Diplomarbeit

Preeclampsia – monitoring of hemodynamic parameter and vasoactive substances (ADMA, SDMA and Endothelin-1)

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Graz, am 13. April 2015

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Graz, am 13. April 2015 *Thomas M. Weber*

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Zusammenfassung

Präeklampsie – Beobachtung von hämodynamischen Parametern und vasoaktiven Substanzen (ADMA, SDMA und Endothelin-1)

Hintergrund und Ziele

Präeklampsie ist eine multifaktorielle Krankheit mit einer derzeit noch weitgehend unbekannten Pathophysiologie. Weitreichende pathologische Vorgänge dürften für die Entstehung der Hypertonie verantwortlich sein. Die beeinträchtigte autonome Kontrolle des kardiovaskulären Systems (Sympathische und Parasympathische Regulation) sowie eine veränderte Baroreflex Sensitivität dürften ihren Teil zu der nicht funktionierenden Kontrolle des Blutdrucks beitragen. Auch eine Inhibition von Stickstoffmonoxid (NO) bei Präeklampsie dürfte den pathophysiologischen Entstehungsprozess der Krankheit beeinflussen.

Patienten und Methoden

72 Frauen wurden in die Studie eingeschlossen. Sie wurden aufgrund ihrer Schwangerschaftswoche (33+6 und 34+0) aufgeteilt und weiters getrennt in Präeklampsie (16) und gesunde Kontrollen (56). Early-onset Präeklampsie wurde definiert bis zur 33+6 Schwangerschaftswoche, late-onset Präeklampsie ab 34+0 Schwangerschaftswoche.

Der Task Force® Monitor (TFM®; CNSystems, Graz, Austria) wurde verwendet um den Blutdruck, die Herzfrequenz und die Thoraximpedanz kontinuierlich zu überwachen. Der kontinuierliche Blutdruck wurde nicht-invasiv vom Finger mit der „vascular unloading technique“ abgeleitet.

Die Herzratenvariabilität wurde von einer 3er-Ableitung des EKG berechnet. Diese reflektiert die Aktivierung des autonomen Nervensystems. SDNN, rMSSD, LF, HF und der Quotient aus LF/HF wurden daraus berechnet und für weitere Beschreibungen verwendet.

Die Baroreflex Sensitivität ist verantwortlich für die Regulation des hämodynamischen Systems. Die Berechnung erfolgte aufgrund eines Anstiegs/Abfalls des systolischen Blutdrucks zusammen mit einem Anstieg/Abfall der Dauer des R-R-Intervall am EKG.

Die „pulse transit time“, die den Zustand der peripheren Gefäße widerspiegelt, wurde berechnet aus der Zeit, die vergangen ist, zwischen der R-Zacke am EKG und dem steilsten Anstieg der Pulskurve am Finger.
Zuletzt wurden ADMA, SDMA und Endothelin-1 (vasoaktive Substanzen) mit der ELISA-Methode gemessen.

Ergebnisse

Alter und Körpergröße zeigten keinen signifikanten Unterschied zwischen Schwangeren mit Präeklampsie und den gesunden schwangeren Frauen. Die Gewichtsdifferenz (vom Anfang der Schwangerschaft bis nach der Geburt) war höher bei Präeklampsie. Der durchschnittliche Geburtstermin war früher bei Frauen mit Präeklampsie (248 Tagen) als bei gesunden Schwangeren (274 Tagen).
Der Blutdruck war höher bei präeklamptischen Schwangeren (systolisch, diastolisch und mittlerer arterieller Blutdruck). Die Herzfrequenz zeigte ein stabiles Level in der Kontrollgruppe, wurde aber deutlich niedriger bei Präeklampsie ($p<0.05$) mit Werten von 87.7±13.2 Schläge pro Minute bei early-onset Präeklampsie und 73.9±12.8 Schläge pro Minute bei late-onset Präeklampsie. Die Atemfrequenz zeigte keinen signifikanten Unterschied zwischen den Vergleichsgruppen.
Die „pulse transit time“ (PTT) zeigte, dass präeklamptische Frauen eine deutlich niedrigere PTT haben als die Gesunden ($p<0.05$).
Eine signifikante Interaktion wurde bei der Barorezeptor Sensitivität ($p<0.05$) zwischen early- und late-onset Präeklampsie und der Kontrollgruppe gemessen.
ADMA- und SDMA-Spiegel waren deutlich erhöht bei präeklamptischen Frauen ($p<0.001$) als bei gesunden Schwangeren. Endothelin-1 zeigte keine signifikanten Unterschiede.
Zuletzt wurde die Korrelation zwischen dem mittleren arteriellen Blutdruck und dem ADMA-Spiegel gemessen. Dies zeigte eine positive Korrelation mit einem Koeffizienten von 0.485.

Schlussfolgerung

Der Einfluss des autonomen Nervensystems auf das kardiovaskuläre System scheint beeinträchtigt zu sein. Die niedrigere Herzfrequenz bei den late-onset

präeklamptischen Frauen könnte aufgrund eines höheren paraympathischen Einflusses zustande kommen. Die Ergebnisse von SDNN, rMSSD und HF lassen darauf schließen, dass die parasympathische Kontrolle des kardiovaskulären Systems in den Vordergrund rückt. Die beeinträchtigte Baroreflex Sensitivität in late-onset Präeklampsie lässt darauf schließen, dass die Schwangere versucht, sich an die veränderten Bedingungen anzupassen. Die erhöhten Plasma-ADMA und SDMA Spiegel zeigen, dass die Inhibition von Stickstoffmonoxid eine Rolle in der Entstehung von Präeklampsie spielt.

Abstract

Preeclampsia – monitoring of hemodynamic parameter and vaoactive substances (ADMA, SDMA, Endothelin-1)

Background and Objective

Preeclampsia is a multifactorial disease with a widely unknown pathophysiological process. Several pathways are considered to be responsible for the severe hypertension in preeclampsia. The changed autonomic cardiovascular control (sympathetic and parasympathetic nervous system) as well as an impaired Baroreflex sensitivity contribute its part to a dysfunctional regulation of the blood pressure. Finally, the inhibition of nitric monoxide (NO) in preeclamptic women is taken into account to influence the pathophysiological process.

Patient and Methods

72 women were included in the study. They were divided according to their week of gestation (33+6 and 34+0) and separated into preeclamptic women (16) and healthy controls (56). By definition, the week of pregnancy 33+6 is early-onset preeclampsia, the week of pregnancy 34+0 is late-onset preeclampsia.

The Task Force® Monitor (TFM®; CNSystems, Graz, Austria) was used to perform continuous hemodynamic monitoring of blood pressure, heart rate and thoracic impedance. The continuous blood pressure was derived non-invasively from the finger using an improved version of the vascular unloading technique.

The heart rate variability, which displays the autonomic cardiovascular control, can be derived from a three-lead ECG. SDNN, rMSSD, LF, HF and the LF/HF-quotient were calculated and used for further descriptions.

The baroreflex sensitivity, which is responsible for the regulation of the hemodynamic system, was calculated with a rise/fall of the systolic blood pressure in addition to an increase/decrease of the R-R-interval.

The pulse transit time, which reflects the current state of the peripheral blood vessels, was indexed by the time elapsed between the closest previous ECG R-wave and the steepest upstroke of the peripheral pulse at the finger.

Finally, ADMA, SDMA and Endothelin-1 (all potent vasoconstrictory substances) were determined using the ELISA-Kit.

Results

Age and height showed no significant difference between healthy and preeclamptic women. The weight difference (initial weight before pregnancy until after delivery) was higher in preeclampsia. The average day of birth was earlier in preeclamptic women than in healthy controls with 248 days and 274 days, respectively.

The blood pressure (BP) showed higher values in preeclamptic women (systolic BP, diastolic BP and mean arterial blood pressure). The heart rate was almost steady in the control group, but decreased in preeclamtic women ($p<0.05$) with 87.7±13.2 beats per minute in early-onset preeclampsia and 73.9±12.8 beats per minute in late-onset preeclampsia. The breathing rate was constant in all groups.

Concerning the heart rate variability, SDNN displayed a significant interaction ($p<0.05$) between early- and late onset PE versus healthy controls. rMSSD showed a significant finding between the groups regarding the gestational age ($p<0.05$), and also a significant interaction ($p<0.01$). The low frequency domains suggested no significant values, the high frequency domains displayed a significant interaction between healthy controls and preeclampsia ($p<0.05$). No significant result was shown in the LF/HF-quotient.

The pulse transit time (PTT) showed that preeclamptic women have a significant lower PTT than the control group ($p<0.05$).

The baroreflex sensitivity displayed a significant interaction between PE/control and the week of pregnancy ($p<0.05$).

ADMA and SDMA showed higher levels in preeclamptic women ($p<0.001$) than in the control group. Endothelin-1 displayed no significant findings.

Finally, we investigated in the correlation of the mean arterial blood pressure and ADMA with a correlation coefficient of 0.485, suggesting a positive correlation.

Conclusion

The influence of the autonomic nervous system on the cardiovascular system seems to be impaired. The lower heart rate in late-onset preeclampsia might be due to a higher parasympathetic influence. SDNN, rMSSD and high frequency domains suggest that the parasympathetic nervous system is upregulated. The changed baroreflex sensitivity in late-onset PE gives evidence that the cardiovascular system of the expectant mother tries to adapt to the high blood

pressure. Elevated ADMA and SDMA levels in preeclampsia show that endovascular inhibition of NO plays a role in the development of PE.

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Glossar und Abkürzungen

ANP - atrial natriuretic peptide

BNP - brain natriuretic peptide

Na^+ - Natrium

HR - Heart rate

SV - Stroke volume

CO – Cardiac Output

NO – Nitric monoxide

SA-node – Sinatrial node

SVR – systemic vascular resistance

PTT – Pulse transit time

BP – Blood pressure

HRV – Heart rate variability

LDL – low density lipoprotein

NOS – Nitric monoxide synthase

BH_4 - Tetrahydrobiopterin

ADMA – Asymmetric dimethylarginine

SDMA – Symmetric dimethylarginine

GFR – Glomerular filtration rate

ET – Endothelin

SNS – Sympathetic nervous system

PE – Preeclampsia

O_2 – Oxygen

IUGR – intrauterine growth restriction

BMI – Body mass index

sFlt-1 – soluble fms-like tyrosine kinase-1

PlGF – placental growth factor

VEGF – vascular endothelial growth factor

TFM® – Task force monitor®

ECG – electrocardiography

SDNN – standard deviation of N-N-Interval

rMSSD – root mean squared successive difference of the R-R-Interval

LF – low frequency domains

HF – high frequency domains

RRI – R-R-Interval

ELISA – Enzyme linked immunosorbent essay

GA – gestational age

SBP – systolic blood pressure

DBP – diastolic blood pressure

MAP – mean arterial blood pressure

BRS – Baroreceptor reflex sensitivity

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1 Introduction

Preeclampsia is a disease with many unknown pathophysiological pathways. The disturbed function of the placenta might be an early cause leading to a disease, which is complicating 2-8% of all pregnancies. Multifactorial pathways activate the endothelial system, which further results in a systemic reaction of the maternal cardiovascular system. [1]

The autonomic nervous system seems to contribute its part to the development of hypertension, although many studies are limited due to a small number of study participants [2]. A higher sympathicotonus in pregnancy leads to an elevated blood pressure. [3]

Another pathway that influences the development of preeclamptic hypertension is the impaired vasodilation. Nitric oxide is the main vasodilator in human. Inhibition of this system can cause systemic vasoconstriction, which mainly influences the blood pressure [1].

1.1 Physiological cardiovascular changes in normal pregnancy

1.1.1 Haemodilution

Shortly after fertilization, a modification of the cardiovascular system takes place. Basically, all these adaptions are to cope with the increased blood volume. It starts about 6 weeks after fertilization, has a peak around the 32nd week with a maximum volume of 4700-5200 mL, and falls to ground level a few weeks after delivery. This means, a rise of about 1200-1600mL (+45%) blood volume at the end of pregnancy is observed. [4]

Main goals of haemodilution[1] in pregnancy are: [5]

- To sustain the exchange of gases and nutrients between mother and fetus at the placenta (due to rheological changes of haemodilution – lower hematocrit, lower plasma viscosity)
- To maintain the systemic blood pressure whilst the uterus and the vascular system expand

[1] The red blood cells rise only about 20%, while the plasma volume rises about 45%, leading to a lower hematocrit. This state is defined as haemodilution. [6]

- To reduce the risk of hypotension and to ensure enough volume for preload (ventricular end-diastolic volume)
- To rise the cutaneous blood perfusion (for a better heat exchange of the expectant mother via skin)

The specific mechanisms that raise the blood volume are not completely clear. It is caused by various factors which are mainly hormone based (e.g. Progesterone). Furthermore, the fluids shift from the extracellular space to the intravasal space. [4,7]

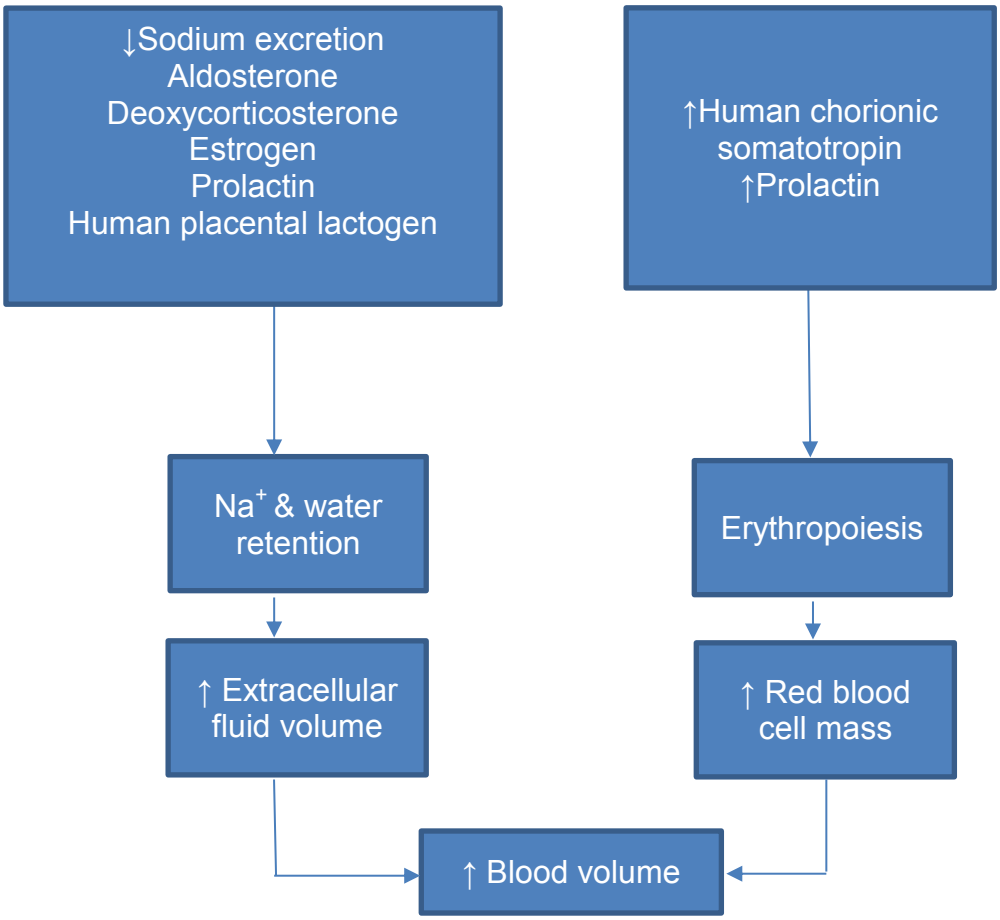
The atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are also affected by the haemodilution. The higher plasma volume stretches the atria of the heart, causing a release of ANP. The physiologic functions of ANP:
1) it dilutes the peripheral vessels
2) diuretic function.

This causes a downregulation of the haemodilution. BNP is released by the ventricles of the heart. It works the same physiologic pathway as ANP, resulting in an increase of the cardiac output, which causes a higher blood flow in the kidney (and is responsible for a higher diuresis). [4]

Other two mechanism that contribute to the physiological haemodilution:

- Progesterone promotes the sodium retention of the kidney (increased reabsorption in proximal and distal tubulus).
- The liver produces more angiotensinogen, which is a substrate of renin, and stimulates the aldosterone secretion at the cortex of the suprarenal gland (that increases the reabsorption of sodium and water in the kidney). [6]

Further mechanisms are displayed in graph one.



Graph 1: Physiology of hypervolemia, adapted from [8]

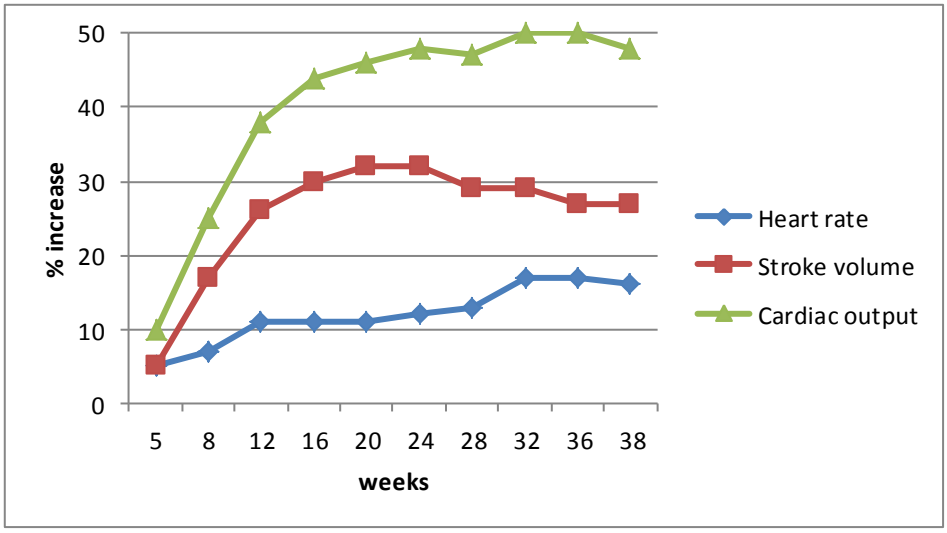
1.1.2 Cardiac physiology

The arterial blood pressure first decreases until about the 20th week of pregnancy, followed by an increase until delivery. Various authors agree that there is only a change in diastolic pressure, whilst the systolic blood pressure is stable. [6]

To maintain the blood circulation, the heart rate (HR) increases from a resting heart rate of 60 beats per minute to 70 – 75 beats per minute. [4] Possible mechanisms are a compensatory response to a decreasing systemic vascular resistance and hormonal influences by the thyreoid glands. [6]

The stroke volume (SV), which is the blood volume pumped out of the left ventricle each cardiac cycle, rises by about 20-30% (normal: 60-100mL). The cardiac

output (CO)[2], which is the blood volume pumped into the aorta every minute, rises at least 30%. [6]



Graph 2: Heart rate, stroke volume and cardiac output in pregnancy [4]

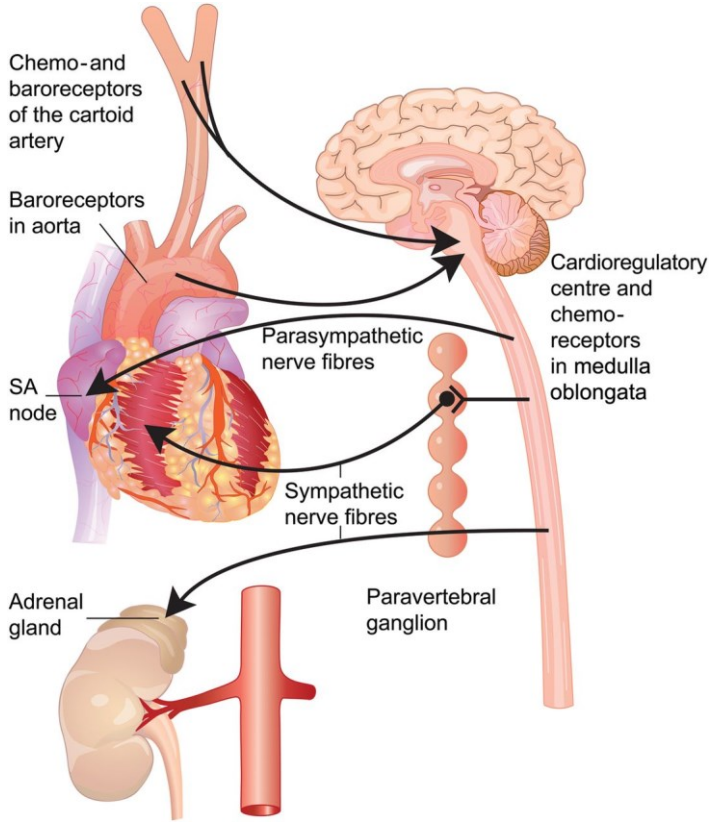
To compensate the elevation of the cardiac output and the abatement of blood pressure, a systemic arterial vasodilation is necessary, followed by a stimulation of the neurohumoral-axis. Through a negative feedback loop, the renin-angiotensin-aldosterone system is activated together with many other hormones (i.e. ANP, vasopressin, noradrenaline). This system works together with the renal hemodynamics, adaptions of the cardial system and the vasoconstriction of veins and arteries. [9]

Several different other mechanisms have been showed to correlate with the vasodilation. Estrogene, progesterone, prostaglandines and prolactine seem to be relevant. Also, prostacyclin seems to attenuate the effect of vasoconstriction of angiotensin II. [4] It is likely that the NO production is enhanced, while a decreased vascular response to norepinephrine (and other hormones) occurs. [10]

[2] CO=stroke volume*heart rate

1.2 Autonomic nervous system

The autonomic nervous system is divided into two main parts: the sympathetic system and the parasympathetic (vagal) system. In healty human, the sympathetic and parasympathetic system operate in balance. [11] Main feedback loops of the autonomic nervous system are displayed in graph three.



Graph 3: Regulation of sympathetic/vagal system on cardiovascular system [11]

In summary, the effects of the activation of the parasympathetic system are a bradycardia, lower cardiac contractility, lower vascular resistance and a lower venous return. The activation of the sympathetic nervous system results in tachycardia, elevated cardiac contractility, high vascular resistance and an elevated venous return. [12]

In pregnancy, the autonomic nervous system seems to be impaired, although the mechanism is still poorly understood. [3,13]

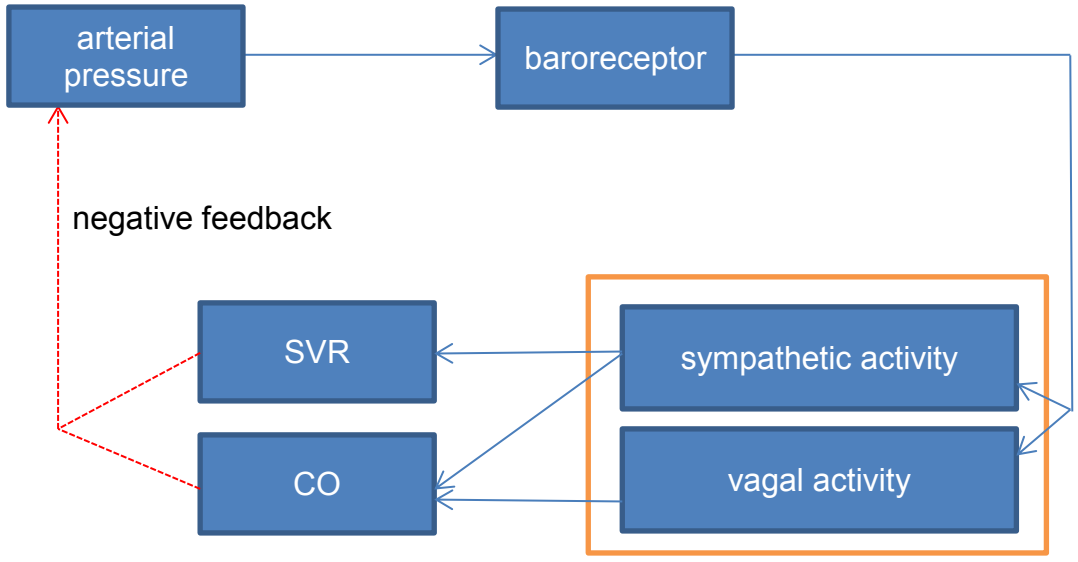
1.3 Baroreceptor

The baroreceptor system regulates the arterial blood pressure by giving informations of the changing blood pressure to the central nervous system.

The two main locations of baroreceptors are in the aortic sinus and aortic arch.

A higher blood pressure activates the baroreceptors. It rises the discharge of vagal neurons and lowers the discharge of sympathetic neurons (which act on the heart and blood vessels). [14] Main mechanisms are displayed in graph four.

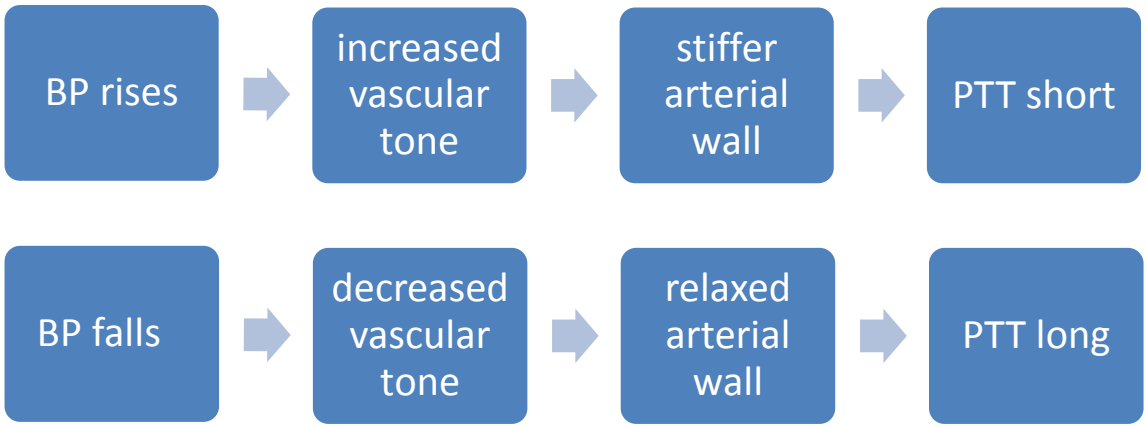
Looking at the time-domains of the autonomic nervous system, it can be said that parasympathetic reaction is an immediate reaction of the autonomic nervous system, while the sympathetic reaction is about 2-3 seconds delayed. This explains why the baroreflex controls the heart rate (beat-to-beat) mainly via a vagal activation. [12]



Graph 4: Negative feedback loop BRS-adaption; SVR is the systemic vascular resistance [14]

1.4 Physiological background of measuring the pulse transit time

It is possible to measure the current condition of the arterial vessels. The pulse transit time (PTT) is the time that the pulse wave needs to travel between two arterial sites. This time domain is directly connected to the blood pressure. [15]



Graph 5: Correlation between blood pressure and PTT [15]

The PTT is not able to reflect absolute values of the blood pressure. It is only possible to predict a change in blood pressure over a short time interval. Studies suggest that the PTT reflects the endothelial function, which is a major factor that contributes to the development of hypertensive disorders. [15]

Smith et al. [16] investigated in the pulse wave arrival time – which also reflects the state of the vessels - of pregnant versus non-pregnant women. They suggested that there is no difference in pulse wave arrival time due to a compensatory vasodilation in pregnancy.

The gold-standard of measuring the arterial stiffness is the carotid-femoral pulse wave velocity. The time is measured that the pulse wave needs to travel from the right carotid artery to the right femoral artery. To calculate the pulse wave velocity, the distance (in meters) is divided by the time domain (in seconds). [17]

1.5 Heart rate variability

The heart rate variability (HRV) is the variation of beat-to-beat intervals (R-R Intervals). [18]

It is reflecting the capacity of an individual to adapt to distress and environmental challenges. [18]

The heart rate on a beat-to-beat basis is not completely regular. The time interval between the R-spikes shows fluctuations due to a complex input of the neurophysiological systems. [18]

The normal HRV is due to synergistic actions of sympathetic and parasympathetic nerval system, mechanosensitive and chemosensitive neurons (including the baroreflex). Other factors that contribute to the heart rate variability are circadian rhythms, body temperature, metabolism and hormones. [19]

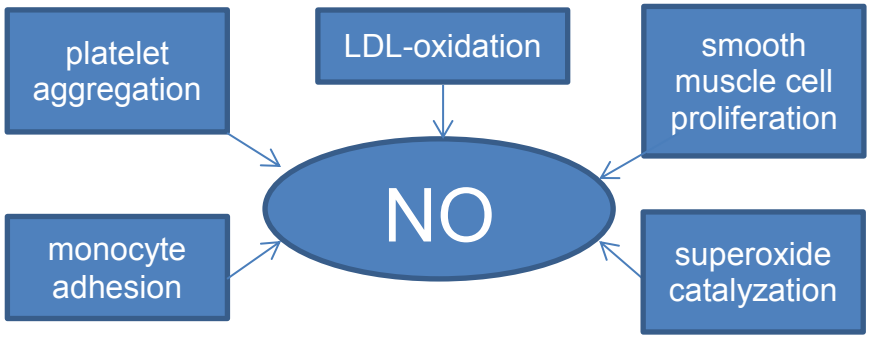
The autonomic control of the cardiovascular system, the changing blood pressure and the respiration produce changes in short-term rhythms of the heart rate variability. [20]

Studies show that the HRV decreases in pregnancy, especially in the second trimester. Reasons for this fall are a combination of an increased resting heart rate, hypervolemia and a higher preload. Nevertheless, there is no change in blood pressure variability. [21]

Several studies show a connection of cardiovascular mortality and an impaired autonomic nervous system. The markers of the heart rate variability are used in clinic to predict the mortality of myocardial infarction patients. [22]

1.6 Endothelial regulation of the cardiovascular system

Nitric monoxide (NO) is a potent vasodilator and responsible for pathologic processes that lead to vascular diseases. [23]

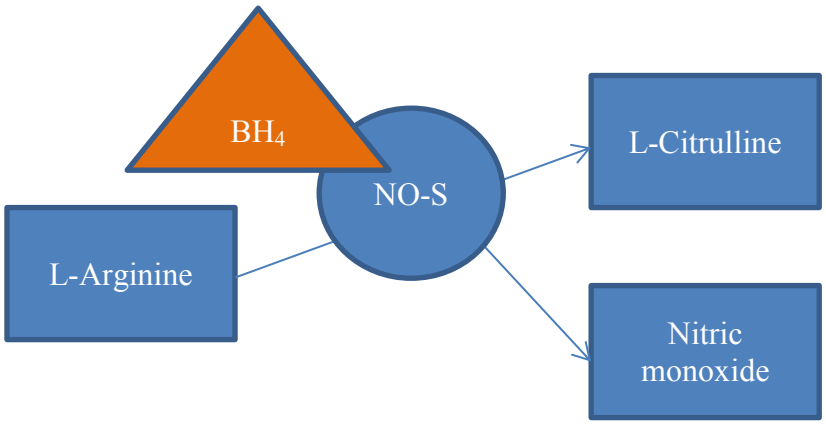


Graph 6: Factors influencing the NO-synthesis, which is responsible for vasodilation [23]

Biochemically, L-arginine is converted into L-citrulline by endothelial nitric oxide synthase (NOS). [24]

Three sub-forms of NOS are known: neuronal NOS (nNOS), inducible NOS (iNOS) and the endothelial NOS (eNOS). For the endothelium regulation, the

eNOS is the main point of interest, because it is the converting enzyme for nitric monoxide in the endothelium. Complex chemical mechansims are responsible to produce continuously NO to keep a basal vasodilator tone. [24]



Graph 7: Conversion of L-Arginine to L-Citrulline and nitric monoxide by NO-S and BH_4 (a co-enzyme) [24]

In uncomplicated pregnancy, the relaxation of uterine arteries induce an increase of vasodilatory mediators (e.g. NO). Mayr et al [25] showed in animal experiments, that the administration of NG-nitro-L-arginine methylester (L-NAME), which inhibits the NOS, causes a “so-called” preeclampsia-like syndrome. The symptoms of the sick rats are comparable to the symptoms of preeclamptic mothers.
Niu et al. [26] show that reduced NOS is strongly associated with increased oxidative stress in endothelial cells. This is well-known to lead to an increased risk for cardiovascular diseases – and preeclampsia. [27]

1.6.1 Inhibition of NOS

Asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) is an inhibitor of all three isoforms of nitric monoxide synthase (NOS). Elevated levels of ADMA are directly related to a higher risk of cardiovascular diseases. [28]

ADMA plasma levels are regulated by two mechanisms: [29]
1) activity of dimethylarginine dimethylaminohydrolase (DDAH), which is a degrading enzyme of ADMA
2) renal excretion

Functions of ADMA: [30]

- Reduced vascular compliance
- Increased vascular resistance
- reduced blood flow
- Promotes atherogenesis

In normal pregnancy, ADMA levels are reduced until the end of the first trimester, but continue to rise until delivery. Holden et al. [32] showed that ADMA levels are elevated especially in the third trimester compared to normotensive pregnancies. Increased ADMA levels at birth help to prepare the uterine muscle fibers for higher contractility at delivery. After delivery, ADMA levels begin to fall to base levels again. Pregnant women with preeclampsia show higher ADMA levels, which might be a factor that contributes to the development of endothelial dysfunction. [31]

Symmetric dimethylarginine
SDMA, which is a symmetric counterpart of ADMA, does not influence the NO synthetase. It is eliminated by the kidneys, which means it directly correlates with the glomerular filtration rate (GFR) and the serum creatinine level. It can be used as a marker of renal and hepatic failure, although its relationship to the risk of developing cardiovascular diseases is not clear yet. [33]

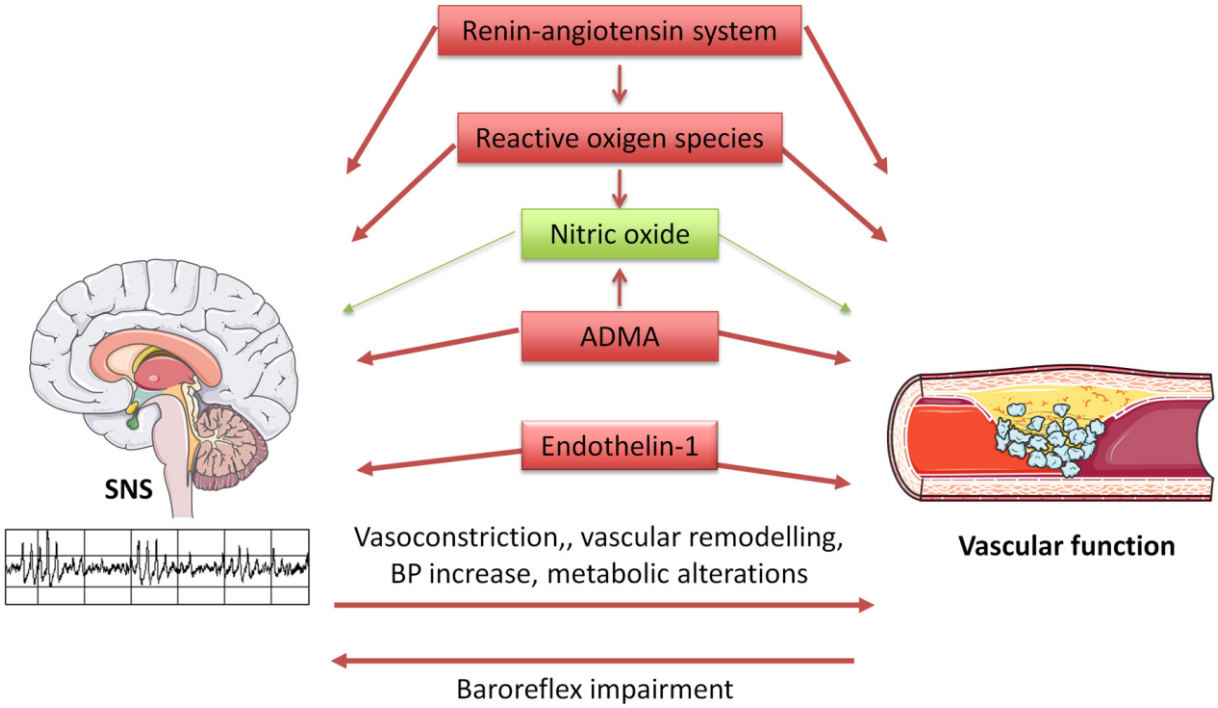
Endothelin
Endothelin, which also can be released from the vascular endothelium, seems to play an important role in the development of cardiovascular diseases.
The Endothelin-system is very complex and yet not completely understood. There are three different sub-types of Endothelin: ET-1, ET-2 and ET-3. We will only focus on ET-1, because its physiological impact on the blood vessels (vasoconstrictory effect) is best known. [34]

Two different kind of receptors for binding are known: ET_A-receptor and ET_B-receptor. The binding of ET-1 together with the ET_A-receptor is thought to be responsible for the extended vasoconstrictory effects of ET-1. [35]
It has been shown in animal studies that the Endothelin system also influences the nitric monoxide-dependent vasorelaxation. [34] There are two mechanisms that could be responsible:

1) decreased NO-production
The eNOS activity, as described above, is negatively regulated by a molecule called Caveolin-1. This means a higher level of Caveolin-1 causes a lower eNOS-activity. In situations like endothelial damage, ET-1 causes a higher level of Caveolin-1, which results in lower eNOS-production and a decreased vasorelaxation. [34]

2) increased NO-degradation
It is thought that eNOS-reactions can cause reactive oxygen species, which can lead to endothelial damage. Tetrahydrobiopterin (BH_4), which is a co-factor in the reaction of eNOS, seems to be a target molecule for reactive oxygen species. Together with NO, the reactive oxygen species produce peroxynitrate, which can oxidize BH_4. Finally, the oxidation of BH_4 leads to an impaired function of eNOS. [34]

1.6.2 Interaction of NO with the sympathetic nervous system
Recent studies [2] suggest that the regulation of the vascular tone could be directly linked between the endogenous endothelial system and the sympathetic nervous system. The biochemial pathways that lead to the regulation of the blood vessels could be almost the same:



Graph 8: Interaction of sympathetic nerve system and vascular function [2]

Graph 8 shows the interaction of the regulation of vascular function. It displays that inhibitors of NO react on both the vascular system and the sympathetic nervous system (SNS). The sympathetic system can directly influence the vascular function (e.g. by vasoconstriction). As a counterpart, the vascular function can directly influence the sympathetic nervous system by an induction of baroreflex impairment. [2]

1.7 Hypertension in pregnancy

By definition, it is possible to distinguish between four categories:

- Preeclampsia
- Chronic hypertension
- Chronic hypertension with preeclampsia
- Gestational hypertension

Chronic hypertension is hypertension that started before pregnancy. Gestational hypertension is defined as an elevated blood pressure (>140/90 mmHg) after 20 weeks of gestation without proteinuria. The following criterias are included in the definition of newly-onset hypertension in pregnancy:

- Thrombocytopenia (<100.000/microliter)
- Serum creatinine over 1,1mg/dL or an elevation of factor two of the serum creatinine if no other renal disease occurs
- Impaired liver function (elevated liver transaminases) [36]

1.8 Preeclampsia

1.8.1 Definition

The definition of preeclampsia (PE) changes almost annually. It is defined as hypertension with a systolic blood pressure over 140 mmHg and a diastolic blood pressure over 90 mmHg on two separate occasions. It occurs at a gestational age of 20 weeks or later. The blood pressure before the 20^{th} gestational week must be normal. Additionally, a proteinuria with more than 300mg per 24 hours occurs. Alternatively, a protein/creatinin-ratio over 0,3 mg/dL is accepted for the diagnosis of preeclampsia. [36]

By definition, a mild and severe form of preeclampsia can be distinguished. The following criterias can occur in severe preeclampsia. One of these symptoms needs to be added: [36]

- Blood pressure higher than 160/110 mmHg on two measurments at least 6 hours apart
- Elongation of diffusion distance (lower O_2 saturation)
- Oliguria
- headache
- Epigastric pain and/or affected liver function
- Thrombocytopenia
- Intrauterine growth restriction (IUGR)

The distinction between mild and severe preeclampsia is a very clinical definition. In the 1980s, several authors published a different definition of preeclampsia, which distincts between early- and late- onset preeclampsia:

Sub-category	Clinical presentation
Early onset PE (= until 33+6 weeks of gestation)	*More often associated with:* • Placental dysfunction • Low placental volume • Intrauterine growth restriction • Abnormal uterine Doppler • Low birth weight • Adverse maternal and neonatal outcome
Late onset PE (= from 34+0 weeks of gestation)	*More often associated with:* • Normal placenta • Larger placental volume • Normal fetal growth • Normal uterine Doppler • Normal birth weight • Better maternal and neonatal outcome

Table 1: Main differences between early- and late-onset preeclampsia [37]

It needs to be mentioned that the above clinical presentation is variable. Findings in early onset PE can also occur in late onset PE. [37]

A severe complication of preeclampsia is the HELLP-syndrome (H-hemolysis, EL-elevated liver enzymes, LP-low platelets). The incidence of women with severe preeclampsia that will develop the HELLP-syndrome is about 10-20%.
The best indicator to predict the illness is the platelet count. Studies show that the best therapy is an early delivery, together with blood pressure control. [1]

1.8.2 Epidemiology and Etiology

Different studies show that the prevalence of PE ranges from 2 to 8% of all pregnancies worldwide. In Africa, the incidence of PE seems to be much higher

than in well-developed countries. About 16% of maternal deaths are linked to complications of severe preeclampsia. [1]

There are many predisposing factors of preeclampsia, some of them are also risk factors for other endothelial diseases, including atherosclerosis. Main risk factors are displayed in table two:

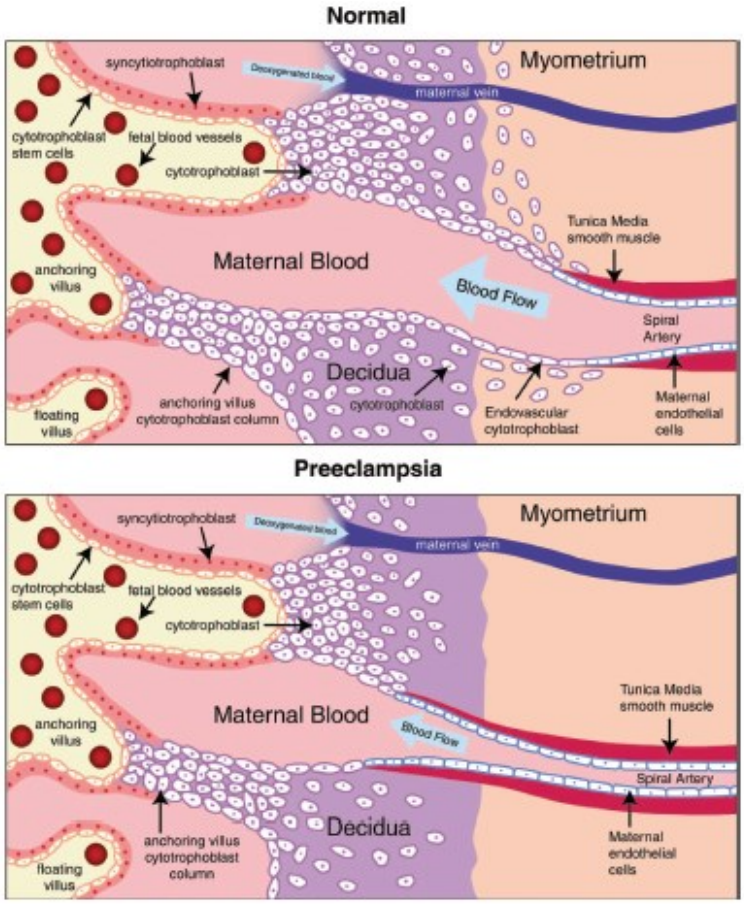
Risk factor	*Relative risk*
Nulliparity	*2.91*
Multiparous women	
Preeclampsia in any previous pregnancy	*7.19*
Age 40 years or older	
Nulliparous women	*1.68*
Multiparous women	*1.96*
BMI of 35kg/m^2 or higher	*1.55*
Family history of preeclampsia	*2.90*
Medical disorders	
Pre-existing diabetes	*3.56*
Presence of antiphospholipid antibodies	*9.72*

Table 2: Risk factors of preeclampsia [1]

1.8.3 Pathophysiology

Main pathophysiologic aspects of preeclampsia remain undiscovered.

Changed uteroplacental blood flow is evidenced to contribute to the development of PE. All women with PE have abnormal placentae. The trophoblastic invasion (main part of uteroplacental blood flow) of the placenta is reduced. This leads to a defective intravascular penetration of the spiral arteries (in normal pregnancy, the spiral arteries expand volume and reduce resistance). The remodelling of the spiral arteries, which is important for a high-flow and low-resistance system, fails. The impaired spiral arteries have a thicker muscular coat than the normal decidua vessels in pregnancy. As a result, the blood flow is reduced. Ischaemic areas in the placenta develop due to the hypoxia. [1]



Graph 9: Placentation in normal pregnancy and preeclampsia [38]

To compensate the poor blood flow in the placenta, the mother increases the blood pressure in order to ensure the blood supply of the fetus. This way, the ischaemic areas are continuously reperfused. Therefore, oxidative stress arises and might be responsible for the dysfunction of the placenta and oxidative stress. [1]

The second part of the pathophysiology includes the release of several bioactive substances into the maternal system. As a result of oxidative stress, several components that stimulate the production of cytokines, are released from the intervillous space. The production of cytokines is followed by a systemic inflammatory response of the maternal system. This is well known for leading to endothelial dysfunction, which could be responsible for the onset of preeclamptic symptoms. [1,17,18]

1.8.4 Diagnostics

Current studies show that sFlt-1, PlGF and VEGF (antiangiogenic factors) have important predictive and diagnostic implications. In clinical practice, the sFlt-1/PLGF-ratio is used. They start to ascend close to the end of the second trimester, making it possible to predict preeclampsia four to five weeks before clinical symptoms occur.

Doppler echography is used as a medical device to evalute the vascular resistance in the uterine artery. A postsystolic incision (=Notch) in Doppler echography might be a sensitive predictive marker of preeclampsia. [1]

1.9 Aim of the study

The aim of the study was to show if the autonomic nervous system can adapt to the hypertensive condition in preeclampsia. The preeclamptic women were divided in two main categories: Early-onset of preeclampsia (<33+6 weeks of pregnancy) and late- onset preeclampsia (>34+0 weeks of pregnancy). We hypothesized that the autonomic nervous system is able to adapt to the elevated blood pressure in preeclamptic women. Since early-onset PE is associated with a higher morbidity and mortality [37], we think that the adaption of the autonomic nervous system to the changed demands is better in late-onset preeclampsia than in early-onset preeclampsia. We used the heart rate variability to evalute sympathetic and parasympathetic changes. Additionally, we examined the baroreflex sensitivity, which is involved in the control of the blood pressure, to investigate the adaption of the cardiovascular system.

Furthermore, we aimed that the general vasoconstriction in PE influences the pulse transit time. We hypothesized that the pulse transit time in preeclamptic women is lower than in normal pregnant women.

Finally, we thought that the nitric oxide system in preeclampsia is impaired. We suggested that higher levels of ADMA and SDMA are found in preeclampsia. Also, we hypothesized that the highest endogenous vasoconstrictor, Endothelin-1, is elevated in preeclamptic women.

2 Material and Methods

2.1 Design of study

This is a retrospective, cross-sectional study with 200 women, who were asked to participate after first trimester screening. After a detailed anamnesis, women with preexisting diseases like insulin-dependent diabetes, cardiovascular or renal diseases, or other pregnancy-related diseases were excluded from the study. All other women were familiarized with the test protocol and the measurements were conducted.

The 16 remaining preeclamptic women were matched with 56 healthy women according to age, height and pregnancy week. Finally, 72 pregnant women were included for analysis. All participants had singleton pregnancies and a normal pregnancy outcome.

An informed consent was obtained by all study participants. The study was performed according to the local ethics comittee's guidelines.

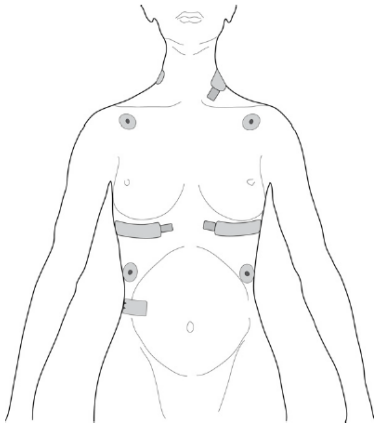
2.2 Measuring methods

The Task Force® Monitor (TFM®; CNSystems, Graz, Austria) was used to perform continuous hemodynamic monitoring of blood pressure, heart rate and thoracic impedance.

The advantages of this method are:

- Non-invasive
- Only one single measurement is neccessary to calculate all needed variables
- Cheap and efficient

A three-lead electrocardiography (ECG), using the CNSystem ECG-electrodes, were placed at the thoracic region to measure the heart rate. Also, two impedance electrodes were placed at the chest and the neck to measure the thoracic impedance.



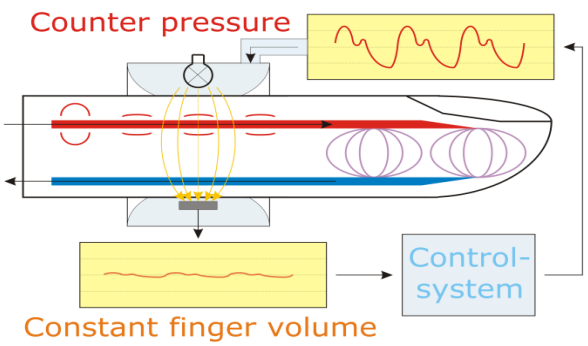
Graph 10: Proper place of measuring electrodes [39]

During the whole procedure, the probands remained in a 15° lateral position. They were asked not to talk or make abrupt movements. After an adaption period of 20 minutes, the ten minutes of measurements, which were used for the data, were performed at rest.

Blood pressure

The continuous blood pressure was derived non-invasively from the finger using an improved version of the vascular unloading technique. A cuff is placed around the finger. The blood volume inside of the artery undergoes a continuous change caused by the pulsation of the artery (heart activity). Theses changes are measured by infrared light. A counter pressure applies on the arterial wall. As the blood volume continuously changes, the pressure from outside adapts to the changing volume inside the artery, which correlates to the arterial pressure. [40]

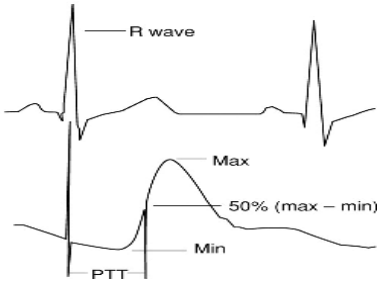
To correct these measurements to absolute values, the oscillometric blood pressure was determined at the contralateral arm.



Graph 11: Vascular unloading technique [40]

Pulse transit time

The pulse transit time (PTT) is the time that the arterial pulse pressure wave needs to travel from the aortic valve to the finger. PTT (in milliseconds) was indexed by the time elapsed between the closest previous ECG R-wave and the steepest upstroke of the peripheral pulse at the finger. [41] Although the gold standard is the pulse wave velocity, we did not measure the distance between the aortic valve and the finger.



Graph 12: Pulse transit time [42]

Heart rate variability

Using the ECG (sampling rate = 1kHz), it is also possible to calculate the heart rate variability (HRV). The Einthoven II lead is used to detect all R-spikes. After an artifact detection (five minute epochs with a 95% valid R-R-Interval are accepted), all further specific results of the heart rate variability can be calculated. [39]

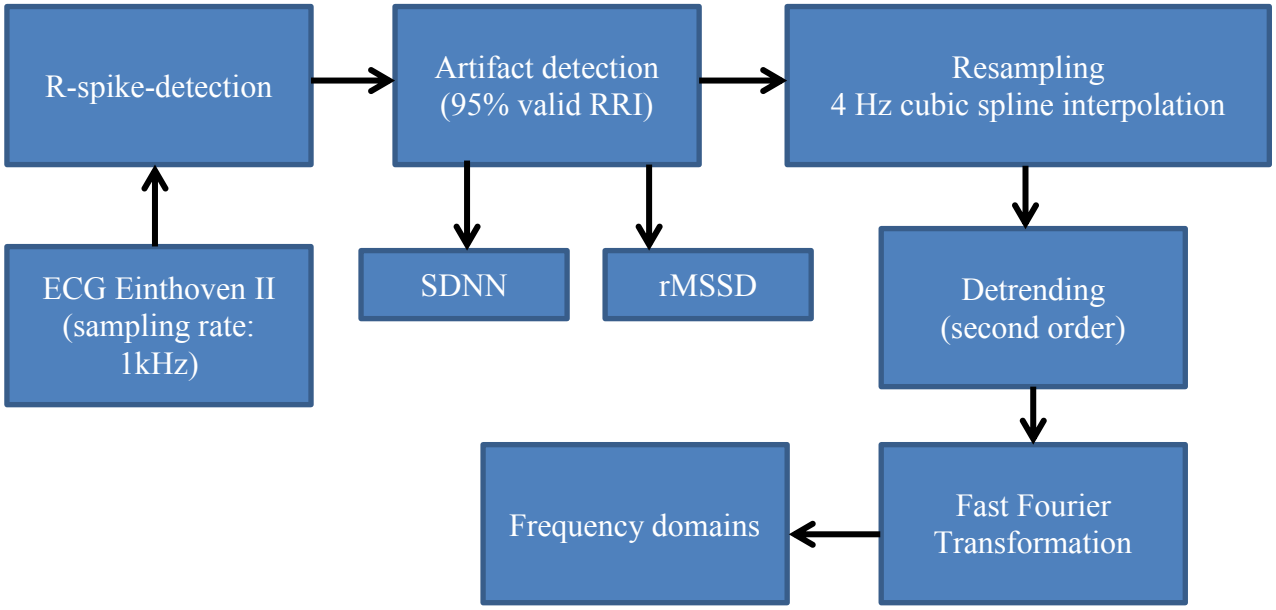
The SDNN, which is the standard deviation of normal-to-normal beat, is usually measured in time intervals of five minutes. It reflects the long-term components of the vegetative nerval system and the associated circadian rythms. SDNN is a parameter that represents the overall activity of the heart rate variability. It is best associated with the prognosis of cardiovascular diseases. [18]

The rMSSD, which is the root mean squared successive difference of the R-R-interval, shows how the heart rate changes from beat-to-beat. It includes the high frequency part and reflects the vagus-tonus. [18]

The low frequency domains $(LF)_{RRI}$, the high frequency domains $(HF)_{RRI}$ and the ratio of LF_{RRI}/HF_{RRI} can also be calculated.

Low frequency was definded as 0.04 – 0.15 Hz, high frequency was defined as 0.15 – 0.40 Hz, according to published recommendations. [18]

The low-frequency domain reflects the influence of sympathetic activity, the high-frequency domain the parasympathetic activity (influence of respiration), and the ratio of LF/HF reflects the balance of sympathetic and parasympathetic activity. [18]



Graph 13: Flow-chart for calculations of heart rate variability values [43]

Respiration frequency

Another measured value is the respiration frequency derived from the thoracic impedance. Low alternating currents with high frequency pass between two electrodes and record the changes (chest movement or volume changes at thorax). During inspiration, the lung fills with air and increases voltage, while during exspiration, the resulting thoracic impedance decreases. These changes are recorded and the respiration frequency is computed. [44]

Baroreflex sensitivity

The baroreceptor reflex sensitivity was calculated using the sequence technique. Three consecutive beats were used with the following definitions:

- Rise in systolic blood pressure in addition to an increase of the RRI
- Drop in systolic blood pressure in addition to a decrease of the RRI

Including criteria:

- Change of RRI at least > 4 ms
- Change of systolic blood pressure >1mmHg

The regression line between the systolic blood pressure and the RRI-values imply on the baroreceptor reflex sensitivity. [43]

ADMA, SDMA and Endothelin-1

ADMA, SDMA and Endothelin-1 are measured with the appropriate ELISA (Enzyme-linked immunosorbent assay)-Kit. The blood samples were obtained using EDTA vials.

First, a pre-treatment of the samples is neccessary: They are pipetted into wells of the reaction plate. Buffer is added to each well, the plate is shaken, buffer is added again and the plate has to be shaken again immediately. After an incubation time of 30 minutes, additional buffer together with water is added, followed by an incubation time of 45 minutes. 50 µl are used for ELISA.

The 50 µl of the pre-treated samples are pipetted into the well of a different plate and 50 µl of antiserum solution is added. After the plate is shaken for a short time, it is incubated at 2-8° for 15-20 hours. Afterwards, the solution is removed and the wells are washed with buffer four times. The enzyme conjugate solution (100 µl) is added to each well, followed by an incubation of one hour at room temperature on a horizontal shaker. Next, the wells are washed again, followed by incubation of 20-30 minutes. Finally, stopping solution is added and the optical density is measured at 450 nanometers within one hour. [45]

2.3 Data processing

In order to process the set of data, the following „constant factors“ were used to make further calculations. The population was divided into two subgroups:

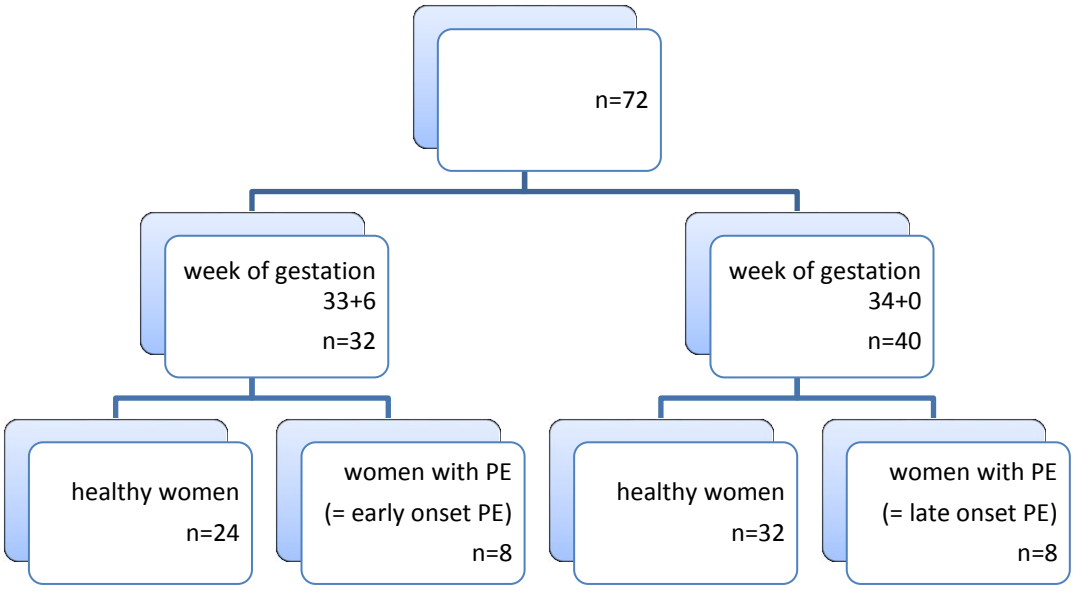
- Week of gestation until week 33+6
- Week of gestation from week 34+0

We decided to use the definition of early- and late onset preeclampsia according to Raymond et al. [37] So we were also able to divide the control group into two subgroups. Other definitions would make it impossible to sub-categorize the healthy women.

Afterwards, these subgroups were divided again into healthy women and women with preeclampsia.

Criterias for preeclampsia were defined as: [36]

- Blood pressure
  - Systolic blood pressure above 140 mmHg
  - Diastolic blood pressure above 90 mmHg
- Proteinuria > 300mg in 24 hours
- Symptoms occur after 20 weeks of gestation



Graph 14: Classification of the study participants

2.4 Clinical management

All study participants received, if neccessary, medical treatment according to the local guidelines. The treatment of preeclampsia includes an interdisciplinary management of the expectant mother. It includes the therapy of the hypertension in order to protect the mother from serious complications, medical prevention of seizure (via magnesium sulphate), fluid management and the time of delivery. [46]
In our study, the drugs used for the treatment of hypertension were Aldometil and Adalat. The active component of Aldometil is Methyldopa. It reduces the

hypertension through a sympatholytic process (which reduces the frequency of the heart). Adalat combines two active components: Nifedipin and Atenolol. Nifedipin is a calcium channel blocker which relaxes the arterial muscel wall. Atenolol is a β_1-antagonist (which also reduces the frequency of the heart). [46,47]
The fluid management is neccessary in order to prevent severe complications. [48]

2.5 Statistics

Microsoft Excel 2010 and SPSS 21 were used for statistic calculations. To analyze the statistical connection between the constant factors and the dependet variable, analysis of variance (ANOVA) is implemented and the f-test is used to determine the significance level. A p-value <0.05 was considered to be significant.

3 Results

For the calculations, the preeclamptic women were compared with the control group (= healthy and normal pregnancy). The acronym “GRP” is used for further descriptions. Also, the week of pregnancy (gestational age = GA) was divided into two subgroups. Group one is up to pregnancy week 33, group two starts from pregnancy week 34.

Preeclampsia	Group 1	early onset preeclampsia (until 33+6)
	Group 2	late onset preeclampsia (from 34+0)
Control group	Group 1	healthy control group (until 33+6)
	Group 2	healthy control group (from 34+0)

3.1 Description of the study population

Demographic values as age, height, weight difference and body mass index difference (BMI) are displayed in table three.

There is no significant age-related difference between the compared groups. The height also shows no significant result.

The F-statistics of the weight difference shows a significant result between preeclamptic and healthy women compared to the gestational age, but there is no significant interaction. The weight difference was calculated as the weight after pregnancy minus the weight before pregnancy.

The BMI of preeclamptic and healthy women were 30.0±6.2 kg/m^2 and 26.4±3.6 kg/m^2, respectively. The BMI difference of women with preeclampsia is higher than in healthy women.

The average day of birth for preeclamptic women was after 248 days of pregnancy, the average day of birth for healthy women 274 days. According to the p-value (<.001), there is a significant difference between the groups, also a highly significant interaction (p<.001) can be shown. Five dates of birth are missing for the control group 1 and four dates of birth are missing for the control group 2.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
Age (years)	29.8±5.5	29.6±7.2	32.6±3.6	31.1±5.8	GA:	*F* = 0.33; *ns.*
					GRP:	*F* = 1.91; *ns.*
Height (cm)	166.8±8.1	167.0±2.8	166.6±5.9	166.2±5.1	GA:	*F* = 0.002; *ns.*
					GRP:	*F* = 0.095; *ns.*
Weight Difference (kg)[3]	13.1±4.9	14.6±3.5	9.6±4.3	13.4±4.5	GA:	*F* = 4.54; *p< .05*
					GRP:	*F* = 3.63; *ns.*
BMI Difference kg/m^2	4.8±2.1	5.2±1.2	3.5±1.6	4.6±1.6	GA:	*F* = 3.86; *ns.*
					GRP:	*F* = 3.63; *ns.*
Average day of birth	232±18	264±14	274±11	274±11	GRP:	*F=19.68; p<.001*
					GA:	*F=51.56; p<.001*
					GA*GRP:	*F=19.66; p<.001*

Table 3: Age, height, weight difference, BMI difference and the average day of birth.

[3] For preeclamptic women, two values were missing for the weight before pregnancy. The missing values were recalculated. The average weight difference in the group was taken and subtracted from the weight after pregnancy. The same procedure was performed with the BMI.

3.2 Cardiovascular and respiratory measurements

3.2.1 Heart rate, blood pressure and breathing rate

The standard cardiovascular results are displayed in table four.

A significant difference occurs between groups 1/2 and the heart rate. Also, the interaction between preeclampsia and healthy compared to the gestational age shows a significant alteration.

The systolic blood pressure, the diastolic blood pressure and the mean arterial pressure show the same pattern. There are no significant findings between the group related to the week of pregnancy and the blood pressure, also no interaction between the gestational age and the PE/healthy in the corresponding values. All blood pressure values (SBP, DBP, MAP) are higher in preeclamptic women than in healthy women.

The breathing rate shows no significant result in either of the F-statistics.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
Heart rate (beats per Minute)	87.7±13.2	73.9±12.8	84.4±9.7	82.9±9.6	GA:	*F* = 6.71; *P< .05*
					GRP:	*F* = 0.91; *ns.*
					GA*GRP	*F* = 4.28; *P< .05*
Systolic Blood Pressure (mmHg)	149.3±13.9	142.5±12.4	107.6±11.5	109.2±15.0	GA:	*F* = 0.456; *ns.*
					GRP:	*F* = 95.08; *P< .001*
					GA*GRP	*F* = 1.20; *ns.*
Diastolic Blood Pressure (mmHg)	90.3±12.3	100.5±9.7	66.8±9.9	67.4±13.4	GA:	*F* = 2.60; *ns.*
					GRP:	*F* = 70.77; *P< .001*
					GA*GRP	*F* = 2.03; *ns.*
Mean Arterial Pressure (mmHg)	108.3±13.1	112.9±9.9	79.3±10.3	80.4±14.3	GA:	*F* = 0.63; *ns.*
					GRP:	*F* = 74.73; *P< .001*
					GA*GRP	*F* = 0.23; *ns.*
Breathing Rate (per minute)	19.8±1.7	19.0±3.0	17.6±3.3	18.2±2.4	GA:	*F* = 0.02; *ns.*
					GRP:	*F* = 3.64; *ns.*
					GA*GRP	*F* = 0.73; *ns.*

Table 4: Heart rate, blood pressure values (SBP, DBP, MAP) and the breathing rate

3.2.2 Heart rate variability values

The results of heart rate variability are shown in table five.

The standard deviation of normal-to-normal beat (SDNN) shows a significant interaction between the gestational age and control versus PE. No significant result in both other F-statistics.

The root mean squared successive difference of the R-R-interval (rMSSD) shows a significant finding between the groups correlating to the week of pregnancy and the rMSSD, also the interaction of the GA and healthy/PE shows a statistically significant result.

Looking at the logarithm of the low frequency (LF), there is no significant result in all calculated F-statistics.

In difference, the logarithm of the high frequency (HF) shows a significant interaction between the week of pregnancy and control/preeclampsia. No significant output in both other sub-results.

The logarithmic ratio of low frequency to high frequency (LF/HF) indicates no significant result in either of the values.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
SDNN (ms)	34.4±11.1	49.7±18.4	37.9±21.7	34.0±11.6	GA:	*F* = 1.49; *ns.*
					GRP:	*F* = 1.72; *ns.*
					GA*GRP	*F* = 4.24; P< .05
rMSSD (ms)	17.6±7.6	40.6±18.5	23.6±23.6	19.5±8.5	GA:	*F* = 4.20; *P< .05*
					GRP:	*F* = 2.74; *ns.*
					GA*GRP	*F* = 8.70; *P< .01*
$\ln(LF_{RRI})$ (ms^2)	5.4±1.1	6.1±0.9	5.6±0.9	5.5±0.8	GA:	*F* = 0.97; *ns.*
					GRP:	*F* = 0.51; *ns.*
					GA*GRP	*F* = 2.44; *ns.*
$\ln(HF_{RRI})$ (ms^2)	4.7±1.0	5.8±1.0	4.9±1.2	4.8±0.9	GA:	*F* = 2.37; *ns.*
					GRP:	*F* = 2.17; *ns.*
					GA*GRP	*F* = 4.14; *P< .05*
$\ln(LF/HF)_{RRI}$	0.7±1.1	0.3±0.6	0.7±0.8	0.7±0.7	GA:	*F* = 0.93; *ns.*
					GRP:	*F* = 1.41; *ns.*
					GA*GRP	*F* = 0.92; *ns.*

Table 5: Main heart rate variability values (SDNN, rMSSD, LF, HF, LF/HF)

3.2.3 Pulse transit time

The pulse transit time (PTT), as displayed in table six, shows that preeclamptic women have a significant lower PTT than the control group, although the interaction of the group referring to the week of pregnancy and healthy vs PE is not significant.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
PTT (ms)	241.4±13.2	242.3±19.5	253.3±14.2	248.7±13.5	GA:	*F* = 0.21; *ns.*
					GRP:	*F* = 4.97; *P<.05*
					GA*GRP	*F* = 0.45; *ns.*

Table 6: The pulse transit time (PTT)

3.2.4 Baroreceptor reflex sensitivity

The baroreceptor reflex sensitivity (BRS), which is listed in table seven, shows a significant interaction between PE/control and the week of pregnancy. In all other F-statistics, no significant findings are given.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
BRS (ms/ mmHg)	9.7±3.4	15.7±8.4	12.1±9.4	10.2±3.4	GA:	*F* = 1.15; *ns.*
					GRP:	*F* = 0.70; *ns.*
					GA*GRP	*F* = 4.47; *P<.05*

Table 7: Baroreceptor reflex sensitivity (BRS)

3.3 Endovascular values

Main endovascular results are displayed in table eight.

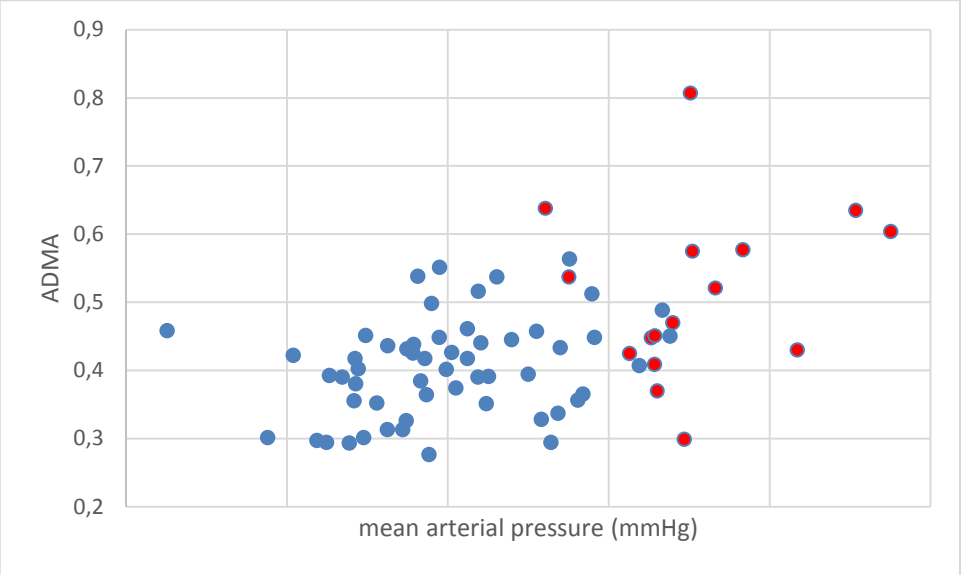
ADMA and SDMA show the same schemata. Both display significant findings between healthy and preeclamptic women (GRP), but no significant result occurs between the group related to the gestational age and ADMA/SDMA. Also, there is no significant interaction between the group depending on the week of pregnancy and healthy/PE. Endothelin-1 does not show a significant result at all.

	Preeclampsia		Control		*F*-statistics	
GA	1 (*n=8*)	2 (*n=8*)	1 (*n=24*)	2 (*n=32*)		
ADMA	0.50±0.12	0.53±0.14	0.40±0.07	0.41±0.07	GA:	*F* = 0.44; *ns.*
					GRP:	*F* = 19.22; *P<.001*
					GA*GRP	*F* = 0.17; *ns.*
SDMA	0.54±0.26	0.51±0.13	0.37±0.09	0.39±0.08	GA:	*F* = 0.02; *ns.*
					GRP:	*F* = 18.36; *P<.001*
					GA*GRP	*F* = 0.69; *ns.*
Endothelin-1	0.59±0.34	0.67±0.39	0.58±0.74	0.60±0.54	GA:	*F* = 0.09; *ns.*
					GRP:	*F* = 0.04; *ns.*
					GA*GRP	*F* = 0.02; *ns.*

Table 8: ADMA, SDMA and Endothelin-1

3.4 Correlation of the mean arterial blood pressure and ADMA

The analysis of the correlation between the mean arterial blood pressure (MAP) and ADMA – as displayed in graph 15 - according to Pearson shows a correlation coefficient of .485, however taken into account that the preeclamptic women might contort the relationship. This result suggests that the MAP is positively correlated to plasma ADMA-levels.



Graph 15: Correlation of MAP and ADMA. The red spots represent the values of preeclamptic women, the blue spots represent the control group.

4 Discussion

Our study suggests that the autonomic nervous system in preeclamptic women is impaired. The measurement of the heart rate variability (with SDNN, rMSSD, LF, HF) indices a change to a more parasympathetic-controlled cardiovascular system in late-onset preeclampsia. Several authors [49,50,51] reference to contrary studies that suggest a more sympathetic control. Due to the fact that the pathophysiology of preeclampsia is not completely understood, our results regarding the autonomic regulation of the cardiovascular system need to be discussed.

The impaired endothelial system seems to play a key role in the further development of preeclamptic symptoms [52]. The results of our study regarding ADMA and SDMA are consistent with several other studies [1,23,27,30], although Endothelin-1 is not altered.

4.1 Main demographic results

The women participating in the study were approximately the same age and height. Older women have a higher risk of developing PE [53], which would result in a higher prevalence of PE. The height might influence the BMI, which is a risk factor for the development of PE [48,53,54]. Since these two variables do not differ, we can compare the study groups regarding age and height. No other risk factors were considered relevant due to a small number of study participants. A history of smoking is yet another interesting variable that influences the incidence of PE [53], however, a bigger study population would be needed to divide the PE groups into smokers/non-smokers.

The weight difference is significant depending on the gestational age. In both PE groups, preeclamptic women gain much more weight than the women in the control group. Comparing the BMIs at the beginning of the pregnancy, the number of women that will develop PE have a higher BMI than the women in the control group. Studies show that overweight and obese women (BMI ≥ 35 kg/m^2) have a relative risk of approximately 1.5 of developing PE. [1] In this study, we confirmed that obese women have a higher incidence of PE.

4.1.1 Day of birth

Compared by the day of birth, women in the control group were pregnant an average of 274 days, which is very close to the normal duration of pregnancy (280 days). Goldenberg et al. [54] suggest that preeclampsia is a common reason for pre-term birth. Many other reasons can cause the difference of 26 days between healthy and preeclamptic women – e.g. smoking, infections or even stress. [54]

The pathologies of the HELLP-Syndrome, including renal failure, are responsible for an elevated risk for the maternal and perinatal mortality. The only treatment that helps the mother is an early delivery. Since the pathology in early-onset preeclampsia is more severe than in late-onset PE, it could explain the earlier delivery in early-onset PE. [55] However, early labor does not help the fetus. For the fetus, the primary prognostic factor for the further physiological development is the gestational age at birth. [55]

4.2 Cardiovascular measurements

In both preeclamptic groups, the systolic BP, diastolic BP and MAP are elevated. These observations define the pathological state of preeclampsia. These elevated values may be responsible for the life-long risk of hypertension and cardiovascular diseases. [36]

The study does not make a distinction between mild and severe preeclampsia. The mother`s severe symptoms can cause a higher stress level for her, which can implicate on the heart rate variability. The high stress level - caused by the neurohumoral activation of the suprarenal glands (cortisol and adrenaline)- continuously activates the sympathetic nervous system, which is responsible for the higher blood pressure and the elevated heart rate. [56]

Considering the heart rate, women with a normal and healthy pregnancy appear to have a slightly lower heart rate before the 34th week of pregnancy. This seems to be physiological due to adaptations of the cardiovascular system in late pregnancy. Another interesting phenomenon occurs with preeclampsia: The HR is approximately 13 beats per minute lower in late-onset PE than in early-onset PE. This observation can have different reasons:

- During late-onset preeclampsia there might be a switch from sympathetic to a more dominant parasympathetic control of the HR. Frequency domains of the HRV (HF, rMSSD) suggest that the parasympathetic activity in late-onset preeclampsia is higher than the sympathetic activity (significant interaction). This phenomenon could be responsible for the lower heart rate. In contrast to our findings, some studies show that the sympathetic activity is higher with preeclampsia. [50, 57] They suggest that the higher basal activity of the sympathetic nerve may cause the vasoconstrictory state of preeclampsia. After delivery, the blood pressure and sympathetic activity were normalized, insinuating a quick recovery, which might be caused by the vegetative nerval system.
- Studies show that the sympathetic nervous system is mainly responsible for the short-term regulation of the blood pressure. [2] A higher parasympathetic activation in late-onset PE could mean that the cardiovascular system is able to adapt to the changed demands. It downregulates the heart rate and also tries to lower the blood pressure. Unfortunately, the parasympathetic nervous system cannot change the blood pressure to a normal level, suggesting that other pathophysiological factors contribute to the hypertension in late-onset PE.
- The elevated baroreflex sensitivity (BRS) in late-onset PE pregnancy can cause lower heart rates via the negative feedback-loop. The high sensitivity of the baroreceptors (due to the elevated blood pressure) modulates the vagal activity, which causes a lower heart rate. [14]
- Visser et al. [48] show with their research that the heart rates in untreated PE-patients (week 28-31) are significantly lower than in treated PE-patients. They suggest that the hemodynamic profile in PE is reflected by the clinical management (administration of drugs, intravenous fluid).

The arterial stiffness in PE is responsible for a lower compliance in large arteries. [58] This leads to a higher afterload in the cardiovascular system. This physiological reaction reduces the cardiac output (via the Frank-Starling-mechanism). A lower CO leads to a higher HR, which has also been confirmed by Rang et al [59]. Unfortunately, this cannot be explained by our results.

4.3 Heart rate variability

Our results show that there is a significant interaction (SDNN, rMSSD, HF) between PE/healthy and the week of pregnancy. No significant result was found in all other values.

The interaction of SDNN and rMSSD supports our findings of a lower heart rate in late pregnancy of preeclamptic women. Since SDNN reflects the total variability, our results show that the HRV is increased in late preeclamptic pregnancy. Faber et al. [60] suggest that SDNN is lower in PE compared to normotensive pregnancies. Other studies show inconsistent results [51,61,62].

Another reason for the higher HRV in late pregnancy might be the medical treatment. Cook et al. [47] showed that rMSSD is increased by about 61 percent during the therapy with beta-blockers by sympathetic blockade. In our study, not all participants received (if it was neccessary) the same treatment. This fact can contort some of our values.

Additionally, SDNN represents 30-40% of day to night difference of beat-to-beat intervals. [63] This might have an effect some of the results as our measurements were performed only during day-time.

The low-frequency (LF) domain of the HRV shows no difference between healthy and preeclamptic subjects, which agrees with the literature [49,60]. It is a mixture of parasympathetic and sympathetic activity, therefore no clear interpretation can be made from this value [18]. Some studies [18,49,64] suggest that the LF represents the sympathetic modulation. A higher LF would result in tachycardia, which does not occur in our study.

Viewing the high frequency (HF) domain, there is a significant interaction. Since it represents the parasympathetic influence on the cardiovascular system, it makes sense that in late PE-pregnancy this value is elevated. Regarding preeclampsia, different studies show conflicting results: either a decrease [49,61] or no change [57,60] in HF.

The LF/HF – representing the sympathovagal balance - indicate no significant result, however, this might be due to the small study population. Our results propose that the parasympathetic control of the cardiovascular system is more important in late pregnancy than sympathetic control.

4.4 Pulse transit time

Our results regarding the arterial pulse transit time show a significant difference ($p<0.05$) between the PE and the control group. Although there appears to be no changes during the course of pregnancy, the PTT is lower in PE. Robb et al. [65] show a higher pulse wave velocity in PE than in normotensive pregnant women, suggesting a higher arterial stiffness in PE. This seems to be consistent with our expectations.

An advantage of PTT over the pulse wave velocity is that no length measurements are neccessary to calculate the values, although the pulse wave velocity is better known in clinical practice.

Besides PE, other conditions can influence the PTT[4]. In our case, the severe hypertension in PE might lead to a media hypertrophy in central arteries (due to the proliferation of the smooth muscle cells). [66] This might result in an impaired production of NO, which plays a key role in the development of PE. [1]

Also, the elevated vascular resistance (due to the impaired vascular compliance) indicates a failure of the cardiovascular system to adapt adequately in case of BP changes. [58] Although it has been shown that plasma volume reduced during PE [67], structural changes of the heart are necessary (left ventricular hypertrophy) to overpower the high resistance in the cardiovascular system. [58]

4.5 Baroreceptor reflex sensitivity

The BRS shows a significant interaction between the week of pregnancy and healthy/PE.

In our findings, the BRS is significantly elevated in late-onset PE. Stabilizing the blood pressure would be a physiological reaction of the cardiovascular system in PE. A higher baroreflex sensitivity allows the cardiovascular system to adapt adequately to short-term changes of the blood pressure. This could result in a "reset" of the BRS, meaning that the baroreflex sensitivity is working on a new level that is adapted to the elevated blood pressure.

The main focus of all circulation changes is to sustain the blood flow from the mother to the fetus. Since preeclampsia is a state of vasoconstriction, the main

[4] Genetic factors, age, gender, blood pressure, smoking, atherosclerosis, diabetes, renal failure, hypercholesterolaemia [66]

goal is to reduce the total peripheral resistance to sustain the uterine blood flow. [1]

Faber et al. [60] report no significant change of the BRS, while other authors [68,69] report a lower BRS in PE.

4.6 Endovascular values

ADMA and SDMA are similarly elevated in PE. Although only ADMA inhibits the NO-S, the role of SDMA in the development of PE is not entirely clear yet.

Böger et al. [27] suggest that elevated ADMA levels are responsible for a higher risk of suffering from cardiovascular diseases. A meta-analysis of over 3 million pregnant women [70] showed that women with a history of PE have an elevated risk of developing severe cardiovascular diseases (e.g. ischaemic heart disease, stroke, venous thromboembolism). Although several different mechanisms influence these pathophysiological events, ADMA seems to play a key role.

Miyazaki et al. [71] found that circulating ADMA levels are influenced by several factors, including the carotid artery intima-media thickness. We have not determined the base-line levels of circulating ADMA before pregnancy, which can possibly result in a lower absolute elevation of ADMA.

Little is known about the pathophysiology of SDMA. Bode-Böger et al. [33] suggest that SDMA could influence the NO-production by limiting the arginine-availability. Further, SDMA is a good indicator of renal function. Since many severe cases of PE underly a renal dysfunction, this may explain the elevated plasma SDMA-levels.

Further research is needed to determine wether overweight and obese women have different pathophysiological pathways that lead to a higher incidence of PE. A possible explanation could be a higher basline-level of ADMA.

We showed a positive correlation of the mean arterial blood pressure and ADMA. The review of Cooke [30] suggests that plasma ADMA-levels are directly related to an adverse outcome of patients with cardiovascular diseases. Further, an elevation of ADMA correlates with the severity of the disease. We were able to show that a higher mean arterial pressure results in elevated ADMA-levels.

Endothelin-1 is not significantly elevated, although Powe et al. [52] think that Endothelin-1 as an endogenous vasoconstrictor is elevated in PE. A mentionable restriction of measuring ET-1 is that it is released towards the basolateral side of the cell, meaning it does not function as a circulating hormone. Considering this phenomenon, plasma ET-1 levels are not reflecting the absolute ET-1 production. Therefore, possible bindings of ET-1 to the receptors can be covered. [35]

4.7 Limitations of the study

We performed our measurements with TFM®, which is a cheap and efficient tool to monitor the cardiovascular system. In the following table, we compared TFM® with the gold standard:

Continuous blood pressure	• Compared to other non-invasive BP-systems (i.e. Finapres™): BP trend is the same, no interruptions between measurements are necessary [72] • Compared to intra-arterial blood pressure monitoring (IAP), the systolic blood pressure was lower and the diastolic blood pressure higher than IAP [73]
R-spike-detection	• Compared to MIT/BIH[5] database, the detection rate of TFM® was 98,9 percent [72]

4.7.1 Further limitations

Our present study contains only 16 women that developed PE, which limits the significance of all results. Although the main cardiovascular parameters correlate with general knowledge, special values (HRV, BRS, PTT) would need a bigger study population for more accuracy and the comparability to other studies.

In our study, two woman had extraordinary high/low values in some of the results. That is one preeclamptic woman and one woman in the control group. They could possibly distort some of the statistically significant result.

[5] A database, which is specialised in arrythmia analysis [74]

Another possible limitation is the type of therapy. Not all of the preeclamptic women received the same therapy, probably due to varying severity of the disease. Medication can influence some of the values, although the difference to untreated preeclamptic women should not be too high. Other influences, e.g. smoking, were not considered to be relevant.

4.8 Conclusion

The influence of the autonomic nervous system on the cardiovascular system seems to be impaired. The lower heart rate in late-onset preeclampsia might be due to a higher parasympathetic influence. SDNN, rMSSD and high frequency domains suggest that the parasympathetic nervous system is upregulated. The changed baroreflex sensitivity in late-onset PE gives evidence that the cardiovascular system of the expectant mother tries to adapt to the high blood pressure. Furthermore, the lower pulse transit time in preeclamptic women could be caused by arterial stiffness, although the method might have its problems. Finally, elevated ADMA and SDMA levels in pregnancy show that endovascular inhibition of NO plays a role in the development of PE, although in our study, Endothelin-1 shows no significant result. We showed a positive correlation of MAP and ADMA.

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