women with preeclampsia had lower cerebral blood volume measure (f) in the caudate nucleus in comparison with both normal pregnant and non-pregnant women (p=0.01 and p=0.03, respectively) (Fig 1A). Additionally, the blood flow measure (fD*) was lower in the caudate nucleus in preeclampsia compared to normal pregnant and non-pregnant women.
(both \( p=0.02 \)) (Fig 1B). No other differences in perfusion-related parameters were observed across the tested brain regions and groups, and there was no evidence of cerebral edema in pregnant women or women with preeclampsia.

When comparing women with severe and moderate preeclampsia, there were no significant differences in perfusion parameters. Further, no difference in perfusion parameters was noted between women with or without antihypertensive treatment, and no correlation between perfusion parameters and cerebral symptoms was found.
Discussion

This is, to our knowledge, the first report using IVIM to study cerebral perfusion and edema in preeclampsia. In four out of five areas examined all IVIM-parameters were similar between study groups. In the fifth, a part of the basal ganglia, we found lower cerebral blood volume (f) and blood flow (fD*) measures in preeclampsia compared to normal pregnant and non-pregnant women. Importantly, we found no evidence of cerebral edema (D) in preeclampsia in our population.

Although PRES has been described since 1996 (4) and has been accepted as part of the pathophysiology of eclampsia (2), it is still unclear how the typical radiological findings of subcortical edema arise. One theory describes a breakdown of cerebral autoregulation due to hypertension resulting in hyperperfusion and vascular edema. An opposing model includes hypoperfusion with ischemia and edema as a result (7). In support of the former theory Zeeman et al measured the blood flow in the main cerebral arteries and found higher velocities in the preeclamptic patients (15). Hyperperfusion was also demonstrated in a study using both CT, MRI and single photon emission computed tomography (16). But since most patients with subsequent eclampsia are either normotensive or have a mean arterial pressure far from exceeding the upper limits of autoregulation prior to seizure, the rationale of the hyperperfusion theory can be questioned. Several MRI studies have also found lower relative cerebral blood flow and significant decrease in cerebral blood flow and volume to back up the alternative etiology for PRES with hypoperfusion as a starting point (17-19).

We included measurements from the parieto-occipital white matter, a region most often affected in PRES (20), but found no evidence of perfusion disturbances in our women with preeclampsia. The only area where groups did differ was, more surprisingly, in the caudate nucleus, which is a component of the basal ganglia. There are some reports on involvement of this area in PRES and it seems more common in eclampsia/preeclampsia (21, 22). The reason remains unclear but perhaps the anatomy of the microvasculature of the basal ganglia with a higher number of non-anastomotic vessels is part of the explanation (22). However, we also present data from the lentiform nucleus, another basal ganglia component, where all groups had comparable results. Thus, our data include multiple comparisons and it cannot be excluded that the observed difference is a type I error.

In PRES the subcortical edema that is the radiological hallmark, has been revealed as predominantly vasogenic by DWI (23). However in a recent study as many as one third had a
cytotoxic edema in addition, a finding which indicates a greater risk of irreversible neurological sequelae (3, 24). How the edema develops and its place in the timeline towards eclampsia is not clear. The endothelial dysfunction seen in preeclampsia is thought to increase the propensity of edema, which is confirmed in animal studies (25, 26). In our study we could not detect any differences in the true diffusion coefficient (D) between groups, although a third of the women with preeclampsia had a severe disease at examination and two thirds had cerebral symptoms with either headache, or visual disturbances, or both.

The method used in this study is both a strength and a limitation. The IVIM technique provides a non-invasive, contrast agent-free method to study the perfusion at the capillary level and has been used for assessment of cerebral perfusion (14, 27, 28). Some concerns have been raised, however, about the robustness of the perfusion indexes measured by IVIM compared with a more established method such as dynamic susceptibility contrast (29). We do find larger estimates of blood volume (f) and blood flow (fD*) measures in grey matter than in white, which is expected and which strengthens the credibility of our results (30), although differences in angioarchitecture between grey and white matter may also contribute to the observed difference in blood flow when using the IVIM technique (14).

Our cohort is comparable in size with other studies on cerebrovascular changes in pregnancy and preeclampsia and is well characterized with information on neurological symptoms. Although one third of women with preeclampsia had by definition a severe disease when MRI was performed, none developed eclampsia before or after examination. Further, at examination the women had to be sufficiently clinically stable to be transported to the MR facility and undergo the one-hour examination. Women with severe neurological symptoms could therefore not be included. None of the study participants consequently was treated with MgSO4. This indicates a low central nervous system involvement with presumably small detectable differences in cerebral perfusion between study groups.

In this cross sectional study we used IVIM MRI to estimate differences in perfusion and edema between women with preeclampsia, normal pregnancy and non-pregnant women. In conclusion, our results show no evidence of hyperperfusion, but some evidence of hypoperfusion in parts of the basal ganglia in the preeclampsia group compared to both normal pregnancy and non-pregnant women. We found no differences in the amount of edema between the study groups.
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**Supplementary table 1.** IVIM measures of cerebral blood volume (f), edema (D), mean transit time\(^{-1}\) (D*) and cerebral blood flow (fD*) in grey and white matter

<table>
<thead>
<tr>
<th></th>
<th>White matter</th>
<th>Grey matter</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(f)</td>
<td>0.035±0.01</td>
<td>0.04±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(D (10^{-3} \text{ mm}^2/\text{s}))</td>
<td>0.78±0.03</td>
<td>0.71±0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(D^* (10^{-3} \text{ mm}^2/\text{s}))</td>
<td>32.22±2.53</td>
<td>36.08±2.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(fD^* (10^{-3} \text{ mm}^2/\text{s}))</td>
<td>1.06±0.17</td>
<td>1.39±0.30</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Numbers are presented as median ± interquartile range
* p-value according to the Wilcoxon’s signed rank test
References:


