Diplomarbeit

Assessment of vascular function changes in women with high risk of preeclampsia

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Graz, am 29.07.2021

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Abbreviations

bpm	beats per minute
cfPWV	carotid-to-femoral pulse wave velocity
COHb	carboxyhemoglobin
EDHF	endothelium-derived hyperpolarizing factor
mmHg	millimeters of mercury
NO	nitric oxide
PlGF	placental growth factor
PWV	pulse wave velocity
sEng	soluble endoglin
sFlt-1	soluble Fms-like tyrosinekinase-1
TGF-ß	transforming growth factor ß
VEGF	vascular endothelial growth factor

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Zusammenfassung

Hintergrund: Präeklampsie ist eine schwere Erkrankung, die bei 2% bis 8% aller Schwangerschaften weltweit auftritt und mit hoher Morbidität sowie Mortalität für Mutter und Kind einhergeht. Pathologische Vorgänge im Gefäßsystem der Mutter führen dabei zu Symptomen wie Hypertonie und Proteinurie. Ebenso dafür bekannt, negative Auswirkungen auf das kardiovaskuläre System zu haben, ist das Zigarettenrauchen. Umso verwunderlicher ist es, dass das Rauchen während der Schwangerschaft das Risiko für das Auftreten von Präeklampsie zu reduzieren scheint. Die genauen Mechanismen hinter diesem Zusammenhang sind bis heute noch nicht vollständig geklärt. Darüber hinaus ist unklar, welche Auswirkung ein Rauchstopp vor der Schwangerschaft auf das Risiko an Präeklampsie zu erkranken haben könnte und wie sich dieser auf die Veränderungen des Gefäßsystems in der Schwangerschaft auswirkt.

Zielsetzung: Ziel dieser Diplomarbeit ist das Erfassen und Bewerten der Gefäßfunktionsveränderungen bei schwangeren Frauen, die ein hohes Risiko an Präeklampsie zu erkranken aufweisen. Unterschiede zwischen Frauen, die vor der Schwangerschaft geraucht haben und strikten Nichtraucherinnen würden auf einen Zusammenhang des Rauchverhaltens vor der Schwangerschaft mit den Veränderungen des Gefäßsystems während der Schwangerschaft hindeuten.

Methoden: Um die Funktion des Gefäßsystems zu erfassen wurde die Pulswellengeschwindigkeit zu drei verschiedenen Zeitpunkten während der Schwangerschaft gemessen. Die Messungen waren Teil von Untersuchungen im Rahmen einer umfassenden Studie an der Abteilung für Gynäkologie und Geburtshilfe an der Medizinischen Universität Graz. Für die vorliegende Arbeit wurden Daten von 31 Frauen mit einem erhöhten Risiko an Präeklampsie zu erkranken untersucht.

Ergebnisse: Die Auswertung der Daten ergab keine statistisch signifikanten Unterschiede in der Veränderung der Gefäßfunktion zwischen ehemaligen Raucherinnen und strikten Nichtraucherinnen. Dennoch wiesen jene Frauen, die vor der Schwangerschaft geraucht hatten, durchschnittlich eine niedrigere Pulswellengeschwindigkeit bei den Messungen im ersten und zweiten Trimester auf als jene, die nicht rauchten. Dies könnte darauf hinweisen, dass das Rauchen vor der Schwangerschaft einen positiven Einfluss auf die Veränderung des Gefäßsystems bei Frauen mit entsprechendem Risiko hat.

Abstract

Background: Preeclampsia is a severe medical condition that affects about 2% to 8% of pregnancies worldwide. It is characterized by a pathological process within the mother´s vascular system which causes symptoms like hypertension and proteinuria. Cigarette smoking is known to have many adverse effects on the cardiovascular system too. Quite controversially, it seems to reduce the risk of preeclampsia. Though many different theories for this rather conflicting relation exist, the exact mechanisms are still a subject of debate. Furthermore, it is still unclear whether women who smoked before pregnancy but then stopped also have a reduced risk of developing preeclampsia and how this affects vascular adaptations in pregnancy.

Aims and objectives: This diploma thesis aims to assess vascular function changes in women with a high risk of preeclampsia. Possible differences between women who used to smoke before pregnancy and non-smoking women would suggest an influence of the women´s smoking habits on vascular function changes during pregnancy.

Methodology: For this purpose, the pulse wave velocity, a marker for vascular function, was investigated at three points in time during pregnancy. The measurements were performed as part of a large prospective study carried out at the department of Obstetrics and Gynecology at the Medical University of Graz. Data from 31 women with a high risk of preeclampsia were gathered and analyzed.

Results: There were no statistically significant differences between non-smoking women and women who used to smoke before pregnancy. However, the women who used to smoke before pregnancy had on average lower values for the pulse wave velocity than the non-smoking women at the measurements in the first and second trimester of pregnancy.

Conclusion: The results might suggest a positive influence of cigarette smoking before pregnancy on vascular function in women with a high risk of preeclampsia. Moreover, it was discovered that vascular function changes in the participants of our study in general differed from observations made in healthy pregnancies. As all women included had an estimated high risk of preeclampsia, this might be an indication of a pathological process within their vascular systems.

1 Introduction

1.1 The cardiovascular system

The cardiovascular system consists of the heart, which works as a pump, and blood vessels acting as conduits that permit circulation of blood throughout the body. Two different circuits need to be distinguished, the pulmonary circulation and the systemic circulation. Within the systemic circulation blood is transported between the heart and the body, while the pulmonary circulation enables blood exchange between the heart and the lungs. In the lungs blood gets oxygenated and carbon dioxide, a product of metabolism, is breathed out. From there, blood flows back to the heart where it is pumped into the periphery of the body. Blood not only supplies tissues with oxygen but also transports nutrition, such as fatty acids and amino acids provided by the digestive system, hormones and cells.

With every heartbeat blood is ejected from the left ventricle and enters the systemic circulation where it is distributed along the arterial tree. Blood flows from the aorta into arteries and smaller arterioles until it reaches the smallest branches of the vascular system, the capillaries. Via the capillaries nutrition and oxygen is exchanged between the blood and the interstitial fluid. At the same time waste products, such as carbon dioxide and urea, get absorbed. Finally, blood flows from the capillaries into venules and veins back to the heart, where it is ejected from the right ventricle and pumped into the lungs to become oxygenated and the circuit starts again (1). A simplistic illustration of the cardiovascular system is shown in Figure 1.

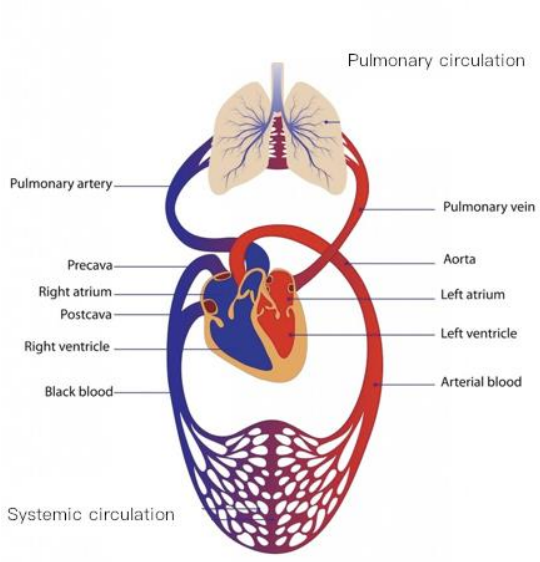

Figure 1: Cardiovascular system. Reproduced from: https://www.memorangapp.com/flashcards/162054/The+Cardiovascular+System+Part+I%3A+Circulation/

In order to supply every organ with sufficient oxygen, blood pressure needs to be strictly regulated. When blood pressure is low, symptoms like dizziness, blurred vision or fainting can occur. Extreme hypotension, for example due to blood loss, can lead to a life-threatening situation which is called a hypovolemic shock. However, when blood pressure is too high, consequences can be fatal too. The most common type of high blood pressure is chronic hypertension, which is a major risk factor for severe diseases such as heart attack and stroke. Prevalence for chronic hypertension is high in Austria, with 16.8% in women and 25.2% in men (2). But there are also other diseases which are characterized by high blood pressure. For example, hypertension sometimes occurs for the first-time during pregnancy. This so-called pregnancy induced hypertension affects mainly young women in their first pregnancy and is a leading cause of morbidity and mortality for both, the neonate and the mother (3). Moreover, high blood pressure is known to increase the risk of cardiovascular disease later in life. To understand the pathophysiology of hypertensive diseases, the body´s hemodynamics and the regulation of blood pressure needs to be understood.

1.1.1 Hemodynamics and arterial blood pressure

When blood is ejected from the heart, it exerts pressure on the walls of the arteries, this force is called blood pressure. Blood flow through the circulatory system can be explained by physical laws and is mainly driven by the rhythmic contractions of the heart and the difference in blood pressure between the arterial and venous system. Due to the heart´s pumping action a pulsatile blood flow is created, with alternating variations of blood pressure. The maximal pressure is called systolic blood pressure and the minimal pressure is called diastolic blood pressure. In a resting adult blood pressure is usually about 120 mmHg systolic and 80 mmHg diastolic, this refers to the pressure in large arteries of the systemic circulation.

The product of heart rate, which is the amount of heart beats per minute (60-80bpm), and the stroke volume, which is the volume the heart ejects with every beat (70-80ml), is called cardiac output (4). It is the amount of blood that is ejected from the heart in a specific time span and shows how effectively the heart is working. Many different factors have an influence on the two parameters that determine cardiac output. These factors are summarized in Figure 2.

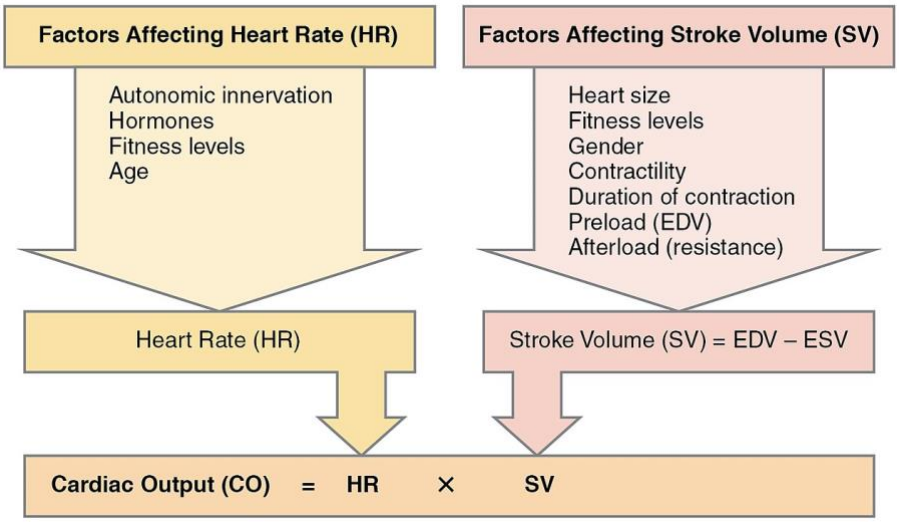

Figure 2: Factors influencing cardiac output. Reproduced from: https://en.wikipedia.org/wiki/Cardiac_output#/media/File:2031_Factors_in_Cardiac_Output.jpg

Blood pressure is not only influenced by cardiac output but also by the total peripheral resistance of the vascular system. Vascular resistance is understood as the force that needs to be overcome when blood is ejected from the heart to create blood flow. Mostly the vessel´s diameter and compliance but also viscosity of the blood and the length of the blood vessel determine vascular resistance. Responding to different stimuli blood vessels can increase in diameter, which is called vasodilation, and as a result vascular resistance is reduced. When the diameter of blood vessels decreases, which is called vasoconstriction, vascular resistance increases. As arteries expand blood pressure is reduced and more blood can flow through them.

As mentioned above, the compliance of a blood vessel contributes to vascular resistance and influences blood pressure. Compliance is the ability of a vessel to distend when transmural pressure is increased (4). It depends on the amount of elastic fibers in the tunica media of vessel walls as well as on the tone of its smooth muscle cell layer. Amongst all arteries, the aorta is the most compliant vessel due to a large amount of elastic fibers in the tunica media. Big arteries like the aorta distend when blood pressure rises during the systole and hence form a reservoir for blood and store the pressure in their elastic walls. During diastole this reservoir discharges, not only ensuring sufficient blood flow to organs while the heart is relaxing, but also helping to reduce fluctuations between systolic and diastolic blood pressure. This phenomenon is called Windkessel effect and is demonstrated in Figure 3.

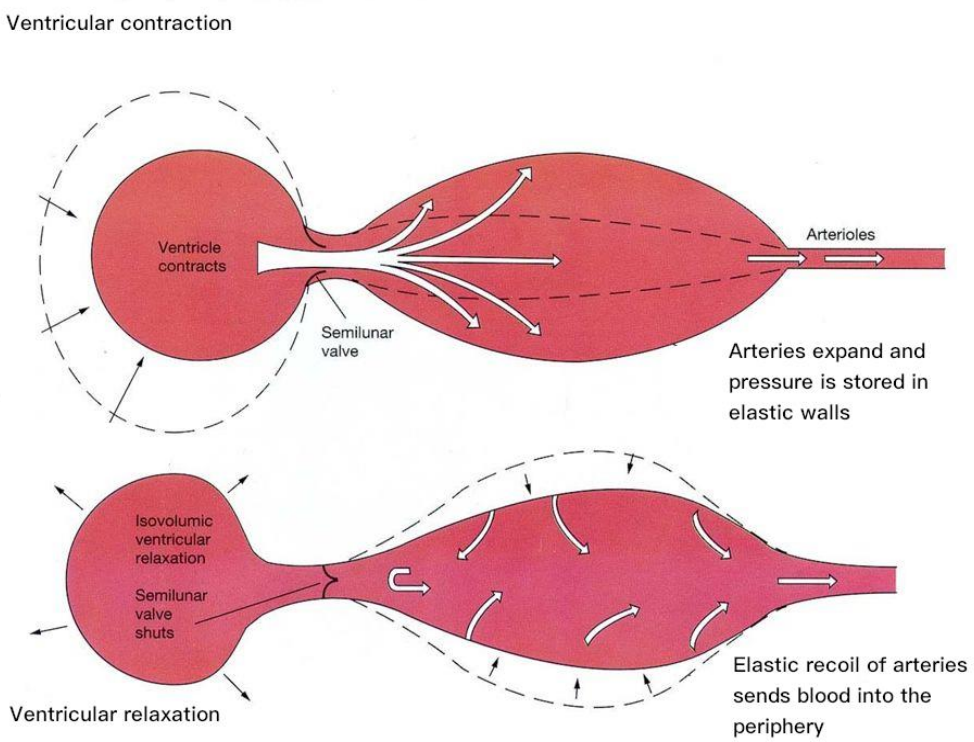

Figure 3: Distension and recoil of arterial vessel walls. Reproduced from: https://slideplayer.com/slide/8768785/

1.1.2 Blood pressure regulation

To secure sufficient blood flow to all organs, blood pressure can be adjusted centrally or the perfusion of an organ itself is regulated independently by local mechanisms. Short term adaptation of blood pressure is mainly regulated via the vegetative nervous system, while long term adaptation is regulated by a change in blood volume due to elimination or retention of electrolytes and water by the kidneys.

Short term regulation of blood pressure:

The vegetative nervous system has, among other important functions, a major impact on short term blood pressure regulation by influencing heart rate and vasomotor activity (5). It can be divided into the sympathetic and the parasympathetic nervous systems, which function in opposition to each other. When stimulated, the sympathetic nervous system prepares the body for a „fight or flight situation" by promoting energy generation. Heart rate increases as well as the contractility of cardiac cells, so an increase in blood flow to skeletal muscles is promoted. At the same time, it causes vasoconstriction leading to an

increase in vascular resistance and cardiac preload. In contrast, stimulation of the parasympathetic nervous system decreases heart rate and causes vasodilation.

Before blood pressure can be adapted, it needs to be recognized by different kinds of receptors sending their information to the reticular formation, which is a part of the central nervous system responsible for cardiovascular control. These receptors can be divided into chemoreceptors, baroreceptors and volume receptors, all reacting in response to different stimuli.

Long term regulation of blood pressure:

The Renin-Angiotensin-Aldosterone system together with two other important hormones, the antidiuretic hormone and the atrial natriuretic peptide, mainly regulate blood pressure by a change in blood volume via the kidneys. When perfusion of the kidneys is low and sodium concentration or osmolarity of blood plasma decreases, these changes are recognized by the juxtaglomerular cells of the kidneys, which start to release a hormone called renin into the blood (5). This causes the activation of the Renin-Angiotensin-Aldosterone system, a hormone system with various effects on blood volume and vascular tone. The most important effects include an increase in sodium and water reabsorption and excretion of potassium ions via the kidneys as well as vasoconstriction, all aiming to increase blood pressure.

The antidiuretic hormone is produced and released by the neurohypophysis. Like the Renin-Angiotensin-Aldosterone system, it promotes an increase in blood pressure by increasing retention of water in the kidneys and vascular resistance. In contrast, the atrial natriuretic peptide is released by muscle cells in the atrium of the heart as a reaction to a volume related distension of the atrium. This hormone promotes sodium and water excretion and vasodilation resulting in a decrease in blood pressure.

1.1.3 Structure of the vessel wall and endothelial function

Arteries and veins consist of three different layers. The outer layer is called tunica adventitia and consists of connective tissue. In larger blood vessels, it contains capillaries and nerves that supply the vessels. Tunica media is the name of the middle layer of blood vessels, which is mainly built of smooth muscle cells. Via contraction or dilation of this muscle layer, vessel diameter changes and thus leads to an increase or decrease in vascular resistance. Tunica media also consists of collagen and elastic fibers. A huge amount of

elastic fibers can be found in large arteries, like the aorta, which are responsible for the vessels´ elasticity. The most inner layer of blood vessels is called Tunica intima, which consists of a monolayer of cells, called the endothelium (6). By lining the luminal walls of blood vessels, endothelial cells form a barrier between the other parts of the vessel wall and the circulating blood. The permeability of the endothelium varies between different organs and regulates the exchange of substances between the blood and the tissue. Various stimuli like hypoxia can cause a change in permeability of the endothelium (7). The structure of the vessel wall is shown in Figure 4.

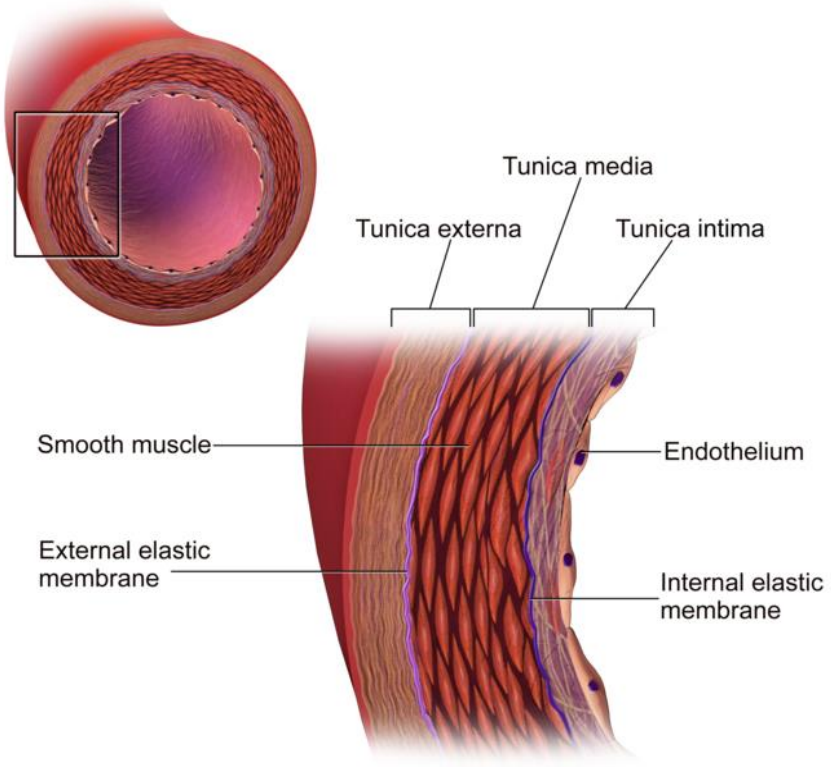

Figure 4: Blood vessel structure. Reproduced from: https://courses.lumenlearning.com/boundless-ap/chapter/blood-vessel-structure-and-function/

Besides its function as a barrier, the endothelium plays an important role in regulating vascular homeostasis. As a response to chemical and physical signals, endothelial cells can produce different factors that influence vascular tone, cellular adhesion or angiogenesis (8). A healthy, resting endothelium physiologically prevents blood clotting by expressing substances like heparan sulfate, nitric oxide or prostacyclin, which inhibit adhesion and aggregation of thrombocytes. Under normal circumstances there is no interaction between the endothelium and leukocytes. As soon as inflammation occurs and the recruitment of immune cells is necessary, the endothelium starts to express adhesion molecules, chemokines and cytokines to interact with these cells as well as platelets (9).

As far as blood pressure is concerned, the endothelium plays a major role in the regulation of vessel tone. The release of vasoactive substances leads to either constriction or relaxation of the smooth muscle cell layer and hence vessel diameter changes.

The most important vasodilator is nitric oxide. It is produced during the process of converting l-arginine to citrulline, which is catalyzed by an enzyme called nitric oxide synthase. There are three different isoforms of this enzyme, called endothelial-, neuronal- and inducible nitric oxide synthase. The first two enzymes produce nitric oxide due to an increase in intracellular calcium levels, while the inducible nitric oxide synthase works independently of calcium concentration. Shear stress, which is the dragging frictional force of blood on the vessel walls, is one of the most important activators of the endothelial nitric oxide synthase and helps to adapt organ perfusion when cardiac output changes (8). After nitric oxide is produced, it diffuses to the smooth muscle cells of the tunica media where it induces vasodilation by causing a decrease of intracellular calcium concentration. Besides its function in the control of blood flow via regulation of vascular tone, nitric oxide plays also an important role in platelet aggregation and leucocyte adhesion (10). Considering all the important functions of nitric oxide, it is not surprising that a disruption of its production has a major impact on the homeostasis of the endothelium and leads to endothelial dysfunction.

Prostacyclin is another potent vasodilator produced by the endothelium. Like nitric oxide it leads to a relaxation of smooth muscle cells and vasodilation. Prostacyclin does not only have an impact on endothelial cells but also binds to receptors of platelets and inhibits platelet activation (9). The impact of prostacyclin always needs to be considered in the ratio to thromboxane, a potent vasoconstrictor working as a counterpart to prostacyclin. A disbalance of these two hormones can cause a disruption in endothelial homeostasis and result in vascular damage. Other important substances contributing to vasodilation which are produced by endothelial cells are summarized under the collective term endothelium-derived hyperpolarization factor (EDHF). These substances were discovered after research showed that relaxation of smooth muscle cells of blood vessels could not be explained by the function of nitric oxide and prostacyclin alone. When released, EDHF activates ion channels, which leads to a hyperpolarization and relaxation of smooth muscle cells. The effect of EDHF can work as a backup when nitric oxide function is impaired and the EDHF pathway, when altered, is believed to contribute to endothelial dysfunction (11).

The body´s strongest vasoconstrictor is endothelin 1. The production of endothelin 1 by endothelial cells is stimulated by shear forces on the vessel wall, angiotensin II or the antidiuretic hormone. An overexpression of endothelin 1, which can lead to hypertension, is usually inhibited by nitric oxide and prostacyclin.

1.2 Arterial stiffness and cardiovascular disease

With cardiovascular disease being one of the main causes of mortality in developed countries, there is a need to assess parameters that can predict the risk for cardiovascular events. Arterial stiffness seems to play an important part in the pathophysiology of cardiovascular disease and therefore, the assessment of this parameter has gained more clinical valuation over the last years. It could help to identify patients with a high risk of cardiovascular events in the future. In fact, arterial stiffness seems to be an independent predictor for cardiovascular morbidity and mortality, as well as a predictor for developing hypertension in young and to this point normotensive patients (12) (13).

Arterial stiffness occurs as a consequence of changes in the consistency of arterial walls, mainly within the tunic media. Due to factors such as aging, genetic predisposition, chronic renal disease and classic cardiovascular risk factors like smoking, hypertension or diabetes mellitus, elastin structures in the vessel wall start to fray and become replaced by collagen proteins (14). This process is often complemented by calcification of the vascular system. Structural changes of the arterial wall and subsequently increasing arterial stiffness cause an increase in hemodynamic load on the endothelium, which damages it. As mentioned above, the endothelium produces vasoactive mediators, like nitric oxide, that contribute to vascular distensibility. When endothelial function is impaired due to its damage, the production of nitric oxide and the vessel´s ability to distend is compromised, which further contributes to increased arterial stiffness (15).

Vascular distensibility in women also seems to be influenced by female sex hormones. Estrogen and progesterone not only cause structural changes in the vessel wall, but also contribute to the regulation of vascular tone through effects on the endothelium and smooth muscle cells by stimulating nitric oxide production (13).

When the capability of the aorta and large arteries to expand is reduced as blood pressure increases, the Windkessel effect, which is important for keeping a steady and even blood flow, is compromised. As a result, the speed of the pulse wave, which propagates through

the arterial tree with every heartbeat, increases. Therefore, reflections of that pulse wave return to the heart earlier during systole, unlike usually during diastole, and the afterload on the heart during systole increases (16). As a consequence, a higher force is needed to pump blood into the vascular system and to maintain a steady stroke volume. This is why pulse pressure, which is the difference between systolic and diastolic blood pressure, increases causing isolated systolic hypertension and more work for the heart, which can result in left ventricular hypertrophy. At the same time, diastolic pressure, which is especially important for the perfusion of coronary arteries, decreases. All these changes in blood pressure have again adverse effects on the vascular system leading to a vicious circle causing further arterial stiffening and consequently resulting in cardiovascular disease. The effects of increased arterial stiffness on hemodynamics are illustrated in Figure 5.

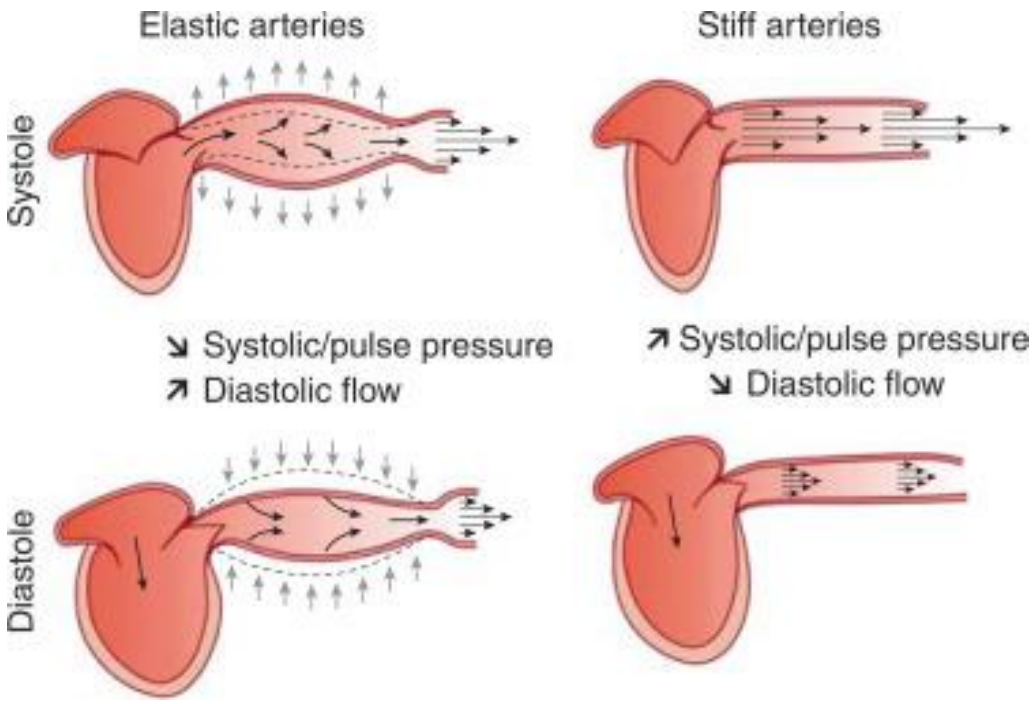

Figure 5: Consequences of arterial stiffness. Reproduced from: (17)

1.2.1 Pulse wave velocity measurement

The most recommended measurement to assess arterial stiffness non-invasively is the measurement of the pulse wave velocity. Pulse wave velocity is the speed at which the blood pressure pulse propagates through the arterial tree (12). When arterial stiffness increases, due to various reasons mentioned above, this speed increases as well. As the elastic capacities of the aorta have the largest impact on hemodynamics by means of smoothing blood flow, lowering pressure pulse and maintaining diastolic blood pressure,

the measurement of the aortic pulse wave velocity is the most clinically relevant (18). In fact, two different meta-analyses including 17 studies showed that the measurement of the aortic pulse wave velocity as a direct measure of the aorta´s stiffness is an independent predictor for cardiovascular disease (19)(20). For measuring the aortic pulse wave velocity, the distance between the carotid artery and the femoral artery is commonly used and recommended, because it covers the aortic system well and both arteries are easy to palpate. The distance can be measured externally, for example with a measuring tape, which is of course just an approximation of the real distance that the pulse wave propagates through the aorta, but with the benefit of being a non-invasive procedure. The carotid-to-femoral pulse wave velocity (cfPWV) is then calculated as the ratio of the distance *d* between the two set points and the time it takes for the pulse wave to travel from one point to the other. The calculation of the carotid to femoral pulse wave velocity is shown in Figure 6.

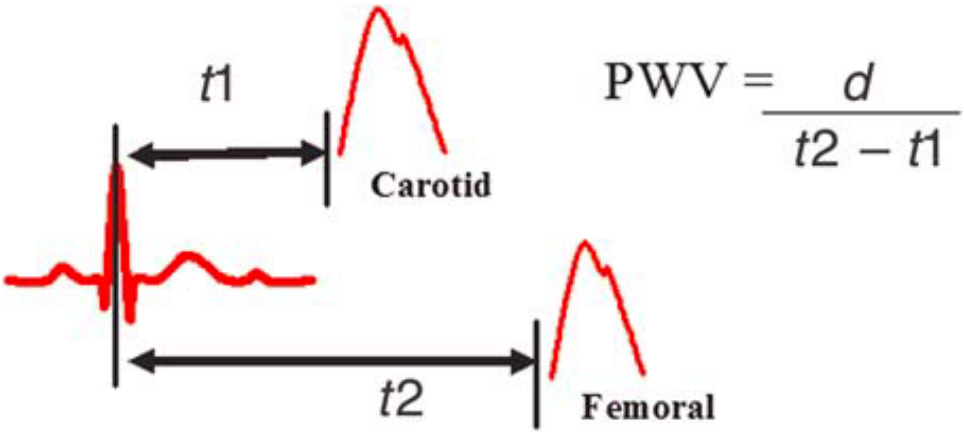

Figure 6: Calculation of pulse wave velocity. Reproduced from: (12)

The measurement of the pulse wave velocity is a simple and non-invasive way to assess a patient´s vascular health status. Because of its user-independency, results are highly reproduceable and it is suitable for investigations on large study populations (21). There is a big variety of different devices used to measure the time when the pulse wave reaches the measuring points at the carotid and the femoral artery. These devices either make use of applanation tonometry, Doppler ultrasound, oscillometry or mechanotransducers (18). All methods seem to achieve good and valid results and can be applied non-invasively.

With these methods, arterial stiffness can be assessed by means of structural and dynamic changes of arterial walls. A pulse wave velocity greater than 10m/s was established as an indicator for the presence of end organ damage of the vascular system (16). However, functional arterial stiffness, as measured by the pulse wave velocity, also highly depends

on blood pressure. Therefore, a fixed threshold value for indicating end organ damage seems controversial. As the aorta´s ability to distend decreases with increasing blood pressure, a rise in blood pressure also contributes to higher pulse wave velocity values (12). Consequently, arterial stiffness is greater in people with hypertension compared to age-matched normotensive individuals. Therefore, devices that automatically adjust the measured pulse wave velocity to blood pressure are preferable.

1.3 Pregnancy

1.3.1 Cardiovascular adaptations in pregnancy

During pregnancy the body of the mother undergoes extensive adaptations to meet the baby´s needs and prepare for delivery. The cardiovascular system of the mother is connected with the cardiovascular system of the baby via the placenta. Sufficient blood flow to the placenta is crucial to supply the growing fetus with nutrition and oxygen as well as to ensure the removal of waste products. To achieve that, physiologic adaptations like an increase in blood volume and cardiac output are made. Cardiac output is mainly increased by a substantial augmentation of stroke volume but also a slight increase in heart rate (22). At the same time, blood volume expands due to increasing plasma volume, which is achieved by a rise in retention of sodium and water through the kidneys (23). The amount of red blood cells also increases during pregnancy, but at a slower rate, therefore, a mild anemia in pregnancy is normal. To prevent hypertension the physiologic increase in cardiac output and blood volume is kept in balance through a drop in peripheral vascular resistance. In fact, there is an even larger drop of vascular resistance in the uterine circulation, shunting more blood to the growing fetus (24). The drop in uterine vascular resistance is based on remodeling of spiral arterioles from high resistance vessels to low resistance ones through a rise of elastin levels and sustained vasodilation. The most important vascular adaptations in pregnancy are summarized in Figure 7.

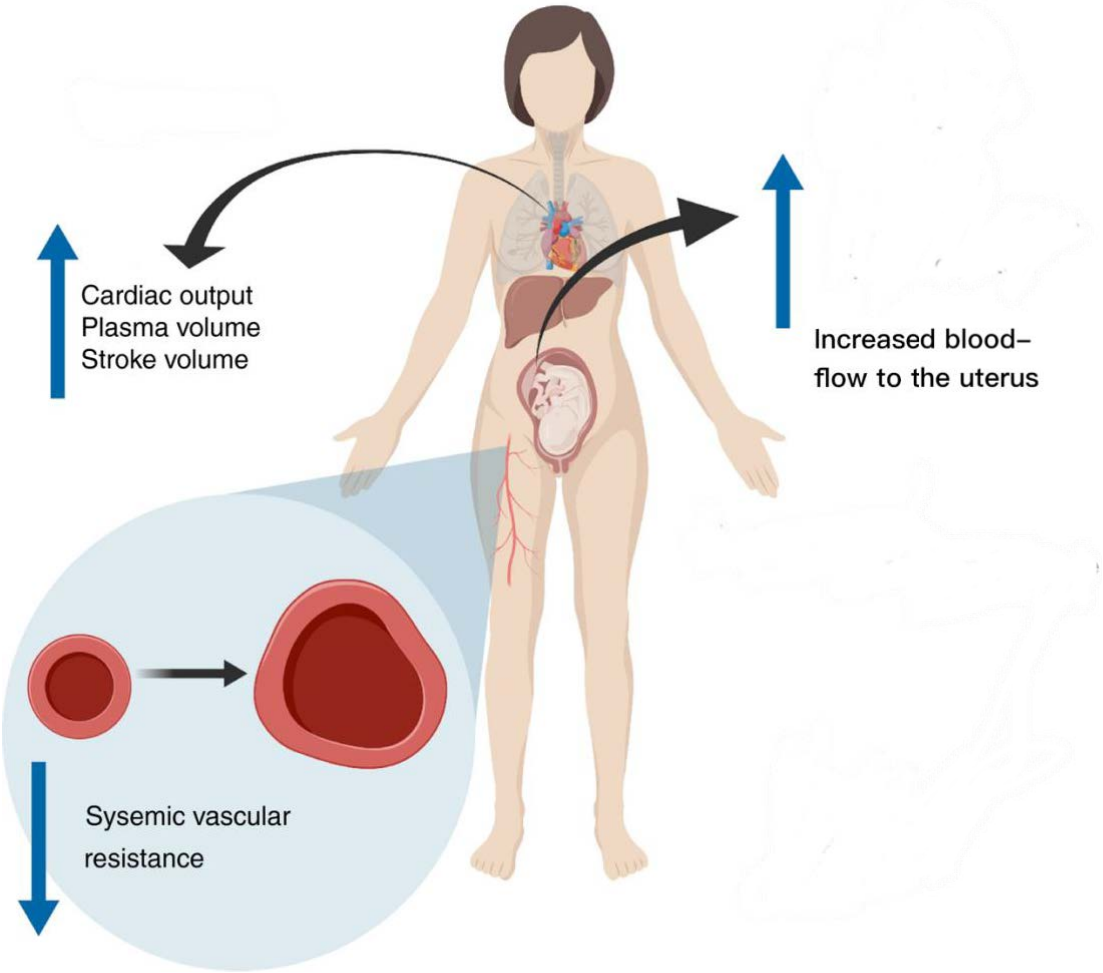

Figure 7: Vascular adaptations in pregnancy. Reproduced from: https://onlinelibrary.wiley.com/doi/full/10.1111/apt.15908

In early pregnancy outward hypertrophy of uterine vessels and angiogenesis, which is the formation of new blood vessels, are the main reasons for the decrease in vascular resistance. However, during late pregnancy, when the new vascular architecture is fully developed, low vascular resistance depends mainly on sustained vasodilation (24). This means a healthy pregnancy is not only dependent on the expansion of vessel size and the formation of new vessels, but also on the endothelial cell´s capacity to produce a sufficient amount of vasodilators. Uterine blood vessels can increase their production of vasodilators by either an increase in expression of vasodilation key mediators or the reorganization of post receptor signaling, so that responses to stimulating signals are greater and longer lasting (24). During pregnancy the expression of the endothelial nitric oxide synthase is up-regulated, and there is an increase in nitric oxide signaling (25). A very important activator for vasodilator production is the elevation of intracellular free calcium concentration. When intercellular signaling increases due to the formation of gap-junctions, calcium responses amplify and vasodilation is promoted (24).

Physical forces such as shear stress increase in pregnancy because of the hemodynamic adaptations mentioned above. It is an important trigger for the vascular system to increase its reactivity to regulate vascular tone. When shear stress is increased, calcium activated potassium channels open up, leading to an influx of calcium into the endothelial cells, which activates nitric oxide synthase, again leading to an increase in nitric oxide production (23). Besides its function in the regulation of vessel tone, an increase in shear stress also stimulates remodeling of arteries.

The steady increase in sex steroids like estrogen and progesterone during pregnancy also stimulates nitric oxide production and hence vasodilation. Additionally, a decrease of endothelial responsiveness to vasoconstrictors like angiotensin II was observed in the pregnant state. The endothelium´s ability to increase self-mediated vasodilation in pregnancy can be observed via the examination of flow mediated dilatation. This parameter reflects the dilation of an artery when exposed to hyperemia (23). The reaction is mainly caused by a release of nitric oxide by endothelial cells. During normal pregnancy an increase in flow mediated dilatation shows the physiologic adaptations of endothelial function to the changing hemodynamics. For a successful hemodynamic adaptation in pregnancy, the implantation and early development of the placenta is extremely important (26). Early disruptions in that development can have severe consequences for the mother and the fetus.

1.4 Preeclampsia

Preeclampsia is a systemic vascular disorder that affects about 2% to 8% of pregnancies worldwide and has severe consequences for both, the mother and the fetus (27). It is usually defined as new onset of hypertension occurring after 20 weeks of gestation combined with proteinuria, a sign of end organ damage of the kidneys (24). However, a significant amount of women face advanced systemic manifestations of the disease without a detectable amount of protein in the urine, which makes the condition sometimes hard to diagnose (28). The only known cure for preeclampsia is termination of the pregnancy with delivery of the placenta, implicating the important role of the placenta in the pathophysiology of the disease.

Most cases of preeclampsia affect young, healthy women in their first pregnancy. However, certain risk factors like obesity, diabetes mellitus, chronic hypertension or renal disease increase the risk for developing the disease (29). By definition, blood pressure in

pregnancy is too high when it rises above 140mmHg systolic or above 90mmHg diastolic and when this is measured at two different times with at least four hours apart. Besides proteinuria and hypertension, other symptoms like thrombocytopenia, liver or kidney dysfunction, pulmonary edema, headaches and blurred vision can occur. Preeclampsia is feared because it can progress rapidly and causes severe complications entailing the death of the mother and the fetus (28).

There is no other definite treatment for preeclampsia than delivery of the baby and the placenta. However, evaluating the right time for delivery can be challenging because the severity of the mother´s condition as well as the maturity of the baby need to be considered. A prophylaxis with low dose Aspirin is recommended for women with a high risk of preeclampsia. It is administered daily after 12 weeks of gestation and a recent Cochrane review shows that the risk can be reduced by as much as 18% (30).

Besides the severity of the acute disease, there is also strong evidence that women with preeclampsia are prone to a higher risk of cardiovascular disease and hypertension later in life. Therefore, further research and a better understanding of the pathophysiology of the disease is needed to identify new therapies and prevention strategies.

1.4.1 Pathophysiology

The pathogenesis of preeclampsia is highly complex and to this day not fully understood. There seems to be no definitive cause for the disease. It is more likely that a number of different factors influence vascular adaptations in pregnancy causing adverse effects on the mother´s vascular system. The pathophysiology of the disease can be divided into two stages and it originates at the very beginning of pregnancy when the trophoblast starts to invade the mother´s endometrium (31). The trophoblast is the outermost layer of cells of the newly developing baby, which later develops into the placenta. In a normal pregnancy the invasion of the trophoblast into the mother´s endometrium causes spiral artery remodeling which transforms the spiral arteries into low resistance, high capacity blood vessels.

In women with preeclampsia the implantation of the trophoblast is abnormal and spiral artery remodeling is incomplete. The important change in adhesion molecule expression, which trophoblasts usually perform, seems to be impaired in preeclampsia and thus trophoblast cells are unable to invade the uterine wall further than to the superficial parts of

the decidua (3). Therefore, maternal uterine blood vessels are not adequately remodeled and remain narrow, which impairs blood flow to the uterus, resulting in placental ischemia. The differences between uterine vasculature in preeclampsia, normal pregnancy and the non-pregnant state are shown in Figure 8.

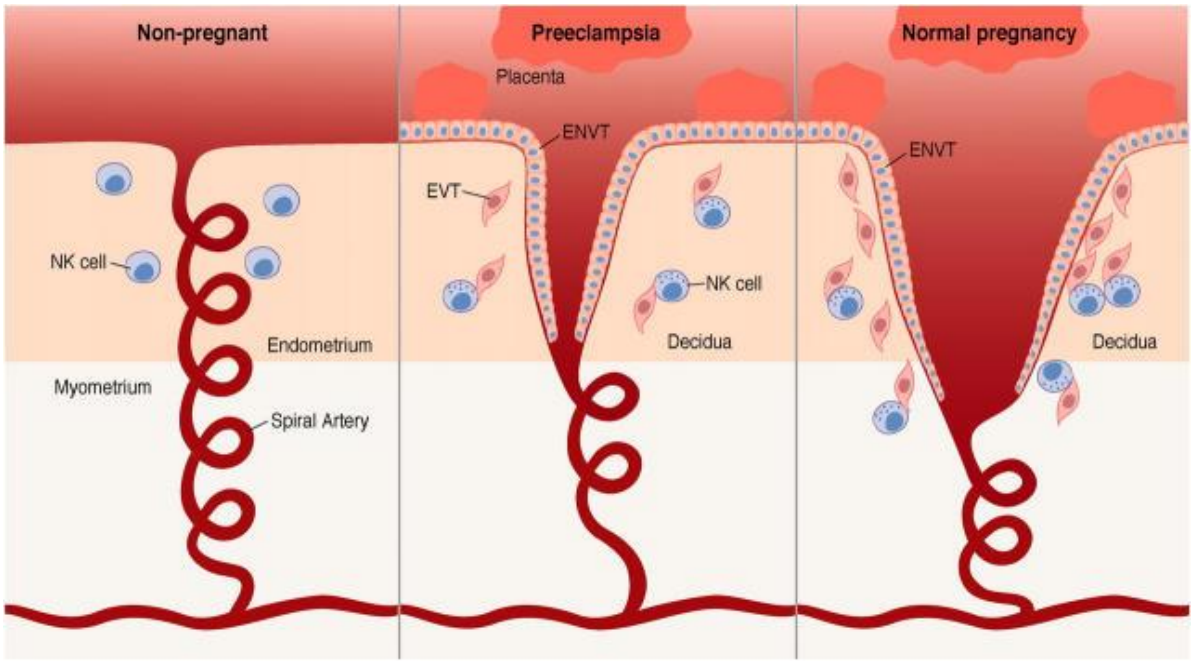

Figure 8: Impaired spiral artery remodeling in preeclampsia. Reproduced from: (32)

Genetic predisposition, preexisting risk factors and increased oxidative stress seem to promote abnormal trophoblast invasion. Moreover, there is strong evidence that an abnormal response of the mother´s immune system to the invading trophoblast plays an important part in the origin of the disease. For example, it was observed that in preeclampsia there is a predominance of the T helper cell type 1 phenotype, which releases proinflammatory cytokines that reduce trophoblast invasion (33). Also, a reduced exposure of the mother´s immune system to paternal antigens seems to play an important part in the pathophysiology of preeclampsia. This was suggested due to the fact that there is an increased risk of preeclampsia in women in their first pregnancies or in a subsequent pregnancy with a change in paternity (31).

The hypoxic state of the placenta triggers the second stage of the pathogenesis of preeclampsia, which is characterized by an excess of antiangiogenic factors like soluble endoglin (sEng) and soluble fms-like tyrosine kinase-1 (sFlt-1) (28). These factors are excessively produced by the hypoxic placenta and reduce the biologic activity of important mediators of endothelial cells. The soluble protein sFlt-1 binds to the proangiogenic proteins vascular endothelial growth factor (VEGF) and placental growth factor (PIGF)

and in this way inhibits their functions. These proteins are important for ensuring endothelial stability and the formation of new blood vessels. SEng binds to the transforming growth factor ß (TGFß), an important growth factor with anti-inflammatory capacities. The inhibition of these signaling proteins subsequently leads to inflammation and systemic endothelial dysfunction causing decreased nitric oxide and prostacyclin production, which impairs vasodilation (28). Additionally, an increased sensitivity to vasoconstrictive agents, like endothelin-1 and angiotensine-2, is observed in preeclampsia and procoagulant proteins are released by the endothelium (34). The two stages of the pathophysiology of preeclampsia are shown in Figure 9 below.

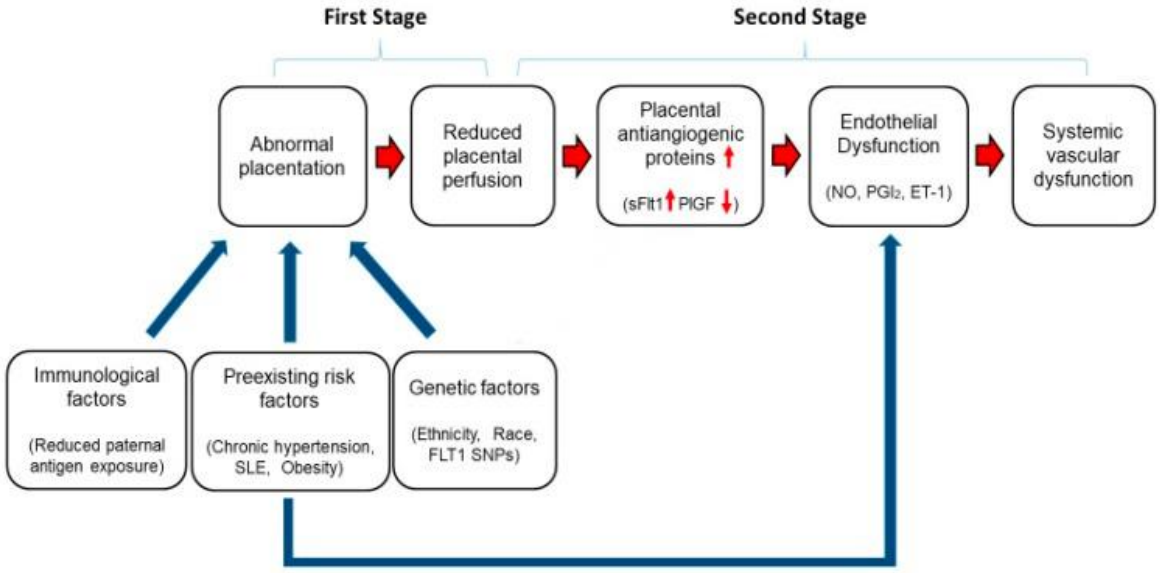

Figure 9: Pathophysiology of preeclampsia. **Reproduced from: (31)**

Generalized endothelial dysfunction and a vasoconstrictive state disrupt the vascular system and its ability to make necessary adaptations for pregnancy. During normal pregnancy arterial blood pressure physiologically decreases because of a drop in vascular resistance due to spiral artery remodeling and sustained vasodilation (24). In preeclamptic pregnancies widespread vasoconstriction and deficient spiral artery remodeling result in hypertension, and damage to the endothelium causes symptoms like proteinuria and edema. Although the pathophysiology of the disease originates from abnormal placentation, it progresses into a systemic vascular disorder based on endothelial dysfunction that severely impairs the mother´s vasculature.

1.4.2 The role of pulse wave velocity measurement in preeclampsia

During normal pregnancy, as maternal blood volume and cardiac output increase, hemodynamic adaptations of the mother´s vascular system prevent an increase in blood

pressure by lowering vascular resistance. This is achieved through angiogenesis and formation of new blood vessels early in pregnancy and sustained vasodilation in the third trimester. An increase in vasodilator production by the endothelium is necessary for maintaining a healthy pregnancy and occurs throughout the systemic circulation (24). This outlines the importance of unimpaired vascular function and a successful remodeling of the spiral arteries in pregnancy.

Non-invasive measurements like the pulse wave velocity (PWV) have proven to be useful for detecting patients with increased arterial stiffness, which seems to be an independent predictor for cardiovascular disease. Over the past few years PWV measurement has also been used to assess vascular function in pregnancy, as arterial stiffness is an indicator for vascular tone regulation and the elasticity of large arteries (18). A study that assessed aortic pulse wave velocity during pregnancy revealed a decrease in arterial stiffness during the second trimester and a slight increase during the third trimester. In the postpartum period PWV returned to the initial values before pregnancy (21). These results were supported by similar findings in other studies investigating changes in PWV during pregnancy (35)(36). This indicates that changes in maternal vasculature during pregnancy are measurable and PWV is a suitable technique for assessing arterial function.

As maternal arterial dysfunction is a key feature of preeclampsia, methods for assessing arterial function like PWV are suitable to determine differences in vascular function compared to normal pregnancies. Multiple studies using the PWV measurement showed that arterial stiffness was significantly increased in pregnancies affected by preeclampsia (37). Moreover, a systematic review including 23 studies revealed that more severe cases of preeclampsia were associated with even higher levels of arterial stiffness. This suggests that there is a direct correlation between the pathogenesis of preeclampsia and arterial stiffness (38).

The increased arterial stiffness in preeclampsia can be explained by increased oxidative stress and inflammation due to the release of antiangiogenic factors by the placenta, which subsequently causes endothelial dysfunction. One study came to the conclusion that PWV was as much as 18% higher in women with preeclampsia compared to values of normotensive pregnancies (39). This is a remarkable result considering the fact that in healthy individuals PWV usually increases by about 6% per decade (39). And this condition does not seem to recede after delivery, in fact, arterial stiffness was found to be increased up to three years after the pregnancy (36). Arterial function testing could also

prove to be useful for predicting the onset of preeclampsia at an early stage, as significantly increased levels of PWV were observed in pregnant women even before the disease became clinically evident. The predictive value of the PWV for the onset of preeclampsia is even higher when it is combined with other parameters like the maternal systolic blood pressure or parameters of endothelial dysfunction, like sFlt-1 (40).

Non-invasive measurement of vascular function using PWV seems to reveal the presence of systemic vascular dysfunction in women with preeclampsia. Furthermore, this technique could help to explore the association between preeclampsia and a risk of future cardiovascular disease. The persistence of arterial stiffness for years after the condition shows that the vascular system is altered compared to women with a normotensive pregnancy. However, it remains unclear whether an increased risk of cardiovascular disease in women who suffered from preeclampsia during pregnancy results from damages to the vascular system caused by the disease itself. As it could also derive from preexisting cardiovascular risk factors causing vascular dysfunction that leads to preeclampsia as well as cardiovascular disease in the future.

1.5 Cigarette smoking

1.5.1 Effects of smoking on vascular function

When cigarette smoke is inhaled, more than 4000 chemical substances enter the body, which are suspected to have negative effects on the vascular system and its function. Approximately 6 million people die every year from the consequences of tobacco consumption, and cigarette smoking is one of the most important causes of cardiovascular diseases like ischemic stroke and coronary heart disease (41). However, the exact mechanisms resulting in cardiovascular disease are not fully understood as the influence of cigarette smoking on the vascular system is highly complex.

One particularly harmful effect of cigarette smoking is the increase in oxidative stress via the absorption of large quantities of free radicals contained in the smoke. This leads to excessive amounts of free radicals in the body and defensive mechanisms are no longer sufficient, which usually keep them at bay. Because of their high reactivity, free radicals lead to mutations which can damage cells or even result in their death. Moreover, they play a major role in the regulation of vascular tone as reactive oxygen species modulate the bioavailability of nitric oxide as they can degrade and inhibit its synthesis (42). So

increased oxidative stress interferes with the vasodilatory and antithrombotic effects of nitric oxide contributing to endothelial dysfunction in smokers (43). Cigarette smoking also seems to promote an inflammatory state, as it was shown that markers of inflammation, like CRP and levels of white blood cells, are increased in smokers and even correlate with the extent of smoking (44). Inflammation of the vascular system is associated with atherosclerosis and therefore increases the risk of cardiovascular disease.

Although cigarette smoke contains many toxic substances, nicotine and carbon monoxide are the two with the most adverse effects on the cardiovascular system. Nicotine is an alkaloid stimulant which is naturally produced in the tobacco plant. It is known to boost the activity of the sympathetic nervous system increasing circulating levels of norepinephrine and epinephrine and rising heart rate and blood pressure (41). Nicotine also causes vasoconstriction in peripheral as well as coronary arteries, promotes inflammation of blood vessels and interferes in lipid metabolism resulting in an increase in serum lipid levels. All these factors contribute to the development of atherosclerosis and subsequently cardiovascular disease. A detailed summary of the adverse effects of nicotine on the vascular system is demonstrated in Figure 10.

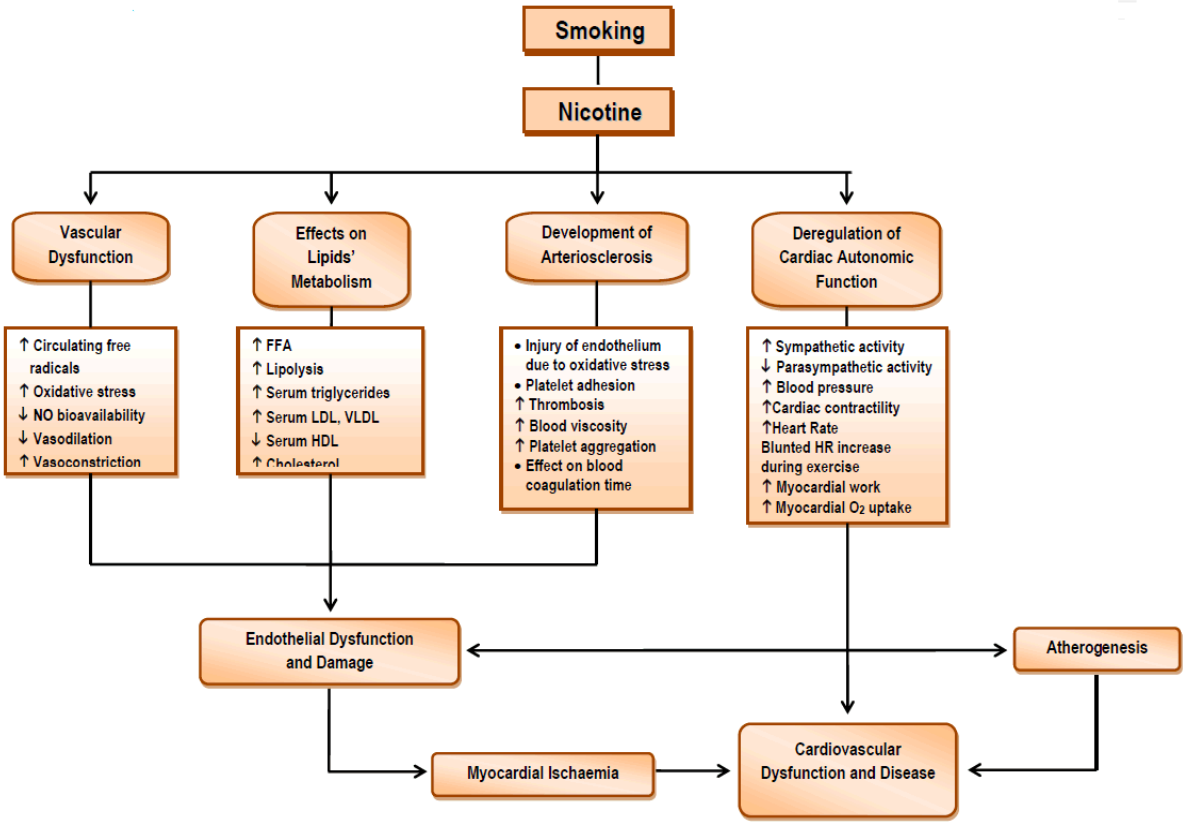

Figure 10: Effects of nicotine on the vascular system. Reproduced from: (41)

In contrast to nicotine, which is contained in the tobacco plant itself, carbon monoxide results from the combustion of tobacco. It binds to the protein hemoglobin in red blood cells with an affinity that is 200 times greater than the affinity between hemoglobin and oxygen. Therefore, levels of carboxyhemoglobin (COHb) increase in smokers and the oxygen supply of the tissue is compromised resulting in a hypoxic state and myocardial ischemia. In fact, carboxyhemoglobin levels were found to be as high as 5-10% in heavy smokers compared to 0.5-2% in non-smokers (41). Chronic exposure to carbon monoxide seems to enhance damages to the endothelium, and by compromising a sufficient oxygen supply to the tissue, vascular permeability increases. As a result, subendothelial edema occur and through deposition of fat in the arterial walls, early atherosclerotic changes occur preparing the ground for cardiovascular disease (41). The effects of carbon monoxide intake via cigarette smoking are summed up in Figure 11.

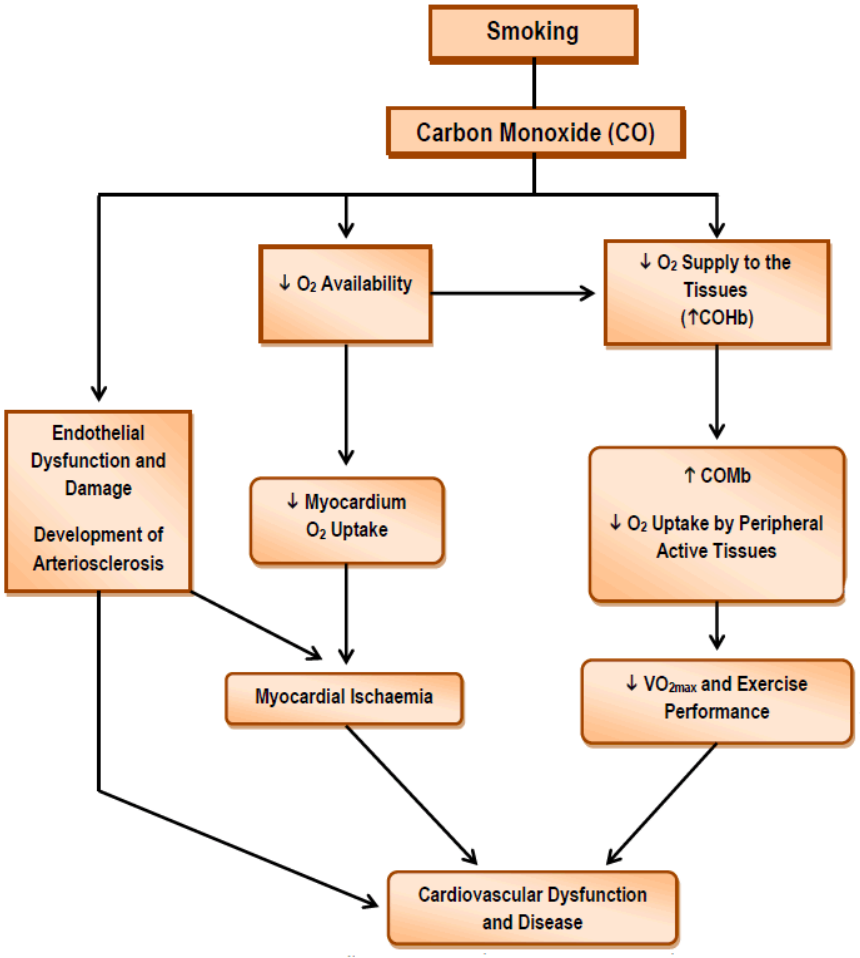

Figure 11: Effects of carbon monoxide on the vascular system. Reproduced from: (41)

Smoking is known to promote damage to the endothelium and causes endothelial dysfunction. The pulse wave velocity is often measured to assess endothelial damage and to detect structural as well as functional changes of arterial walls. Studies investigating acute effects of cigarette smoking on arterial stiffness showed consistent results by revealing an acute increase in arterial stiffness in non-smokers and even to a greater extent in smokers (45)(46). The majority of studies investigating the relationship between chronic smoking and arterial stiffness by using the pulse wave velocity measurement confirmed a positive correlation between these two parameters (47). However, some studies did not find a significant difference in arterial stiffness between chronic smokers and non-smokers, so this relationship is more controversial than the one between arterial stiffness and acute smoking (48).

Several mechanisms caused by cigarette smoking seem to contribute to increased arterial stiffness. As mentioned above, smoking increases oxidative stress and promotes a proinflammatory state. Inflammation leads to calcification of arterial walls and vascular remodeling. These structural changes are also supported by an increase in blood pressure due to cigarette smoking and its damage to the vessel wall, which promotes deposition of collagen, calcium and extracellular matrix in the arterial wall (49). Furthermore, the alteration of lipid metabolism leads to atherogenesis and increased intima media thickness. Insulin resistance also seems to be enhanced by cigarette smoking, and the association between arterial stiffness and mean daily glucose values has been shown in previous studies (49). Chronic smoking also damages the kidneys, which causes accumulation of collagen and calcification in elastic arteries further compromising their ability to distend.

However, these damages to the arterial walls causing increased arterial stiffness, seem to be somewhat reversible as it was discovered that former smokers had better endothelial function than current smokers. The effects of smoking on arterial stiffness are summed up in Figure 12.

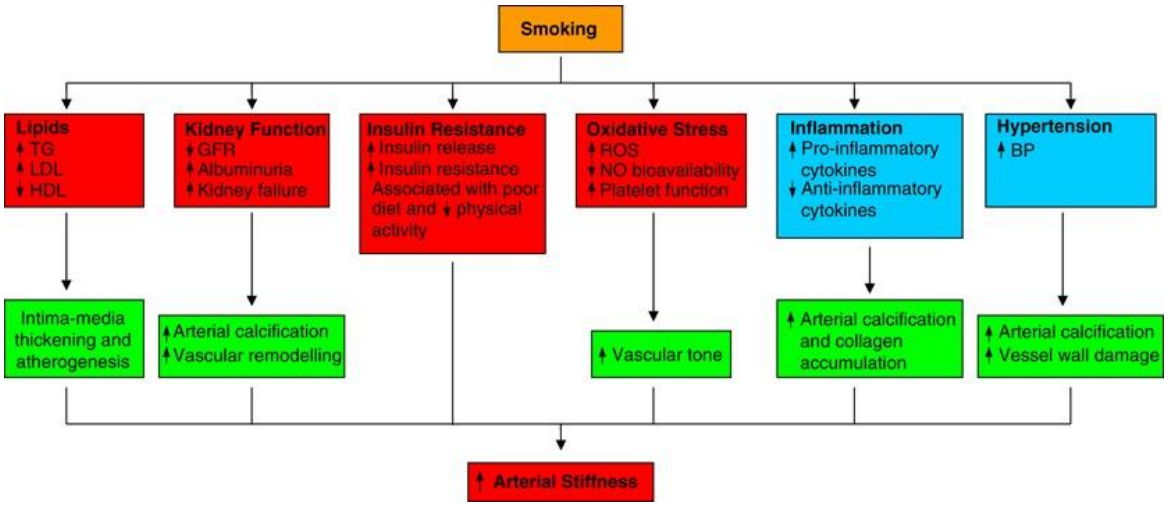

Figure 12: Pathogenesis of arterial stiffness caused by cigarette smoking. Reproduced from: (49)

1.5.2 Preeclampsia and the effects of cigarette smoking

Cigarette smoking during pregnancy is known to have adverse effects on the development of the fetus and causes severe complications during pregnancy. These include preterm delivery, premature rupture of the membranes and placental abruption (50). The risk of being born with a low birth weight is almost twice as high in babies born from mothers who smoked during pregnancy, compared to babies of non-smoking mothers. Hence, they are more likely to develop serious health problems and often face intellectual disabilities (51). Even deformities in the fetus can occur due to cigarette smoking during pregnancy. Nicotine contained in cigarette smoke promotes vasoconstriction in placental blood vessels, so the oxygen supply of the baby is impaired. Additionally, poisonous carbon monoxide enters the baby´s blood stream and replaces oxygen molecules by binding to hemoglobin with greater affinity decreasing the oxygen supply of the fetus even further. Therefore, it is strongly recommended to quit smoking before pregnancy. If this is not possible, a reduction in the amounts of cigarettes smoked per day was shown to be beneficial.

Despite all the negative effects of cigarette smoking, there are some diseases where cigarette consumption seems to decrease the risk of the onset of the disease. Preeclampsia was shown to be one of those disorders. In fact, the overall risk reduction for preeclampsia due to cigarette smoking during pregnancy was discovered to be up to 50% compared to non-smoking pregnant women (50). There even seems to be a dose-response pattern, which means that heavy smoking further reduces the risk of developing the disease. Numerous studies, conducted all over the world have shown consistent results regarding cigarette

smoking during pregnancy and a decreased risk of preeclampsia (50). Since then, great effort has been put into the discovery of the underlying biochemical mechanisms that lead to this rather paradoxical effect as it could provide a better understanding of the pathophysiology of preeclampsia and help to develop new treatments and prevention strategies.

As described previously, pathogenesis of preeclampsia is a two staged process starting with abnormal placentation and resulting in a disbalance of pro- and anti-angiogenic factors, which causes endothelial dysfunction. Pro-angiogenic growth factors play an important role in the process of spiral artery remodeling and maternal vascular function in general. The excess of anti-angiogenic factors observed in preeclampsia is hence a plausible explanation for vascular dysfunction in preeclampsia. It is hypothesized that pregnant women show a special threshold for angiogenic disbalance, and preeclampsia occurs when it is exceeded (52). Cigarette smoking seems to influence concentrations of pro- and anti-angiogenic factors towards a more balanced ratio, which reduces the risk of the onset of the disease. Circulating levels of proangiogenic factors like the vascular endothelial growth factor (VEGF) and the placental growth factor (PlGF) are both decreased in preeclamptic women. However, cigarette smoking seems to increase placental expression of VEGF and therefore may let blood levels of VEGF rise enough to reduce the risk of preeclampsia (50). Furthermore, concentrations of the antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1), which is increased in preeclamptic women and antagonizes PlGF and VEGF, were discovered to be significantly lower in pregnant women who smoked compared to non-smokers in both, normal pregnancies and preeclamptic ones (53). Other protective effects of cigarette smoking against preeclampsia might be attributed to its immunosuppressive power as preeclampsia is thought to be caused by an altered immunologic response of the mother´s immune system to the allogeneic fetus.

A large study was conducted to discover whether nicotine was the main ingredient in cigarette smoke that contributed to the protective effect against preeclampsia (54). In this study, the risk of preeclampsia was investigated in a group of women who smoked during pregnancy and compared to the risk of a group of women that used Swedish snuff, a smokeless tobacco, where nicotine is absorbed via the oral mucosa and rises nicotine blood levels as high as cigarette smoking but without the consumption of products of combustion. Women who smoked during pregnancy had a reduced risk for preeclampsia,

whereas snuff users did not. These findings suggest that rather components of combustion than nicotine mediate the protective effect of cigarette smoking. Additionally, in vitro studies showed that the exposure of endothelial cells to carbon monoxide, which is a major component of combustion, decreased the release of sFlt-1 further supporting the findings of the study on snuff and cigarette smoking (55).

Most studies that determined a protective effect of cigarette smoking on preeclampsia risk refer to cigarette smoking during pregnancy. However, awareness of the predominantly negative effects of smoking during pregnancy entails a high rate of women who stop smoking before they become pregnant or during early pregnancy. Studies evaluating the risk of preeclampsia in these groups of women showed inconsistent results. Some revealed that women who quit smoking before, or early in pregnancy, especially when they were heavy smokers, also had a slightly decreased risk of developing preeclampsia. Though these results were mostly not statistically significant (56)(57). Others found no evidence that smoking before pregnancy reduced the risk of preeclampsia (58).

2 Aims and objectives

Preeclampsia is among the most severe pregnancy complications responsible for high morbidity and mortality in both, the mother and the fetus. Since the protective effect of cigarette smoking during pregnancy on the risk of preeclampsia became evident, numerous studies have focused on this rather conflicting relation.

However, little is known about the effects of cigarette smoking before pregnancy on the cardiovascular system and its impact on the pathogenesis of preeclampsia. Therefore, this diploma thesis aims to assess vascular function in women with a high risk of preeclampsia and the influence of cigarette smoking before pregnancy on these parameters.

For this purpose, the pulse wave velocity, a marker for vascular function, was assessed in women with a high risk of preeclampsia several times during pregnancy. The participants were divided into two groups, the group of non-smokers and the group of women who used to smoke before they got pregnant. So far, many studies have dealt with the association between cigarette smoking and the risk of preeclampsia, but this is the first study to investigate the effect of cigarette smoking before pregnancy on vasculature and vascular function changes during pregnancy. The results of this study could provide a better understanding of the complex association between cigarette smoking, its impact on vascular function during pregnancy and the risk of preeclampsia.

3 Material and methods

The data processed in this diploma thesis were collected as part of a prospective study on the influence of nicotine consumption on the risk of developing preeclampsia. This study was performed at the department of Obstetrics and Gynecology at the Medical University of Graz. An ethics permission was obtained before the recruitment of study participants could be started (EK: 31-541 ex 18/19).

3.1 Participants

Inclusion criteria: For this study the only inclusion criteria was an estimated high risk at the first trimester-screening for preeclampsia. The risk was calculated by an algorithm considering maternal risk factors, biophysical parameters and maternal biomarkers.

After a positive first trimester screening for preeclampsia, a dose of 150mg acetylsalicylic acid was prescribed to prevent the onset of the disease.

Exclusion criteria: The only exclusion criteria for the study was the discontinuation of the prescribed acetylsalicylic acid intake.

Potential participants in the study were informed about the study protocol and the possibility of exiting the measurements whenever desired. All women were asked to give written informed consent before participating in the study. The participants had to undergo a thorough clinical assessment of their vascular function and cardiovascular health by an experienced physician prior to the testing.

Patients were divided into two different groups based on their self-reported smoking status. One group consisted of women who used to smoke before pregnancy but then stopped, and the other group consisted of non-smoking pregnant women.

3.2 Study protocol

Data for this diploma thesis were obtained from pulse wave velocity measurements of the participants at three different points in time during pregnancy. The first measurement was carried out between the 13th and 17th week of gestation, followed by measurements at around week 24 of gestation and after week 37 of gestation. All measurements were performed in the same room at the Department of Obstetrics and Gynaecology at the

Medical University of Graz. Special attention was paid to ensuring a quiet and comfortable environment for the participants, so that the measured values were not distorted by disruptive elements such as loud noise. Room temperature was kept at approximately 24 degrees Celsius.

The participants were requested to avoid stressful activities such as doing sports or exercising and to refrain from consuming stimulating substances like coffee 24 hours prior to the appointments. On the day of the measurements, the participants were asked to have only a light breakfast and to empty their bladder before the testing. A short medical history was taken from every participant with special focus on signs and symptoms of pregnancy complications like pregnancy induced hypertension. After that, blood pressure was measured non-invasively in a sitting position, followed by the measurement of the pulse wave velocity.

3.3 Data collection

Pulse wave velocity was measured in a supine position using Vicorder. This is a vascular testing device that detects the time of arrival of the pulse wave at two measuring points oscillometrically. It is connected to a computer which then calculates the pulse wave velocity as the ratio of the distance between the two set points and the time it takes the pulse wave to travel from one point to the other. Vicorder automatically adjusts the patient´s pulse wave velocity to their blood pressure.

Before the measurement could start, two blood pressure cuffs had to be attached to the patient. To calculate the carotid-femoral pulse wave velocity, one cuff was placed around the participants´ neck with the sensor above the carotid artery and the other one was positioned as high as possible around the left thigh. A demonstration of the right adjustment of the blood pressure cuffs is shown in Figure 13.

Figure 13: Positioning of the blood pressure cuffs.
Picture taken by the author

Before the cuffs were inflated, the distance between the cuffs was measured with a measuring tape. This value, together with the participants´ blood pressure, had to be entered into the dialog box on the computer, so that the pulse wave velocity could be calculated and adjusted to the blood pressure. The cuffs were inflated to 65mmHg and the oscillometric signals were obtained in real time. The measurement lasted until the quality of the waveform on the screen was valid. After completing the measurement, the mean value of the pulse wave velocity was automatically calculated.

3.4 Data analysis and statistical methods

Data from 31 different women at high risk of developing preeclampsia were collected. Of those, 28 women were submitted to the first pulse wave velocity measurement between week 13 and 17 of gestation. The second measurement, at around the 24th week of gestation, was performed on thirteen women and seven women underwent the third measurement after the 37th week of gestation, as shown in Table 1. At this point it has to be noted that two women who took part in the second measurement did not take part in the first one and two women who participated in the third measurement did not participate in

the second one. The participants were divided into two groups based on their smoking status as described above. Due to the small sample size of the third measurement, it was not considered for statistical analysis. However, a qualitative analysis of five women who participated in all three measurements was carried out and is presented in section 4.3.

Table 1: Number of participants per group and measurement

	1st measurement	2nd measurement	3rd measurement
Former smokers	n = 13	n = 6	n = 3
Non-smokers	n = 15	n = 7	n = 4
Total	n = 28	n = 13	n = 7

The statistical analysis was performed by using IBM SPSS Statistics version 27. Whenever the assumption of normality was required, data were tested by using Kolmogorov-Smirnov and Shapiro-Wilk tests as well as Q-Q-plots. In all considered cases normality was given. Two different analyses were performed. Firstly, data collected from measurement one and measurement two were compared of those women who participated in both measurements. For this comparison, data were not divided into two groups but viewed as one, to investigate whether there was a significant change in the development of the pulse wave velocity between the first and the second trimester of pregnancy. The differences between measurements one and two were tested for normality before a paired t-test to compare the mean values was applied. Secondly, pulse wave velocity between the two groups was compared for the first and the second measurement respectively. To ensure the comparability between the groups, homogeneity with respect to the parameters age and body mass index was investigated beforehand. In both cases t-tests for unpaired samples were used. Due to the variability in the sample sizes and subjects between the measurements and the resulting missing values, it was not possible to perform an analysis of variance, therefore t-tests were used.

4 Results

4.1 Participants and baseline characteristics

The baseline characteristics of the 31 women as a whole as well as divided into the two groups of former smokers and non-smokers are shown in Table 2. There were no statistically significant differences between the two groups concerning the participants´ age or their body mass index. This also applied to the subgroups of the 28 women who underwent the first measurement and the thirteen women who took part in the second measurement. Most women were in their first pregnancy.

Table 2: Baseline characteristics

	Overall collective	**Former smokers**	**Non-smokers**
Number	31	15	16
Age (mean ± std)	32.81 (± 5.07)	31.80 (± 6.03)	33.75 (± 3.94)
BMI (mean ± std)	26.755 (± 6.15)	27.54 (± 6.36)	26.11 (± 6.11)
Pregnancy			
1	n =13 (41.94%)	n = 5 (33.33%)	n = 8 (50.0%)
2	n = 10 (32.25%)	n = 5 (33.33%)	n = 5 (31.25%)
3	n = 6 (19.35%)	n = 3 (20.0%)	n = 3 (18.75%)
4	n = 2 (6.45%)	n = 2 (13.34%)	n = 0

4.2 Development of the pulse wave velocity

To be able to investigate the development of the pulse wave velocity throughout pregnancy, mean values for all three measurements were calculated. These values are summarized in Figure 14. It shows that the participants´ pulse wave velocity slightly increased between the period of the first and second measurement and decreased again between the second and the third measurement. However, as the number of women participating in the different measurements varied and some women who participated in the first measurement did not take part in the second one, and vice-versa, these mean values do not represent the exact longitudinal development of the pulse wave velocity.

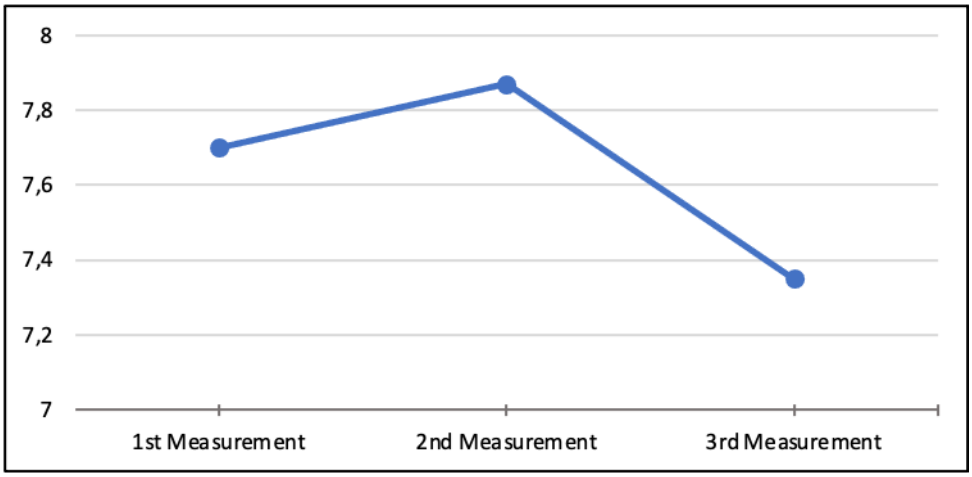

Figure 14: Mean pulse wave velocity measured in m/sec. The blue line corresponds to mean values of the pulse wave velocity of the full sample at the three different measurements.

To investigate whether there is a statistically significant difference between the mean values of the first and the second measurement, data could only be used from women who underwent the first as well as the second measurement. This applied to eleven participants. Even though their pulse wave velocity increased between the first and the second measurement, the difference was not statistically significant with the p-value shown in Table 3. As the number of participants undergoing the third measurement was too small, no statistical analysis of the development of the pulse wave velocity between the second and the third measurement was possible.

Table 3: Mean pulse wave velocity (PWV) of the eleven women who underwent both the first and second measurement. The p-value corresponds to a paired t-test with a 5% level of significance.

	1st Measurement	2nd Measurement	p-value
PWV (mean ± std)	7.44 (± 0.986)	7.92 (± 1.508)	0.162

To identify possible differences between the two groups based on the participants´ smoking habits, mean values of the pulse wave velocity between the groups for all the three measurements were compared. As demonstrated in Figure 15, within the group which included women who used to smoke before pregnancy the mean pulse wave velocity was initially lower and remained roughly at that level at the second measurement, whereas the mean pulse wave velocity of the group which included the non-smoking women increased by 0.31 m/sec. Mean values calculated at the third measurement decreased in both groups and converged towards a similar value.

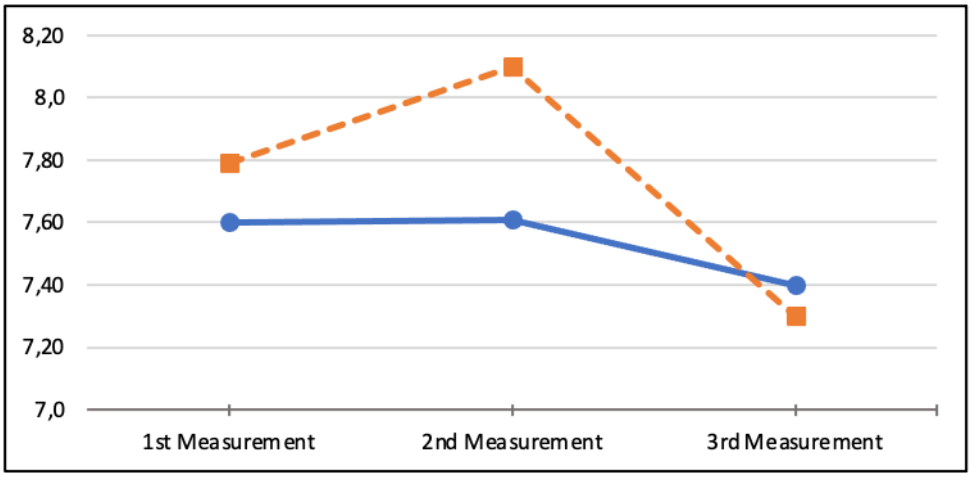

Figure 15: Mean values of the pulse wave velocity in m/sec for women who used to smoke before pregnancy (continuous line) and non-smoking women (dotted line).

However, the statistical analysis of the mean values did not show any significant differences between the two groups concerning measurement one and measurement two, as shown in Table 4. For measurement three not enough data were available to be able to examine the statistical level of significance between the two groups.

Table 4: Comparison of the mean pulse wave velocity (PWV), age and body mass index (BMI) between the group of women who used to smoke before pregnancy and the group of non-smoking women of the first and the second measurement. The p-value corresponds to an unpaired t-test with a 5% level of significance.

	Former smokers	Non-smokers	p-value
1st Measurement			
Number	13	15	
Age (mean ± std)	31.69 (± 6.25)	33.67 (± 4.065)	0.325
BMI (mean ± std)	27.192 (± 6.435)	26.1 (± 6.33)	0.655
PWV 1 (mean ± std)	7.6 (± 1.051)	7.793 (± 1.275)	0.668
2nd Measurement			
Number	6	7	
Age (mean ± std)	29.33 (± 7.202)	33.29 (± 3.773)	0.231
BMI (mean ± std)	24.967 (± 5.812)	23.886 (± 3.322)	0.682
PWV 2 (mean ± std)	7.617 (± 0.856)	8.1 (± 1.754)	0.553

4.3 Case studies of selected participants

Five women underwent all three measurements throughout their pregnancy. Two of these, patient 1 and patient 2, used to smoke before pregnancy. Patient 1 is a 37-year-old woman in her first pregnancy. A body mass index of 21 was measured at the beginning of her pregnancy, which is referred to as normal weight. There were no relevant pre-existing medical conditions and no long-term medication besides the newly prescribed Aspirin because of her high risk of developing preeclampsia. The patient´s pulse wave velocity values were continuously increasing throughout pregnancy between the first and the second measurement.

Patient 2 is a 26-year-old woman in her third pregnancy with a body mass index of 34.7, which is referred to as class 1 obesity. She suffers from chronic hypertension and is treated with antihypertensive medication because of it. Concerning the patient´s pulse wave velocity, it is significant to mention that her value at the first measurement was much higher compared to patient 1. It remained high during pregnancy with only showing a small decrease at the third appointment. However, the difference to patient 1 was getting smaller throughout pregnancy as the pulse wave velocity of patient 1 was continuously increasing. The pulse wave velocity of patient 1 and patient 2 is shown in Figure 16.

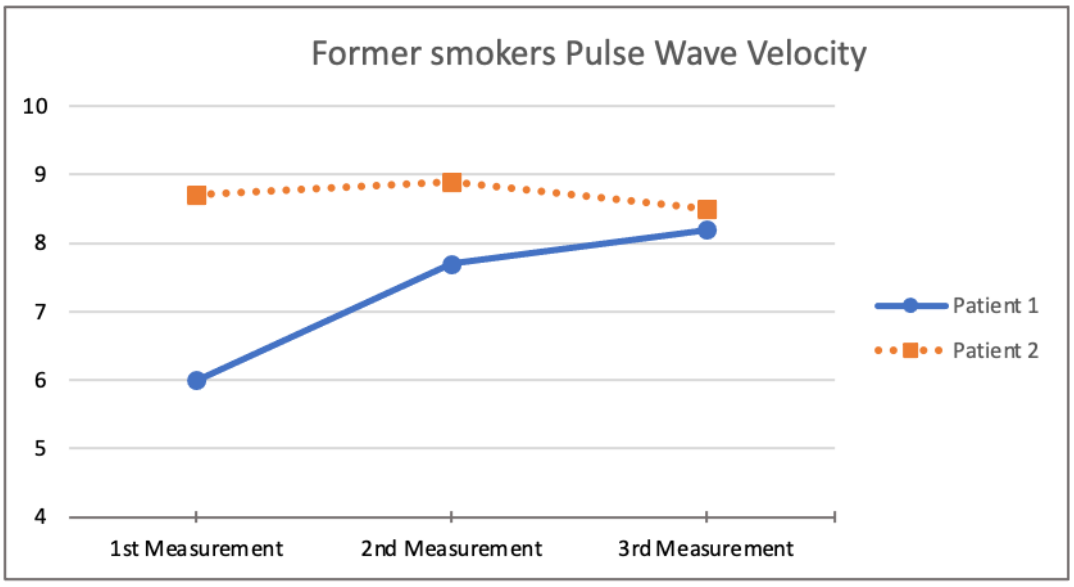

Figure 16: Pulse wave velocity of patient 1 and patient 2, both women who used to smoke before pregnancy.

Three non-smoking women took part in all three measurements throughout their pregnancies. Patient 3 is 35 years old and pregnant for the second time. She is of low weight with a body mass index of 18.4 and suffers from hypothyroidism. Therefore, she is

medicated with Thyrex 50mg. Pulse wave velocity values increased between the first and the second appointment and decreased again towards the third one.

Patient 4 is a 37-year-old woman in her first pregnancy and has a body mass index of 26.1. She suffers from diabetes mellitus type 1 and has to inject insulin on a regular basis. Throughout pregnancy her values of the pulse wave velocity remained at the same level but showed an increase towards the third measurement.

The last patient from this group, patient 5 is a 33-year-old woman in her first pregnancy who suffers from hypothyroidism and is, like patient 3, medicated with Thyrex 50mg. With a body mass index of 22.8, she is of normal weight. Between the first and the second measurement, pulse wave velocity values increased to a great extent. However, until the third measurement the value decreased again. Values of pulse wave velocity of patients 3, 4 and 5 are shown in Figure 17.

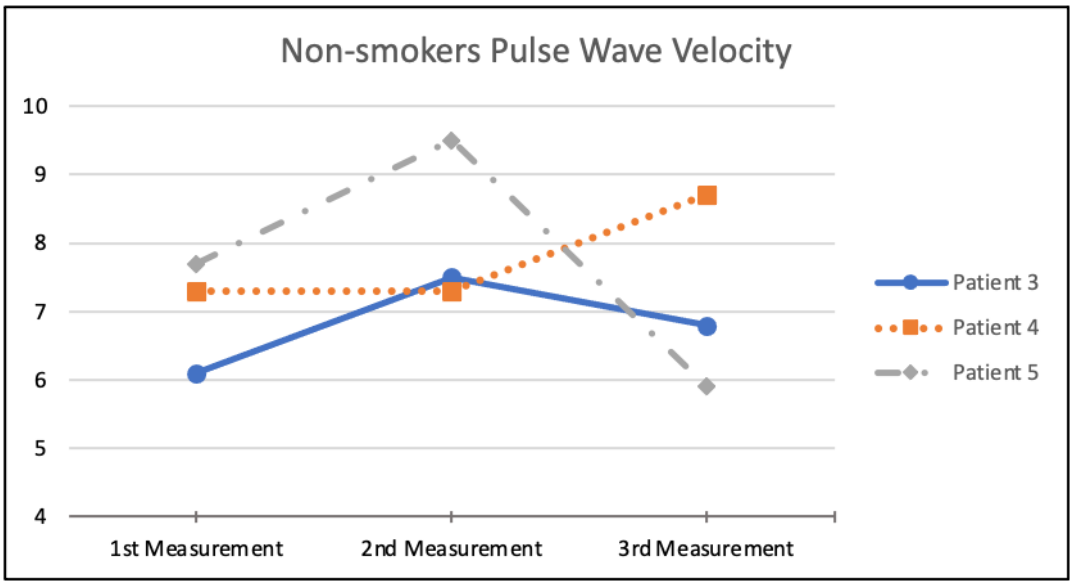

Figure 17: Pulse wave velocity of patients 3, 4 and 5 of the group of non-smokers

5 Discussion

The pulse wave velocity of the participants of our study increased on average between the first and the second measurement. This tendency could be interpreted as an indicator for a pathological process within the vascular system as studies investigating vascular adaptations in healthy pregnancies show that the pulse wave velocity usually decreases between the first and the second trimester (36). The major inclusion criteria for our study was an estimated high risk at the first trimester-screening for preeclampsia. This means that all women who participated in our study were prone to possible disruptions in vascular adaptations during pregnancy. Hence, this could be an explanation why our observations are more in line with studies investigating arterial stiffness in pregnancies complicated by preeclampsia. Oyama-Kato et al., for example, showed an increase in arterial stiffness in proportion to the progress of the pregnancy, and other studies investigating the same parameters were in line with these findings (21). However, the slight decrease of the mean pulse wave velocity towards the third measurement observed in our study cannot be explained by this assumption. As only seven women took part in the third measurement after week 37 of gestation, the validity of this value is limited. Still, it could be speculated that this development is the result of a sufficient therapeutical intervention, which corresponds to the fact that none of these women was diagnosed with preeclampsia.

The non-smoking women who participated in our study had on average a higher mean value of the pulse wave velocity at the first measurement than the women who used to smoke before pregnancy. This difference became even bigger towards the second measurement as the pulse wave velocity of the non-smoking women continued to increase and the one of the non-smoking women remained at the same level. This was interesting to see as cigarette smoking is usually known to increase arterial stiffness. Binder et al., for example, investigated the stiffness index, which has been proved to positively correlate with the pulse wave velocity, in a group of young smokers who had been smoking for five to ten years and compared it to young, non-smoking individuals (47). In those who smoked increased arterial stiffness was observed, which indicates that adverse effects on the vascular system even occur in young smokers. So, the question is why the women in our study who used to smoke before pregnancy had lower mean values of the pulse wave velocity than the non-smoking women. There are several possible explanations for this observation. Firstly, in our study no distinction was made between light and heavy smokers

within the group of women who used to smoke before pregnancy. Furthermore, the exact amount of cigarettes per day was not inquired. It is hence possible that most women within our group were lighter smokers than the ones participating in the study of Binder et al., where the average participant smoked about 13 cigarettes a day, and therefore no impact on the vascular system was measurable in our study (47). Secondly, we measured the pulse wave velocity between the carotid and the femoral artery which covers the speed of the pulse wave as it propagates through the aorta. Binder et al. investigated the stiffness index of the radial artery. So, it can be assumed that the impact of cigarette smoking on arterial stiffness is greater in small arteries than in larger ones. Thirdly, the unexpected discrepancy between the groups in our study could be attributed to the influence of cigarette smoking before pregnancy on the vascular system and the risk of preeclampsia. All women in our study showed an increased risk of developing preeclampsia. The increase in the pulse wave velocity between the first and the second measurement within the group of non-smoking women might indicate a pathologic process within the vascular system even though the disease did not manifest clinically. Smoking during pregnancy is known to decrease the risk of the onset of the disease. In our study we assessed the influence of smoking before pregnancy on the pathogenesis of preeclampsia. One could speculate that the lower pulse wave velocity values of the group of women who used to smoke before pregnancy are a sign of the protective effect of cigarette smoking before pregnancy on the risk of preeclampsia.

The data acquired from the participants who took part in all three measurements throughout pregnancy provide a possible insight in vascular function changes in five different cases of women at high risk of preeclampsia. By taking a closer look at the participants´ history, it became evident that vascular function changes during pregnancy are highly individual and also depend on factors like lifestyle, medication and pre-existing medical conditions. In contrast to findings regarding the whole study cohort where differences between prior smokers and non-smokers were observed, when comparing the data of the five women who took part in all three measurements, no remarkable differences between former smokers and non-smokers were noticed. However, what is striking is the fact that vascular function changes in all five women differ clearly from developments that are usually observed in healthy pregnancies. Many studies investigated the changes of vascular function throughout healthy pregnancies and their observations were mostly in line with each other. Pulse wave velocity was repeatedly shown to decrease during the second trimester and increase again throughout the third trimester and delivery (36)(35).

None of the five women in our study showed such a decrease in the second trimester, quite the contrary, four women showed an increase towards the second measurement at around week 24 of gestation, and one did not show a change in pulse wave velocity at all. These developments are quite consistent with observations of pregnancies complicated by preeclampsia, where the pulse wave velocity increases in proportion to the progression of the pregnancy (21). All women taking part in our study were identified to have an increased risk of developing preeclampsia. Even though none of the women was diagnosed with preeclampsia, these findings might indicate a pathologic process compromising vascular function changes during pregnancy.

Comparing the two patients who used to smoke before pregnancy, the first thing that becomes apparent is the difference of the pulse wave velocity measured at the first appointment between 13 and 17 weeks of gestation. Both values, 6 m/s for patient 1 and 8.7 m/s for patient 2, are still within the normal range according to the established threshold of 10 m/s by Van Bortel et al. (59). However, when considering the findings of Vlachopoullos et al., it has to be taken into consideration that even an increase of 1.0 m/s has a major impact on the cardiovascular system and was shown to increase the risk of cardiovascular events by 14% (60). So a difference of almost 3 m/s is quite remarkable and the question is why these two women show such different results.

In previous studies, increasing age was identified as one of the main determinants of arterial stiffness. Per year, a human´s pulse wave velocity increases approximately by 0.1 m/s (61). Unlike these findings, patient 2, who showed higher values of the pulse wave velocity, is in fact eleven years younger than patient 1. So, in this case, the difference in age does not seem to be the reason for the discrepancy. However, a possible explanation could be the chronic hypertension patient 2 was diagnosed with. High blood pressure per se is known to alter arterial stiffness as a vessel`s ability to distend decreases with increasing blood pressure. Furthermore, structural changes of vessel walls were observed in patients with chronic hypertension, which also contribute to arterial stiffness (61). Another possible explanation could be the big difference in the patients´ body mass index. With a BMI of 34.7, patient 2 is not far from the threshold of obesity class II. Current literature shows inconsistent results for the relationship between obesity and arterial stiffness. As obesity is often associated with cardiovascular diseases, and arterial stiffness is an independent predictor for the onset of such diseases, a positive relation seems plausible. However, a large study by Desamericq et al. came to the conclusion that arterial

stiffness did not differ between subjects according to weight (62). It was stated that previous studies, reporting a positive relationship, were likely biased by the presence of other cardiovascular risk factors that promote arterial stiffness, like diabetes mellitus, which are often associated with obesity. Additionally, it was suggested that the surface measurement of the distance between the carotid and the femoral artery in obese patients, which is required for the calculation of the pulse wave velocity, could lead to an overestimation of the arterial length. Towards the second measurement, the pulse wave velocity of patient 2 increased by 0.2 m/s and finally decreased towards the third one after week 37 of gestation. This slight improvement of vascular function towards the end of the pregnancy could be explained due to the positive effect of smoking cessation on vascular function. Some studies investigating the effects of smoking cessation on the vascular system claim that positive changes of vascular function can be detected in the months after cigarette abstinence (63). Rehill et al. even stated that such changes in arterial elasticity could be seen in subjects as early as four weeks after quitting smoking (64). However, other studies did not find a significant improvement in arterial stiffness after six months, or even two years after smoking cessation (65)(66). So, the effect of quitting smoking on the vascular system is still a subject of debate.

The pulse wave velocity of patient 1 was continuously increasing throughout pregnancy and at the third measurement almost reached a value as high as the one of patient 2. This development corresponds with changes in pulse wave velocity observed in pregnancies complicated by the onset of preeclampsia (38). However, patient 1 did not show the features such as new onset of hypertension occurring after 20 weeks of gestation combined with proteinuria, which are required for the diagnosis of the disease. Still, the changes of pulse wave velocity could suggest pathologic changes of the patient´s vascular system, though not severe enough to become clinically evident.

Out of the three women who stopped smoking before pregnancy, two suffered from hypothyroidism and one from diabetes mellitus type one. Both diseases are known to affect vascular function and increase arterial stiffness (67). Higher levels of fasting blood glucose were shown to be closely related to increased arterial stiffness (61). Especially the duration of the disease and the degree of therapeutic control are decisive for the influence on the vascular system. The increase of the pulse wave velocity of patient 4 towards the third measurement could be interpreted as a sign that the antidiabetic therapy during pregnancy was not sufficient enough. During pregnancy the requirement of thyroid hormones

increases, so women with pre-existing hypothyroidism need to increase their dose of substituted hormones (68). If hypothyroidism is inadequately treated, it is associated with negative pregnancy outcomes and hypothyroidism in general was shown to contribute to arterial stiffness (67). Both women in our study who suffered from hypothyroidism showed quite similar developments of their pulse wave velocity with an increase during the second trimester and a decrease towards the third measurement. However, one can only speculate whether these developments have something to do with their pre-existing medical condition.

5.1 Limitations

All statements, theories and interpretations made and set up in this diploma thesis need to be considered with reservation as they are based on observations made on only a small number of study participants. Due to the outbreak of the Covid-19 pandemic, the study from which the data for this diploma thesis were obtained was interrupted and the recruitment of participants had to start all over again. Therefore, fewer participants than initially planned were included in the study. However, it should be noted that the study from which the data for this diploma thesis were taken is still ongoing. Eventually, enough women will be included to make statistically significant and valid statements about the influence of cigarette smoking on the vascular system and the risk of preeclampsia.

Another limitation of the study is the fact that the participants´ smoking status, which determined the classification into the different groups, was only based on self-reported information. As especially smoking during pregnancy is socially unaccepted and highly stigmatized, it is quite likely that some women might not have reported their smoking habits correctly. Moreover, no distinction between heavy and light smokers was made, which could further distort the observations concerning the influence of smoking before pregnancy on the vascular system.

Like many other studies which investigated vascular changes during pregnancy, this study also began its observations in the first trimester of pregnancy and not right from the time of conception. Therefore, earlier adaptations in vascular function remained unnoticed, which could lead to the fact that changes in vascular function might be underestimated later in pregnancy. The results of this study were compared with many others which had investigated similar issues. The statements of this diploma thesis which were made by comparing our results with these studies are limited due to the facts that many different

factors influence vascular function, different methods to assess the pulse wave velocity exist and study protocols are not standardized.

5.2 Conclusions

So far many studies have investigated the influence of cigarette smoking during pregnancy on the risk of preeclampsia, but little is known about the effect of smoking before pregnancy. Pulse wave velocity measurement is a suitable and proven technique to assess a patient´s vascular health status as an increase is highly associated with subsequent cardiovascular diseases.

The data presented in this diploma thesis suggest an influence of the participants´ smoking habits before pregnancy on their vascular function. As all the women included in the study had a high risk of developing preeclampsia, it was interesting to see that the ones who had smoked before pregnancy had, on average, lower pulse wave velocity values. Whether this effect can be really attributed to the influence of cigarette smoking on vascular adaptations in pregnancy, which determine if a pregnancy is healthy or complicated by preeclampsia, needs to be further investigated.

Having taken a closer look at the development of the pulse wave velocity throughout pregnancy in five different cases of women at high risk of developing preeclampsia gave interesting insights into individual changes of vascular function in pregnancy. It also showed that to be able to interpret data correctly, a patient´s history should always be considered thoroughly.

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