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LUND
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Abstract

Arterial stiffness is an independent risk factor for cardiovascular adverse events, and is traditionally assessed by pulse wave velocity in combination with analysis of the pressure pulse wave in peripheral arteries. Cardiovascular maladaptation to pregnancy and arterial stiffness are associated with hypertensive complications and fetal intrauterine growth restriction in pregnancy. We aimed to investigate arterial stiffness in normal and complicated pregnancy as assessed by photoplethysmographic digital pulse wave analysis (DPA) of the volume pulse wave, a suggested fast, manageable and operator-independent method for arterial stiffness assessment, potentially suitable for screening.

In five separate studies we investigated DPA indices' I) repeatability and associations to reference method indices, II) longitudinal associations with gestational age and distributions in normal pregnancy, III) alterations in early pregnancy and controlled ovarian hyperstimulation for assisted reproduction, IV) associations to uterine artery Doppler velocimetry abnormalities and V) alterations by flavonoid-rich chocolate consumption in pregnant women with uterine artery blood flow velocity changes.

The findings confirm that DPA is an easy and accessible method for arterial stiffness assessment in populations with varying vascular status. DPA arterial stiffness indices show gestational age-dependent changes in normal pregnancy, are affected by hormonal therapy, and are also associated with abnormalities in the uteroplacental circulation, which potentially could be modulated by vascular endothelial-mediated vasodilation.

A normal cardiovascular adaptation is essential for successful pregnancy outcome, and an arterial stiffness increase might precede onset of preeclampsia. Whether DPA is a valuable method for prediction or early detection of complications in pregnancy remains to be investigated.

Key words: digital pulse wave analysis; maternal arterial stiffness; pregnancy; uterine artery blood flow; cardiovascular function

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“The pulse ranks first among our guides; no surgeon can despise its counsel, no physician shut his ears to its appeal. Since, then, the information which the pulse affords is of so great importance, and so often consulted, surely it must be to our advantage to appreciate fully all it tells us, and to draw from it every detail that it is capable of imparting.”

F.A. Mahomed 1872

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Abbreviations

AI	aging index
AIx	augmentation index
APG	acceleration photoplethysmography
BP	blood pressure
CO	cardiac output
COH	controlled ovarian hyperstimulation
DI	dicrotic index
DPA	digital pulse wave analysis
DPB	diastolic blood pressure
DVP	digital volume pulse wave
EEI	cardiac ejection elasticity index
FMD	flow-mediated vasodilatation
FSH	follicle stimulating hormone
HR	heart rate
IVF	in vitro fertilization
IUGR	intrauterine growth restriction
LV	cardiac left ventricle
MAP	mean arterial pressure
NO	nitric oxide
OSI	ovarian sensitivity index
PE	preeclampsia
PI	pulsatility index
PP	pulse pressure
PPG	photoplethysmography
PWA	pulse wave analysis
PWV	pulse wave velocity
RAAS	renin-angiotensin-aldosterone system
SPB	systolic blood pressure
TVR	total vascular resistance
UAS	uterine artery score
UtA	uterine artery

List of papers

This thesis is based on the following original papers, which will be referred to by their roman numerals.

I. **E. von Wowern, G. Östling, P. M. Nilsson, P. Olofsson**.
Digital photoplethysmography for assessment of arterial stiffness: repeatability and comparison with applanation tonometry.
PLoS ONE 2015;10(8):e0135659.

II. **E. von Wowern, K. Källén, P. Olofsson**.
Arterial stiffness in normal pregnancy as assessed by digital pulse wave analysis by photoplethysmography- a longitudinal study.
(*submitted manuscript*)

III. **E. von Wowern, P. Saldeen, P. Olofsson.**
Arterial stiffness during controlled ovarian hyperstimulation and early pregnancy in women exposed to assisted reproduction.
(*submitted manuscript*)

IV. **E. von Wowern, J. Andersson, M. T. Howie, I. Dalene Skarping, P. Olofsson.**
Association between uterine artery Doppler blood flow changes and arterial wall elasticity in pregnant women.
J Matern Fetal Neonatal Med. 2016 Oct 26:1-6.

V. **E. von Wowern, P. Olofsson.**
Can flavonoid-rich chocolate modulate arterial elasticity and pathological uterine artery Doppler blood flow in pregnant women? A pilot study.
J Matern Fetal Neonatal Med. 2017 Jun 28:1-6.

Preface

Palpation of the pulse has been a part of evaluating health since ancient times, however it was first in the middle of the 18th century the circulation and phenomenon of blood pressure arose in western scientific literature. In 1733 Stephen Hales performed the first experimental measurements of blood pressure on a horse by connecting a tube to a punctured artery and observing the blood rising for every pulse stroke. A hundred years later, Etienne Jules Marey constructed the first sphygmograph for registering the arterial pulse wave. Hereafter, different hemodynamic aspects have been of great interest in medical science due to the vast impact of cardiovascular morbidity and mortality on human health.

Sphygmomanometry of the upper arm has been the predominant technique for non-invasive assessment of the vascular system since the late 19th century. In recent decades, along with technical progress, modern research has focused on improved and complimentary methods for prediction of cardiovascular morbidity, such as arterial stiffness assessment. The pulse wave travels from the heart throughout the arterial tree, is reflected in the periphery, and can be registered in a peripheral artery. The elasticity of the arterial wall, and the resistance in the successively branching vessels, determine the pulse wave velocity and the characteristics of the pulse wave contour, which can be analyzed in order to estimate vascular status and endothelial function.

Pregnancy means major changes in the cardiovascular physiology in order to meet the demands of the developing fetus and prepare the woman for delivery. Cardiac output, blood volume and arterial compliance increase already in early pregnancy. There is now growing acceptance that pregnancy complications such as preeclampsia, that previously was considered to originate from the placenta alone, is closely related to the ability of the maternal cardiovascular system to accommodate the hemodynamic demands of pregnancy, and is dependent on cardiac, vascular and endothelial function. Hemodynamic assessment in normal and complicated pregnancy seems to be of value for improved pathophysiological understanding, prediction, diagnosis, disease characterization and optimized treatment of hypertensive disorders and fetal growth restriction in pregnancy.

The purpose of this theses was to investigate maternal arterial stiffness in normal and complicated pregnancies using digital photoplethysmography for pulse wave analysis of the volume pulse wave contour.

Introduction

The vascular tree

The circulatory system constitutes of the heart and the successively branching arterial tree which, after supplying the most peripheral tissues with oxygen and nutrients in the capillary bed, merges in to larger venous vessels and ultimately brings the blood back to the heart. The arterial diameter decreases from about 2-3 centimeters in the aorta to some thousandths of a millimeter in the capillaries.

The arterial walls consist anatomically of three separate layers. The most internal is called the *intima* which is formed by connective tissue with an inner lining of the monolayered *endothelium*. Outside of the intima is the *media* containing smooth muscle cells, and the most outer layer called the *adventitia*, a connective tissue layer containing the vessel's own blood supply and innervation. The anatomical layers structurally differ depending on the size and function of the artery, giving different parts of the arterial tree different properties. With distance from the heart the elastin/collagen ratio changes, so that the vessel walls of central arteries contain a larger proportion of elastic fibers, while collagen is predominant in the periphery (Figure 1). The walls of smaller arteries also contain more smooth muscle cells than the aorta, while the capillaries are highly permeable with no smooth muscle cells in the vessel wall, consisting only of endothelium and the basement membrane.

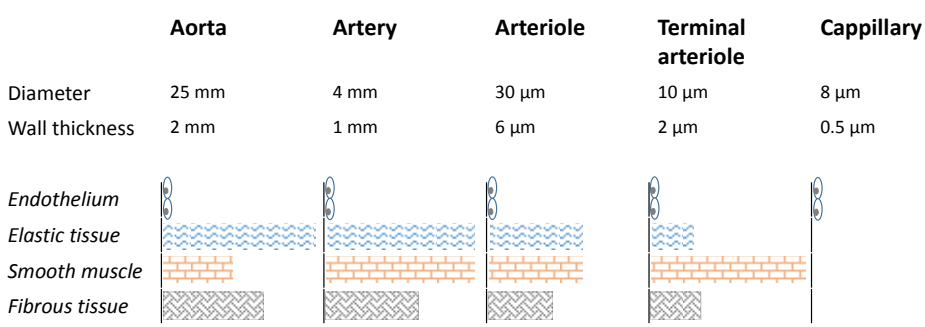

Figure 1.
Schematic illustration of the components of the arterial wall.
Freely modified from Koeppen B.M. and Stanton B.A., Berne & Levy Physiology, 6th Edition

While the main function of the aorta and large arteries is distribution of blood, the smaller arteries distributing blood within organs, together with arterioles, are highly innervated *resistance vessels*. They are the primary site of humoral and autonomic control of arterial blood pressure and blood flow under normal physiologic conditions. On the venous side of gas and nutrient exchange, the capillaries merge into venules of increasing diameter and smooth muscle content, regulating capillary pressure. Larger veins serve as *capacitance vessels* which hold the major blood volume, actively regulates regional blood volume, and hereby alters venous return and cardiac output.

Mechanical properties of arteries

The mechanical properties of arteries are essential to human hemodynamics. For every pulse stroke there is a three dimensional mechanical deformation of the artery wall in the circumferential, longitudinal and radial direction. The circumferential movement is however dominating. The vessel distends as a result of the stress applied to the wall by increase in pressure. Vascular *compliance* (C) is defined as "the total quantity of blood that can be stored in a given portion of the circulation for each rise in mmHg"[1], hence the change in volume (ΔV) divided by change in pressure (ΔP).

$$C = \frac{\Delta V}{\Delta P}$$

Compliance is to some extent dynamic due to the viscous properties of the vessel wall, and thus dependent on the rate by which volume change occurs.

Due to the elastic structure of central arteries, the thoracic aorta is more compliant while arterial stiffness increases towards the periphery. Aortal compliance accommodates the stroke volume in systole, evening out systolic and diastolic pressures, and allows continuous blood flow and propagation of the pulse along the arterial tree. Active constriction of muscular arteries, *vascular tone*, can reduce vessel diameter significantly and increase wall stiffness, effecting the resistance of flow and intravascular pressure. Equivalently, decreased vascular tone increases vessel compliance [2, 3].

Arterial pressure varies throughout the arterial tree. The progressive decrease in compliance distal to the heart causes a systolic pressure amplification, leading to increasing systolic pressure towards the periphery [4]. Aortic pressure is therefore not always accurately reflected in the brachial artery, which commonly is measured for blood pressure assessment.

For every heart beat a volume of blood is ejected from the left ventricle of the heart in systole, and then propagated along the arterial system in the peripheral direction.

As the arterial tree branches, the pulse wave encounter progressively higher resistance, causing a reflective pulse wave in the direction towards the heart with approximately the same velocity. At some point along the arterial tree, the forward and reflecting pulse waves merge. In an ideal setting of compliant arteries, the reflective wave will return to the proximal aorta in diastole, thus enhancing coronary blood flow and smoothening out and reducing the energy impact on the microcirculation [5].

The normal aging process involves replacement of the predominantly elastic tissue and remodulation of collagen content in the arterial media of large arteries such as the aorta. This results in decreased compliance, and subsequently increased systolic blood and pulse pressure, directly by stiffer vessel walls and indirectly by augmentation of the systolic pulse wave by wave reflection from the periphery. The morphological changes of the arterial wall are accentuated by the atherosclerotic process and risk factors such as hyperlipidemia and diabetes [6, 7]. Also genetic predisposition seems to play a role in the development of arterial stiffness [8].

The endothelium

The endothelium is a metabolically active organ of complex features critical for circulatory regulation and hemostatic maintenance. Several substances secreted by the endothelium regulates vasodilation and vasoconstriction, the most important vasodilator being the gas nitric oxide (NO). NO is released as a response to mechanical stress on the vessel wall and is also mediated by acetylcholine which can be used to test endothelial function. Other vasomotor active substances are e.g. prostacyclin and the vasoconstrictors angiotensin-II, endothelin-1 and thromboxane A2. In addition to regulating vascular tone, the endothelium also plays an important role in e.g. platelet activation, coagulation and vascular inflammation. Established cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes and smoking, cause oxidative stress on the endothelium leading to impaired endothelial function with decreased release of NO resulting in vasoconstriction, thrombogenicity and inflammation [9, 10].

Regulation of the circulation

The primary purpose of the circulation is to supply the tissues with oxygen and nutrients to meet their metabolic requirements which can change almost instantaneously depending on stress and activity. In order to adapt to these dynamic

conditions there is an advanced intrinsic regulatory system involving the heart, vascular bed, peripheral tissues and nervous system.

Cardiac output (CO) is a function of stroke volume and heart rate (SV x HR). Through the sinoatrial node the autonomic nervous system is the most important regulator of heart rate, and changes in heart rate is normally the major determinant for rapid adjustments of cardiac output. Stroke volume in turn is affected by cardiac contractility, *preload* and *afterload*.

Preload is defined as end-diastolic left ventricular wall stress, which is determined by ventricular volume or pressure just prior to systole. *Afterload* is the pressure the left ventricle has to overcome during systole. Preload and afterload are influenced by a vast number of physiological factors such as blood volume, vascular resistance, body posture and the venous system. Afterload can be viewed as the composition of a steady (total vascular resistance), and a pulsatile (arterial compliance), component.

Total vascular resistance (TVR), also called systemic vascular resistance, is calculated from mean arterial pressure (MAP), central venous pressure (CVP) and CO (TVR = (MAP-CVP)/CO), where CVP is so low it is usually disregarded, and MAP is the dependent variable [3]. CO and TVR are therefore important determinants of arterial pressure. TVR is determined primarily by vascular tone, i.e. constriction or relaxation of vessel wall smooth muscle cells, which in turn is regulated by several neurohumoral factors (such as the renin-angiotensin-aldosterone-system; RAAS), but also blood viscosity, and vascular intrinsic factors of the endothelium as described above.

The veins hold approximately 70% of the blood volume in the systemic circulation which serves as a reservoir for rapid regulation of blood flow [3]. CO can be increased by an increased CVP and venous return to the heart, increasing preload, in response to factors such as sympathetic activation, respiration and muscle activity. As a slower response, the kidneys may increase blood volume by water and sodium retention through the activation of the RAAS.

Arterial stiffness

In the clinical setting, systemic vascular status is commonly estimated by indirect markers such as brachial systolic and diastolic blood pressure, hyperlipidemia, hyperglycemia and signs of organ damage. *Arterial stiffness* is a dimensionless entity describing altered mechanical properties of the vascular tree, and thus a more direct assessment of arterial wall integrity.

Arterial stiffness has proved to be a strong independent risk factor for cardiovascular events in a variety of populations [11-15].

As described, aging, augmented by genetic and life style factors acting on the arterial wall, involves morphological changes leading to increased arterial stiffness and subsequently an increase in pulse pressure, applying further stress on the arterial wall. The reduced pressure-buffering capacity of elastic arteries increases the pulsatile energy transmitted to the microcirculations, causing damage in vital organs such as the brain, heart and kidneys [16].

Noninvasive assessment of arterial stiffness

Arterial stiffness can be measured noninvasively locally, regionally and systemically, depending on modality and stiffness indices. Local stiffness, i.e. conditions in a specific investigated vessel, generally involves measuring pressure (e.g. brachial pulse pressure) and volume changes (e.g. carotid distensibility), while regional and systemic arterial stiffness generally is determined by analyzing the propagation and wave reflections of the arterial pulse throughout the vessel tree.

There are several indices of arterial stiffness which have shown to be of clinical interest, the simplest being pulse pressure;

$$\text{Pulse Pressure (PP)} = \text{Systolic Blood Pressure} - \text{Diastolic Blood Pressure}$$

PP reflects the buffering capacity of elastic arteries and thus pressure impact on target organs. Increased central PP is an established marker for cardiovascular risk, however, PP measured at the brachial artery level might not reflect central conditions and is directly related to mean arterial pressure, and hence dependent on blood pressure changes. The stroke volume/pulse pressure (SV/PP) ratio describes the elasticity dependent volume-pressure relationship but can then no longer be obtained by a simple a sphygmomanometer [5].

Applanation tonometry

Applanation tonometry is performed by holding a pressure sensor to large superficial arteries (e.g. carotid, femoral and radial arteries) and registering the pressure pulse transit time or the pressure pulse wave contour. By determining the distance between two measuring points, the *pulse wave velocity (PWV)* in meters/second may be calculated. PWV is proportional to the elasticity of the examined artery by the equation;

$$v = \sqrt{\frac{Eh}{2R\rho}}$$

v = wave velocity, E = Young´s elastic modulus, h = wall thickness, R = vessel radius, ρ = density of fluid [5]

As the arterial walls stiffen, the pulse wave velocity increases. Distance measures and time point determinations, and theoretically blood viscosity, constitute potential sources of error. Measurements have to be performed under controlled subject conditions in order to minimize bias.

Carotid-femoral PWV measures arterial stiffness of the aorta but does not provide information on vascular status in peripheral arteries or wave reflection. By applanation tonometry of the radial artery the pressure pulse curve can be obtained, and the aortic pulse pressure curve estimated by a transfer function. Aortic *augmentation index (AIx)* may then be calculated from the pulse wave contour as;

$$AIx\ (\%) = \frac{Augmentation\ pressure\ (AP)}{Pulse\ pressure\ (PP)}$$

where augmentation pressure is the pressure added to the forward going pressure wave (F), created by left ventricular ejection, by the reflected pressure wave (R) from the periphery (Figure 2).

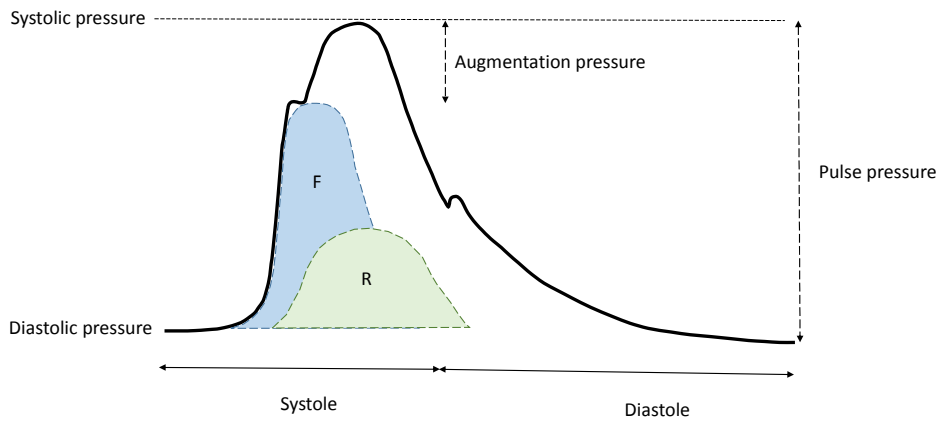

Figure 2.
Schematic aortic pressure pulse waveform derived from applanation tonometry of arteria radialis.

AIx depends on heart rate, PWV, left ventricular outflow, aortal compliance and the amplitude and timing of the reflected wave [5, 17]. Also gender and body height might influence AIx [18]. In stiffer vessels the amplitude of the reflective wave increases and it returns faster. In individuals with more elastic arteries the contribution of the reflected wave is greater than the contribution of PWV, with the opposite relationship in individuals with stiffer arteries [17]. For every 10 bpm increase in heart rate AIx decreases by approximately 4% [19], why common commercial applanation tomometry systems like the SphygmoCor™-system automatically adjusts AIx to a fixed heart rate of 75 (AIx-75). By the use of a mathematical transfer function aortic AIx is estimated from peripheral AIx at the measuring site. The use of transfer

functions has been questioned in terms of accuracy and applicability to different populations, however AIx has proved to be related to endothelial function [20], inflammatory markers for subclinical atherosclerosis [21], and to reflect vasoactive therapy effects independently of PWV [22, 23]. Also, increased AIx is associated with cardiovascular morbidity, although as a less established risk factor than PWV [24-26]. Primarily, AIx seems to be a marker for cardiovascular risk in younger subjects rather than older [26].

In summary, PWV is a measurement of central arterial stiffness, while AIx also informs on wave reflections and thus reflects peripheral vascular conditions and endothelial function.

Applanation tonometry for carotid-femoral PWV in combination with AIx is considered the "golden standard" measurement of arterial stiffness [18]. However, the method has not achieved routine use in common clinical practice, probably due to being cumbersome and somewhat time consuming.

Pulse wave analysis by digital photoplethysmography

Already in the 1930's the technique of registering the pulse in the finger by transmitting light through the tissue, photoplethysmography (PPG), was developed. An infrared photodiode transfers light of a wave length of about 900 nm to a receiver on the opposite site of the finger, and based on the Lambert-Beer law of absorbance of light in the medium of which it is travelling, the technique uses differences in hemoglobin content in systole and diastole to form a pulse curve, hence derived from the volume pulse wave in the finger. The amplitude of the digital volume pulse wave (DVP) is affected by all factors influencing the perfusion of blood in the examined vascular bed such as temperature and activity of the sympatic nervous system, however the shape of the volume curve seems to be mainly influenced by the conditions of the systemic circulation, as is the pressure pulse curve [27, 28]. The DVP curve obtained by PPG is characteristically biphasic with a more or less distinguished incisura, or 'dicrotic notch'; the first phase characterized primarily by systole, and the second phase by diastole and wave reflection from the periphery. As for the pressure pulse wave, in stiffer vessels with earlier return of the reflective wave, the early systolic component of the DVP is to a greater degree augmented and the incisura is smoothened or vanished (Figure 3, upper panel to the right compared to upper panel to the left). In the mid 1900's it was discovered that vasodilation by e.g. alcohol and nitrates resulted in a more pronounced drop of the incisura, also expressed as the 'dicrotic index', and subsequently a relationship between the DVP waveform and coronary heart disease was established with a more triangular waveform shape in older patients with history of cardiovascular morbidity [29]. Millaseau et al. showed in 2000 that the volume and pressure pulse waves were related by a transfer function stable under different conditions[27], and suggested in 2002, after investigating the DVP derived 'stiffness index' association to carotid-femoral PWV, that digital volume pulse wave analysis could be used for assessment

of arterial stiffness [30]. Although the physiological determinants and interpretation of the DVP are complex, its simplicity is intriguing as a clinically applicable method for evaluating vascular status and endothelial function.

Since pulse contour changes reflect arterial properties, the PPG curve can be mathematically remodelled by second derivation to further accentuate and locate inflection points, e.g. in early and late systole. This second derivation PPG, *acceleration photoplethysmogram (APG)*, was first reported in Japanese by Ozawa in 1972 (no reference), and consists of four systolic waves named *a*-*d* and one diastolic wave named *e*, all expressed as quotas of the *a*-wave [31] (Figure 3, lower panel). The APG-quotas have been studied in relation to physiological and clinical characteristics.

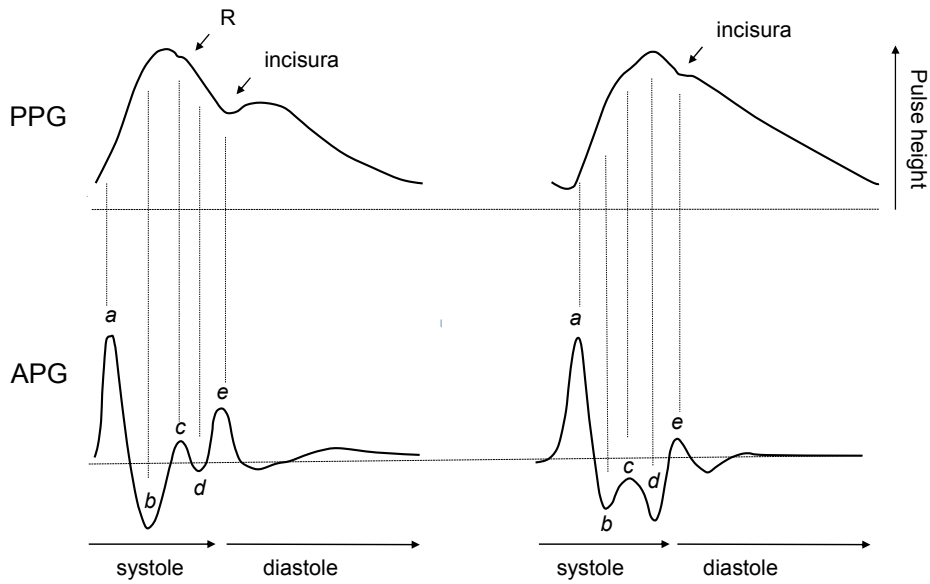

Figure 3.
Schematic crude digital photoplethysmographic (PPG) volume pulse curve (upper panel) and its corresponding second derivative acceleration photoplethysmogram (APG) (lower panels). R, reflective wave. Panels to the right show increased arterial stiffness compared to panels to the left.
von Wowern et al., J Matern Fetal Neonatal Med. 2016 Oct 26:1-6.

The ***b*/*a*** ratio stands for the acceleration of blood into the aorta by ejection from the left ventricle and is hence an indicator of left ventricular power and/or central artery compliance. A less negative *b*-wave develops by age, and has shown to reflect large artery stiffness [32, 33]. Indeed, *b*/*a* is positively correlated to the Framingham risk score [34] and reduced distensibility of the carotid artery, as well as progressively to severity of arteriosclerotic disease [35]. In the study by Takazawa et al. [32] there was no effect of vasoactive agents on the *b*/*a* ratio.

The ***d*/*a*** ratio is believed to represent the intensity of the reflective wave from the periphery augmenting the aortal pressure, and decreases by increased vascular tone or arterial stiffness of peripheral arteries due to structural vascular wall alterations [32]. *d*/*a* has also shown a negative correlation to the Framingham risk score[34], and in congruence, *d*/*a* decreases with advancing age [31, 33]. *d*/*a* has been shown to reflect the effect of vasoactive agents and could therefore potentially be useful for evaluation of therapeutic interventions, and as an index for cardiac afterload [32].

The ***c*/*a*** ratio and ***e*/*a*** ratio decrease with increased arterial stiffness and age [32, 33].

Based on the APG-waves Takazawa et al. [32] constructed the APG *aging index (AI)*, defined as

$$\mathrm{AI} = \frac{b - c - d - e}{a}$$

not to be confused with the pressure wave derived augmentation index (AIx). AI has been suggested as a valuable tool for evaluation of vascular age and screening for arteriosclerotic disease [36]. AI increases with age and is by its definition an index of global arterial stiffness.

A problem in the research field of pulse wave analysis by photoplethysmography is the use of different indices of measurements, but also different terminologies for the same indices. This has been brought to attention by Dr M. Elgendi, and the terminologies recommended by him in 2012 (PPG and APG) has thus been used in this thesis [37].

Importantly, the DPA method cannot differentiate between increased vascular tone regulated by humoral and physiologic mechanisms, and increased arterial stiffness secondary to alternations of the arterial wall due to age dependent remodelling or pathophysiological processes. However, since the more peripheral and muscular resistance vessels are the primary regulators of vascular tone, it is reasonable to believe that this is mainly a consideration for interpretation of DPA indices representing peripheral arterial conditions and wave reflection.

Ultrasound and Magnetic Resonance Imaging

Estimates of local arterial stiffness can be obtained by analyzing the geometrical changes in vessel diameter in diastole in relation to systole using ultrasound or echotracking devices. Superficial arteries such as the carotid, brachial or femoral arteries are commonly examined. This method has the advantage of giving a direct measurement of compliance derived from volume and pressure changes, however, the technique requires skilled operators, and the conditions in the examined local arteries might not reflect conditions in the aorta or other vital vessels. Indeed, carotid stiffness is not of predictive value for cardiovascular events in all populations, and along with the methodological aspects, local measurements are not recommended

for epidemiological research, but rather has a function in pathophysiological and pharmacological studies [18].

Magnetic Resonance Imaging (MRI) enables measurements of local stiffness of the deeper lying aorta, and provide accurate and reproducible measurements of aortal compliance and PWV [38]. However, most previous research and epidemiological studies have used other techniques, and the expensive, cumbersome and time consuming method of MRI is an obvious disadvantage in the clinical setting.

Assessment of endothelial function

Endothelial function can partly be assessed by pulse wave analysis as described above, but classically endothelial function is examined by ultrasonic measurements of vasomotor action in response to vasoactive stimuli. *Flow-mediated vasodilation (FMD)* is the most common method in literature. It is performed by ultrasound of the brachial artery where the blood flow is occluded for a short period of time by a pressure cuff, and the NO-mediated vasodilatory response to the reactive hyperemia when pressure is released, is measured [39].

Gender aspects on vascular function

It is well known that the risk of cardiovascular disease is lower in women than men until menopause, after which the incidence increase significantly in women. Although the exact physiological mechanisms are not completely understood in detail, extensive research has focused on beneficial effects of female sex hormones on the arterial system. Female sex hormone receptors are present in various cell types, including endothelial- and smooth muscle cells, and estrogen and progesterone have shown to exert effects such as vasodilation, as well as modifications of the endothelium, vascular wall, lipoprotein profile and blood coagulation [40, 41]. For example, estrogen stimulates growth of endothelial cells and reduce contractility in vascular smooth muscle cells [40, 41], and in vitro increases elastin deposition in the vascular wall leading to a major increase in the elastin/collagen ratio compared to testosterone [42]. In vivo, aortal compliance in women is greater, and age-dependent degeneration of the vessel wall is postponed, compared to men [43, 44].

Gender has shown to influence AIx, conflictingly indicating higher values in women [45, 46]. However, this might be attributed to differences in height and heart rate [5, 18].

Several studies have investigated arterial compliance changes during the menstrual cycle [47-51]. When measured locally, most results indicate higher arterial compliance in the ovulatory phase compared to the luteal phase, as well as correlations to sex

hormone levels and reduced FMD in the luteal phase [47, 50, 51]. Studies of regional arterial stiffness in the menstrual cycle by applanation tonometry has been somewhat diverging from local measurements, with decreased arterial stiffness in terms of lower or unchanged AIx in the luteal phase compared to the periovulatory phase [49, 52], while PWV in central and peripheral arteries show no cycle dependent variations [47, 51]. This may be due to differences in the influence of menstrual cycle hormone changes on central and peripheral vessels [51], or methodological issues such as different techniques, measurements obtained at different locations, time intervals and sample sizes.

Considering the beneficial effects of female sex hormones on the arterial system, the effect of hormone replacement therapy (HRT) on the risk of cardiovascular disease in post-menopausal women has been widely investigated. Women administered HRT have been reported to have increased arterial compliance and lower AIx, but not differ in PWV, compared to untreated controls [53, 54]. Large randomized controlled trials of HRT treatment have not confirmed observational reports of lower incidence of cardiovascular events, sometimes even reporting negative effects on cardiovascular morbidity and mortality [55]. However, current evidence support decreased cardiovascular risk with HRT given within the first decade after menopause, but no beneficial effect later, and also increased risk of stroke and thromboembolism in both groups [55]. Thus, HRT is not recommended for cardiovascular protection.

In *in vitro fertilization* (IVF), multiple oocyte maturation is achieved by controlled ovarian hyperstimulation (COH) by administration of gonadotropins. Ovarian response to COH and pregnancy success rate is individual and dependent on multiple factors [56, 57], but in general, COH results in supraphysiological estradiol concentrations of about tenfold of the normal menstrual cycle [58]. After embryo transfer, estradiol levels increase or decrease depending on whether pregnancy is achieved or not, while progesterone, after previously being suppressed, seems to rise in both pregnant and non-pregnant individuals [59]. The luteinization process has been associated with activation of the RAAS and sympathetic nervous system in IVF-patients [60], and there is also some evidence for post-ovulatory hemodynamic alterations [61]. A complication to COH with high estradiol levels is the ovarian hyperstimulation syndrome (OHSS), characterized by increased vascular permeability, ascites, hypovolemia, and circulatory dysfunction, but the exact pathophysiological mechanism is not yet fully understood. Studies of arterial stiffness parameters in the assisted reproduction therapy setting have not been found while writing this thesis.

Cardiovascular physiology of pregnancy

Pregnancy means major physiological changes in most organ systems, including the cardiovascular system, to accommodate adequate blood flow to the uteroplacental unit, and meet the metabolic and physiological demands of the mother and the growing fetus. Except for hemodynamic alterations described in detail later, ventilation increases by 40% due to increased oxygen demand, and renal blood flow with a 40-50% increase in glomerular filtration rate (GFR) [62]. Failure to adapt to the pregnant state might lead to maternal complications and morbidity, as well as adverse pregnancy outcome, such as fetal loss or intrauterine growth restriction.

A solid knowledge of the physiological changes of pregnancy is vital for the ability to assess, diagnose and treat women in pregnancy.

Maternal hemodynamic changes

Pregnancy is characterized by a hyperkinetic low resistance circulation evident already in the first weeks of pregnancy, prior to the existence of the fetal-placental unit [63, 64]. Overall, hemodynamic changes generally progress until mid-pregnancy, thereafter reaching a plateau or a slight change towards pre-pregnancy values in the third trimester (Figure 4).

Cardiac output

CO increases by 30-45% in pregnancy, starting early and reaching peak values around mid-pregnancy or by the end of the second trimester [65, 66]. There is less consensus on whether CO changes or plateaus in the third trimester. In labor, CO increases further, and then rapidly decreases after delivery, successively normalizing in the first months postpartum [62].

CO is the product of heart rate (HR) and stroke volume (SV) which both increase in pregnancy. Whether the raising CO is primarily due to an increase in HR or SV, secondary to increased blood volume, is debated [66]. Pregnancy also induces structural changes of the maternal heart with an increase in left ventricular mass [66].

Blood volume

In normal pregnancy plasma volume increases by about 50% with large individual variations beginning early with the most rapid change in the first trimester continuing until the third trimester [67]. This is likely to be triggered by the early hemodynamic changes with systemic and renal vasodilatation, creating an underfilled hypovolemic circulation that stimulates activation of the RAAS to increase water and sodium retention [63, 65]. The vasoconstrictive response to the RAAS-activation is however significantly reduced [68]. This is achieved by alterations of the RAAS components, downregulation of the angiotensin II receptor

vasoconstrictor AT1R and upregulation of the vasodilator AT2R [69], as well as actions of other vasodilators such as VEGF-A, NO, prostaglandins and others [70]. Normally, pregnancy adaptation overall results in a state of hypo-osmotic hyponatremic hypervolemia. Red blood cell mass also increases, but to a lesser extent, manifesting as the physiologic anemia of pregnancy.

Blood pressure, total vascular resistance and arterial compliance

Mean arterial pressure (MAP) falls slightly in the first two trimesters, likely as a response to vasodilation and decrease in vascular resistance (TVR) that is not fully compensated by the increase in CO [62]. After 26-28 gestational weeks, MAP increases towards term. The change in MAP is considered to be mainly attributed to changes in diastolic pressure, while there is less consensus on changes in systolic pressures throughout pregnancy [65, 66]. The change in TVR also occur primarily in the first trimester, reaching lowest values sometime in the second trimester, and with minor increase towards term [66, 71].

In parallel with first trimester TVR changes, arterial compliance of the aorta, as well as other elastic vessels, is increased in pregnancy up to 30% systemically (predominantly in the first trimester), and wave reflections are decreased [66, 72, 73]. This may be due to increased vascular distensibility based on reduced vascular tone as an effect of estrogen and/or shear stress induced vasodilation, and has physiologic value in allowing an increase in intravascular volume without raising MAP [72].

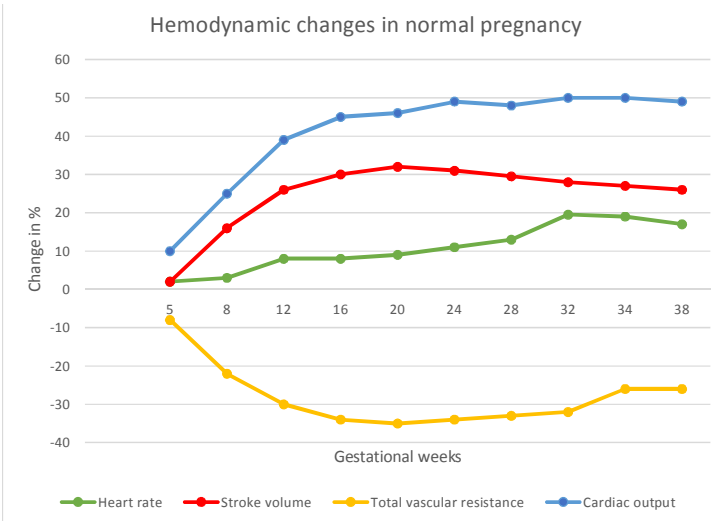

Figure 4.
Change of hemodynamic parameters with gestational age.
Freely modified from Ruys et al., J Cardiol 2013;61:107-112.

The uteroplacental circulation

The main blood supply to the uterus is provided by the uterine arteries (UtA) originating from the internal iliac vessels. The UtAs branch further into arcuate arteries, radial arteries and ultimately the spiral arteries that supply the functional layer of the endometrium. In pregnancy, the spiral arteries are invaded by trophoblasts and undergo major alterations such as lumen dilatation and replacement of muscular and elastic tissue, and become less responsive to vasoconstriction [74]. The UtAs double in diameter by mid-pregnancy, and mean blood flow velocities increase progressively until late third trimester [75]. In all, this results in a high-flow low-resistance uteroplacental circulation, supported by maternal hemodynamic adaptions to accommodate the increased demands of blood supply.

Long term effects of pregnancy on the cardiovascular system

Less is known about the time aspect of normalization of hemodynamic alterations postpartum. Although there seem to be a gradual return of most hemodynamic parameters to pre-pregnancy values within two weeks after delivery, studies have shown persistent changes in cardiovascular measurements up to one year postpartum with higher CO, and lower TVR and MAP, compared to baseline values [76-78]. In addition, studies on nulliparous versus parous healthy women indicate that the magnitude of hemodynamic changes are greater in subsequent pregnancies than the first pregnancy, with an inverse relationship to pregnancy intervals [77, 79]. This has led to the theory that pregnancy might have a cardiovascular remodelling effect with a transient improvement in arterial compliance, which might have significance for the risk of hypertensive complications of subsequent pregnancies as well as cardiovascular morbidity in later life [78, 79].

Pulse wave analysis and arterial stiffness in normal pregnancy

Hemodynamic adaption in the terms of arterial stiffness has also been studied longitudinally and cross-sectionally in normal pregnancy, using pulse wave analysis by applanation tonometry [49, 80-83], and in few studies, photoplethysmography [84, 85]. AIx shows a U-formed relationship with gestational age from first to third trimesters, independently of MAP and HR, reaching nadir sometime in the second trimester [80-83, 86]. This change in central conditions estimated by AIx is not always evident in brachial BP [86]. Alterations of PWV in normal pregnancy is less clear but seem to follow the same pattern as AIx, at least in the third trimester, although to a lesser magnitude [82, 83, 87]. Studies on PPG in pregnancy are scarce but show similar gestational effects on PPG indices of arterial stiffness as AIx [84, 85].

In conclusion, indices of arterial stiffness throughout pregnancy seem to reflect gestational changes of arterial compliance, and provide information on adaptive changes that might not be reflected in standard peripheral BP measurements.

Hypertensive pregnancy complications and preeclampsia

Hypertensive complications of pregnancy are a major cause of perinatal morbidity and mortality worldwide, especially in low income countries. In the developed countries, in spite of generous pregnancy surveillance and available modern health care, gestational hypertension and especially preeclampsia, still bring challenges to obstetricians in terms of predicting, diagnosing, and treating women and their unborn babies.

Gestational hypertension means pregnancy induced hypertension (≥140/90 mmHg) after 20 gestational weeks. *Preeclampsia* is gestational hypertension with the addition of significant proteinuria (≥300 mg/24 h), in the more severe form accompanied by signs of multiple organ affection such as impaired renal- and liver function, coagulopathy and seizures [88]. In the last years an updated classification has been presented due to the variability and diagnostic reliability of proteinuria. The International Society for the Study of Hypertension in Pregnancy (ISSHP) definition of preeclampsia includes cases of gestational hypertension with signs of organ dysfunction and/or intrauterine growth restriction, even in the absence of proteinuria [89].

Clinical presentation of preeclampsia

Preeclampsia (PE) occurs in about 2-7% of healthy nulliparous women, most commonly at the end of pregnancy or around the time of delivery [88]. PE manifested before 34 gestational weeks, so called early-onset preeclampsia, tends to be more severe with increased risks of complications. The main clinical risk factors for PE are previous PE, multiple pregnancy, pregestational chronic hypertension or diabetes, obesity, a family history of PE, or pre-existing renal, autoimmune or thrombofiliac disorders. A previous non-PE pregnancy reduces the risk in next pregnancy, while extreme maternal age (<20 or >40 years) increases the risk as well as longer pregnancy intervals and assisted conception. Also, paternal factors and maternal ethnicity affect the risk of PE.

PE in its severe form might lead to fatal complications such as seizures, cerebral hemorrhage, disseminated intravascular coagulopathy, placental abruption and circulatory collapse. The course and symptoms of disease are often diverse and may be ambiguous and vague, why clinical evaluation is sometimes difficult. At present, there is no curative treatment for PE but delivery and removal of the placenta, which might involve challenges in balancing the risks of maternal complications and fetal

prematurity. Early-onset PE is commonly associated with placental insufficiency and intrauterine growth restriction (IUGR), while late-onset PE is less frequently associated with IUGR and placental pathology [90].

Etiology of preeclampsia

PE, also called "the disease of theories", has been the subject for extensive research, still the exact pathophysiological mechanisms of PE is unknown. However, the so called "two-stage model" of the etiology of preeclampsia has reached wide acceptance. The first stage of the model is characterized by abnormal placentation with defective invasion of the trophoblasts and development of the spiral arteries, leading to hypo-perfusion of the placenta resulting in ischemia, tissue damage, oxidative stress and release of free radicals and cytokines [91]. Evidence suggests this defective process is also dependent on maternal (and paternal) immunological and hormonal factors [91]. Several factors and particles are then released into the maternal circulation causing endothelial damage and a defective immunological response to pregnancy in susceptible mothers [88]. Endothelial dysfunction plays a major role in the pathogenesis of preeclampsia, resulting in vasoconstriction with generally reduced organ perfusion, fluid retention and hemostatic dysfunction [92-94]. This in turn characterizes the maternal disease, the second stage of the two-stage model. Studies have shown decreased NO expression and endothelial integrity [95] in PE, and an exaggerated maternal systemic intravascular immunological response to pregnancy [92]. PE seems to be a condition of multifactorial origin in which both maternal and placental factors play a role. Since endothelial dysfunction is characterizing for both defective placentation and maternal constitutional risk factors for PE, the endothelium has been suggested to be the site were these factors convey [93]. One theory is that early-onset PE might be due to defective placentation and an abnormal hemodynamic adaption to placental insufficiency, while late-onset PE is related to maternal cardiovascular or endothelial dysfunction [96, 97].

Maternal hemodynamics in preeclampsia and intrauterine growth restriction

In recent years there has been an increasing interest in the association between cardiovascular maladaptation to pregnancy and PE with or without IUGR. An insufficient plasma volume expansion is associated with hypertensive pregnancy complications and IUGR [67]. Studies concerning alterations of cardiovascular parameters such as CO and TVR in PE have shown conflicting results [98-101]. This could be due to differences in technical methods, severity of disease and study populations, in particular maternal obesity [102]. Also, high and low CO have been reported, depending on measurements in preclinical phase of PE or manifested disease, respectively [103]. Time of onset could also explain differences in results. Early- and late-onset PE, although not clearly separated entities, differ in

pathological characteristics as earlier described, and differences in hemodynamic parameters have also been reported with low-CO/high TVR in early-onset PE, and the opposite in late-onset PE [102].

IUGR with or without PE seems to be associated with an increased TVR and low CO [104-106]. Different hemodynamic profiles of PE have been suggested depending on the presence or absence of concomitant IUGR. With first trimester hemodynamic evaluation, Khaw et al.[107] reported that women who later develop PE without IUGR had increased CO as a result of increased preload, and the increase in CO, rather than TVR, was responsible for the higher MAP. However, women with IUGR but no PE had low CO and high TVR, probably due to either vascular dysfunction or abnormal intravascular volume expansion. Women who developed PE in combination with IUGR had hemodynamic profiles more similar to the IUGR-group.

Endothelial function, believed to be a key mechanism in the pathophysiology of hypertensive complications, has been investigated in pregnancy in regards to PE and IUGR. Endothelial dysfunction, determined as low FMD, is present in high risk women who subsequently develop PE, as well as in preeclamptic women compared to normotensive controls [108]. Endothelial dysfunction persists up to at least three years postpartum after preeclamptic pregnancy, and there are also reports of an association between the magnitude of FMD impairment and severity of disease [109]. In addition, preeclamptic women with a compromised uteroplacental circulation seem to have more pronounced endothelial dysfunction than preeclamptic women with normal blood flow in the uterine arteries [110]. Endothelial dysfunction assessed by FMD in regards to IUGR has been less studied, but Iacobaeus et al. found an association between FMD vasodilation in the first trimester and fetal birthweight in a cohort of healthy non-smoking women [111]. However, the association was endothelial independent, leading the authors to conclude that it was the maternal vascular dilatation capacity, rather than impaired endothelial function alone, that was related to fetal growth.

PE is also associated with structural and functional changes of the maternal heart both in the preclinical phase, in manifested disease and postpartum [96]. Normal pregnancy is associated with eccentric left ventricular (LV) hypertrophy secondary to volume overload, while women with PE seem to develop a concentric hypertrophy similar to changes characterizing essential hypertension in non-pregnant individuals, likely as a response to pressure overload [112]. In line with this, women with, or destined to develop, PE show increased LV wall thickness and diastolic dysfunction, which signals a significant strain on the heart in addition to the ensuing load of pregnancy [96]. Myocardial contractility and relaxation may also be affected [96]. The extra burden on the maternal heart must be considered in clinical care of women with PE, who as a result might have adverse or more severe reactions to obstetrical complications and interventions, and are sensitive to volume overload. This cardiac remodelling might also be relevant for the future increased risk of

cardiovascular morbidity evident after preeclamptic pregnancy (described further in later sections).

In relation to cardiovascular changes in normal and complicated pregnancy, the venous system cannot be forgotten since it holds the blood volume reserve and is an important regulator of CO. Although less thoroughly investigated, alterations in the venous compartment such as decreased venous distensibility and capacitance in preeclamptic women have been reported, and might be essential in the understanding of hemodynamic profiles in complicated pregnancy [113].

Greater knowledge of hemodynamic dysfunction and its role in pathophysiology in PE and IUGR could potentially be valuable for characterizing disease, prognostic evaluation and optimizing care and medical treatment accordingly. Cardiac and TVR measurements have been observed to predict maternal and fetal adverse outcome in normotensive [99] and hypertensive pregnancies [114], to be associated with birth weight [115], and to be of interest in evaluating response to therapy [116, 117].

Association between arterial stiffness and preeclampsia

As vascular adaption and endothelial function play roles in the pathophysiology of PE, and as PE like arterial stiffness are strong predictors for cardiovascular disease, the association between PE and arterial stiffness has gained increasing interest. Cross-sectional and longitudinal studies have investigated arterial stiffness during and after complicated pregnancy using different measurement methods with fairly unanimous results. In 2012, a systematic review and meta-analysis by Hausvater et al.[118] of the association between PE and arterial stiffness concluded that arterial stiffness is increased before, during and after preeclamptic pregnancy, and is higher in PE than in gestational hypertension. In the meta-analysis they found PE to be associated with an increase of 1.04 m/s in carotid-femoral PWV and 15.1% AIx. Oxidative stress and inflammation, as well as alterations in lipid profiles, NO levels and the RAAS, were presented as potential underlying mechanisms, which are all factors acting on the vessel walls.

Data on the association between PE and arterial stiffness assessed by DPA is scarce. Indices derived from the PPG curve have in two previous studies reported increased arterial stiffness in manifested PE [85, 119], but to date no studies on vascular status in PE assessed by APG were found.

Prediction of preeclampsia

Maternal characteristics and medical history can only predict about a third of PE cases [120] and prenatal care is generally organized to detect manifestations of PE widely in the pregnant population. Diagnostic methods for prediction of individual risk for PE have been the focus of extensive research aiming to prevent complications, optimize care and undertake therapeutic actions. Treatment with low

dose aspirin has proved to reduce the risk for PE and IUGR if started in first or early second trimester [121], hence efforts have been made in recent years to construct algorithms for early prediction based on different markers such as maternal constitutional factors, maternal MAP, biochemical markers and uterine artery Doppler ultrasound (further described below) [122, 123]. Recently, the ASPRE trial, using first trimester screening with such a multimarker algorithm, showed a detection rate of 77% for pre-term preeclampsia [124]. However, prediction algorithms are not generally validated or implemented in clinical practice in Sweden to this date.

Arterial stiffness is increased in women destined to develop PE [125], and Khalil et al. concluded that AIx might be a valuable tool for improving prediction models for PE in early pregnancy [126]. However, in current obstetrical practice brachial blood pressure is generally the only estimate of maternal cardiovascular function outside the uteroplacental unit in clinical risk assessment.

Uterine artery Doppler blood flow velocimetry

In response to trophoblast invasion in normal pregnancy, uteroplacental arteries undergo structural changes described previously, in order to maximize blood flow to the placental bed. Failure of normal trophoblast invasion and vascular adaption, such as in PE, result in increased resistance to blood flow in the uteroplacental circulation. The increased resistance in the vascular bed may be detected in the uterine arteries (UtA) by Doppler ultrasound blood flow velocimetry (described in more detail in Methods section), and can be used to predict risk for PE and IUGR in first and second trimester [127-129]. UtA Doppler changes have a higher sensitivity in second trimester if used alone, and for predicting severe disease [127], but can be used in combination with other markers for prediction of PE in the first trimester [122].

Although UtA blood flow changes are associated with placental vascular abnormalities and complicated pregnancy, the positive predictive value is still low which has raised the question of influence of non-placental factors on the UtA velocity waveform. The pathophysiological determinants of the waveform characteristics are not fully elucidated, and generalized maternal arterial and endothelial function outside the uteroplacental circulation have been suggested as background factors of UtA changes [97, 130]. Maternal HR shows an inverse relationship to UtA pulsatility index (PI) [131], and there is some evidence for an association between increased arterial stiffness, expressed as PVW and AIx, and UtA blood flow changes [125, 132], raising the question if UtA Doppler blood flow velocity changes may reflect insufficient maternal cardiovascular performance.

Long term maternal risks of preeclampsia

It is now a well-established fact that women who develop PE in pregnancy have an increased risk of cardiovascular morbidity and mortality in later life. Preeclamptic pregnancy is associated with a fourfold increase in the relative risk of hypertension, and a twofold increased risk for ischemic heart disease, stroke and thromboembolism within 10-15 years postpartum [133]. Overall mortality is also increased in women with a history of PE after 15 years [133]. In addition, hypertension appears earlier in later life than in women without pregnancy complications, and the severity of PE is associated with the risk increase for cardiovascular events [134].

The explanation might be found in the persistent cardiac and vascular structural and functional changes evident after PE. In a study by Melchiorre et al.[135], 40% of women with a preeclamptic pregnancy had asymptomatic heart failure stage B defined as left ventricular hypertrophy or dysfunction one year postpartum. Cardiac impairment was more prevalent and pronounced following preterm PE, and 40% had developed essential hypertension within two years postpartum [135]. The findings were supported by Ghossein-Doha et al.[136], who found a fourfold independent risk increase for subclinical heart failure stage B in women with a history of PE 4-10 years after pregnancy. Women with cardiac abnormalities might also have an increased risk for recurrent PE in next pregnancy compared to women who have no or mild cardiac dysfunction in the non-pregnant state [137]. Furthermore, women with previous PE display endothelial dysfunction independently of other vascular risk factors [108, 138], and persistent increased arterial stiffness [118] at least the first years after pregnancy. It is yet to reveal whether active management and prophylactic interventions before the appearance of clinical symptoms of cardiovascular disease could improve the future health of women affected by PE in pregnancy.

Cacao

The cocoa bean, also called cacao bean or simply cacao, is the seed of the cacao tree, *Theobroma cacao*. The tree has been cultivated for at least a thousand years in Central- and South America. Cacao had a significant status in ancient Maya and Aztec civilizations as a promoter of health, believed to have been brought down to earth from heaven by the gods [139]. In the 16th century cacao entered Europe and was modified into sweet chocolate with added sugar and spices. It was originally food for royalty, but successively spread to be the popular and readily available food item it is today. In recent decades, beneficial effects of flavonoids, found in high quantities in cacao, on cardiovascular health have come to interest, and cacao-rich chocolate has again earned a reputation as a salubrious part of the diet.

Flavonoids

Flavonoids, a large subgroup of polyphenols found in plants, are nutrients acquired by fruit and vegetable intake, but also plant-derived food items such as cacao, tea and red wine. Flavonoids have antioxidant and enzyme regulating effects, and may also modulate cell signalling pathways and gene expression [139].

Flavonoids can be divided into subclasses dependent on molecular structure (flavanols, flavanones, flavones, isoflavones, flavonols, anthocyanidins), of which the flavanols epicathecin and cathecin are present in large quantities in cacao and account for the high antioxidant capacity of dark low-processed chocolate [139].

In addition to their antioxidant properties, there are several other mechanisms through which flavanols might exert effect. Flavanols seem to promote vasodilatation, acutely by reduced enzymatic NO inactivation and long-term by increasing expression of endothelial NO synthase (eNOS), platelet inhibition through reduced glycoprotein IIb/IIIa expression, increase the high-/low-density lipoprotein ratio (HDL/LDL), and inhibit angiotensin-converting enzyme (in vitro) [140]. In addition, flavonoids might have anti-inflammatory effects through modulation of cytokines [139].

Effect of cacao on cardiovascular health

The first epidemiological evidence of a beneficial effect of cacao was seen in the Kuna Indians, a native Central-American culture that consumes massive amounts of cacao in their diet, and is a population with very low cardiovascular mortality and a rare resistance to age-dependent increase in high blood pressure [140]. Following this finding, numerous heterogeneous studies, including meta-analyses and randomized controlled trials (RCT's), showed that cacao had a lowering effect on blood pressure and cardiovascular morbidity [141-143]. In a Cochrane review in 2012 including 20

RCT's [144], a small but significant effect of flavanol-rich cacao (on average 545.5 mg of flavanols daily) on systolic and diastolic blood pressures was found. The effect seemed to be short term, being significant in studies of less than 2 weeks duration. However, these studies also had a flavanol-free control arm, whereas low-flavanol control groups were more common in long term studies. If there are clinical implications of these findings, or if it truly is the flavanols and not other components of chocolate/cacao products exerting the effect, is yet to be further elucidated.

The underlying mechanism of the beneficial effects of cacao seem to lie in improvement of endothelial function. Hence, the effect of cacao on FMD has been investigated. In a study by Hermann et al. [145] on smoking men, dark chocolate with high cacao-content (74%) had a significantly improved effect on brachial artery FMD two hours after consumption, lasting eight hours, while white chocolate with low cacao-content (4%) showed no effect. Increased FMD after flavanol-rich cacao ingestion has also been shown in healthy non-smokers [146, 147], and in a dose-dependent manner with different flavonoid-doses [148].

Effects of cacao on arterial stiffness and wave reflection

A few studies address the effect of cacao on arterial stiffness and wave reflection. Vlachopolous et al. reported that habitual cacao consumption was associated with lower AIx, PWV and PP in healthy individuals [149]. Peripheral pressures were not significantly different why the authors suggested that the protective effect of cacao might be underestimated when only systolic and diastolic brachial pressures are considered. Decreased PWV, AIx, and PP after cacao consumption have also been reported in interventional studies [148, 150]. Grassi et al. [148] investigated the effect of cacao with flavonoid doses from 0-800 mg on PWV, and found the decrease to be dose-dependent, with all tested flavonoid-doses giving significant reductions.

Studies using volume pulse wave contour analysis to investigate vascular effects of cacao are scarce, but reduced "stiffness index" [147] and increased pulse height, indicating vasodilation [151], after cacao ingestion have been reported.

Effects of cacao in pregnancy

Due to the seemingly protective effects of cacao on the cardiovascular system in the non-pregnant population, the interest in potential effects in pregnant women, and on pregnancy complications, has awakened. In an epidemiological prospective study of approximately 1700 women, self-reported chocolate consumption in the last trimester, and theobromine concentration (a component of chocolate acting as an antioxidant) in the umbilical cord blood at birth, were inversely correlated to risk of preeclampsia [152]. The results were not supported in a later retrospective similar study [153], but confirmed by another prospective observational study with self-reported chocolate consumption measures [154]. However, these studies were all observational with heterogeneous populations, and did not account for type of chocolate product consumed, or different chocolate components, in addition to the limitations of

possible confounding factors in such studies. In 2012, di Renzo et al. [155] performed a RCT in which 44 healthy women at the end of the first trimester were given a daily 30 g chocolate supplement with 70% cacao content until delivery. Compared to controls, women who had consumed chocolate during pregnancy, had lower diastolic and systolic BP as well as lower levels of liver enzymes. In a pilot RCT investigating acute and long term effects of app. 1-3 moths of chocolate flavanols on FMD and BP in healthy pregnant women, no significant differences between the intervention and control groups were found [156]. The study was small, and the control-group received a low-flavanol chocolate as placebo, compared to high-flavanol chocolate with equivalent theobromine content in the intervention group, which could have affected the outcome of the study.

In an effort to investigate the mechanism behind the suggested inverse relationship between cacao and preeclampsia, Bujold et al. [157] studied the effect of chocolate flavanols and theobromine on uteroplacental blood flow by Doppler blood flow velocimetry. Women with elevated UtA pulsatility index (PI) in the first trimester were randomized to supplements of either high-flavanol high-theobromine chocolate, or low-flavanol low-theobromine chocolate. After 12 weeks there were significant improvements in the UtA PI's supporting the protective effect of chocolate on preeclampsia, however, decreased resistance to blood flow was seen in both groups, leading the authors to speculate that other components of chocolate than flavanols and theobromine might influence the response.

There have also been concerns about possible negative effects of flavonoids in pregnancy. High doses of polyphenols have been associated with fetal defects in animal models [158], and to early ductus arteriosus constriction in the third trimester [159].

In conclusion, there are scientific findings supporting beneficial effects of cacao on the cardiovascular and metabolic system, also in the pregnant population, but the possible protective effect for hypertensive pregnancy complications, and the underlying exact mechanisms, are yet to be further investigated and clarified.

Aims of studies

The overall aim of this thesis was to investigate maternal arterial stiffness in normal and complicated pregnancy using digital pulse wave analysis by photoplethysmography.

Specific aims were:

I. To investigate repeatability of arterial stiffness parameters measured by digital pulse wave analysis, and their correlation to arterial stiffness parameters measured by applanation tonometry.

II. To investigate the influence of gestational age on digital pulse wave analysis arterial stiffness parameters in normal pregnancy, and establish reference values adjusted for gestational age.

III. To investigate the effect of controlled ovarian hyperstimulation and early pregnancy on arterial stiffness assessed by digital pulse wave analysis.

IV. To investigate the association between pathological uterine artery Doppler blood flow velocity changes and maternal arterial stiffness measured by digital pulse wave analysis.

V. To investigate the effect of flavonoid-rich chocolate on uterine artery blood flow measured by Doppler ultrasound blood flow velocimetry, and maternal arterial stiffness assessed by digital pulse wave analysis, in second and third trimester pregnant women with abnormal uterine artery Doppler blood flow indices.

Methods

This thesis was based on studies using five separate study groups: four study groups of women and one of mixed gender. Digital pulse wave analysis (DPA) by photoplethysmography was used for arterial stiffness assessment in all studies.

Digital pulse wave analysis by photoplethysmography

DPA was performed with a Meridian DPA™ photoplethysmography (Salcor AB, Uppsala, Sweden) in all included studies (Figure 5). A finger clip with a light emitting diode, and a light receiver on the opposite side, registers the volume pulse curve in the finger and the pulse curve is then analyzed by a computer software program. A registration takes 70 seconds during which the subject is restrained from moving or speaking. Pulse wave recognition is presented as good if >80% of pulse waves are recognized. In order to get an optimal signal a minimum pulse height (PH) was usually acquired, and the actual PH with a marked normal interval is displayed as the measurement is performed. The Meridian DPA™ then automatically provides 16 arterial stiffness parameters (Table 1) based on the PPG or APG curve (or both combined) (Figure 6). The physiological background of specific Meridian DPA™ parameters were obtained by the manufacturer's manual (Meridian DPA, Meridian Co., Ltd., Korea) and personal correspondence with Mr. Saint Myeong and Mr. Steve Kim at the Meridian Company. Physiological interpretation of APG indices have been described in previous literature (see Introduction), however full understanding of the physiological determinants of the APG is still lacking.

Figure 5.
Meridian DPA™.

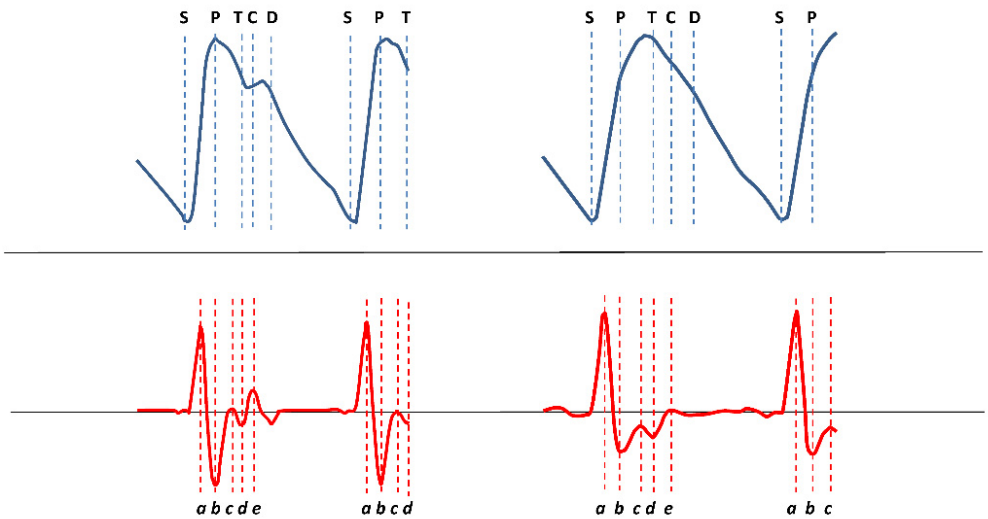

Figure 6.
Authentic Meridian DPA™ photoplethysmographic curves (upper panel) and their corresponding acceleration photoplethysmographic curves (lower panel), from a healthy 33- year old woman (left panel) and a healthy 66-year old man. S = start of systole, P = percussion/forward wave, T = tidal/reflected wave, C = incisura/end of systole, D = dicrotic wave (result of the aortic valve closing).
E. von Wowern et al., PLoS ONE 2015;10(8):e0135659.

Table 1.
Variables provided by the Meridian DPA ™ system.

Pulse height (**PH**)	P - S	Amplitude of the pulse, an indicator of perfusion, stroke volume, vasomotor tone and volume loading [160]. Affected by all factors that affect perfusion and is thus a sensitive but not specific measure of circulatory change.
Ejection time compensated (**ETc**)	$\frac{S-C}{\sqrt{HR/60}}$	Time in milliseconds for the left ventricular ejection of blood adjusted to heart rate (HR) by the Bazett formula. Affected by left ventricular performance and aortic valve disease.
Elasticity index (**EI**)	P/T	An indicator of left ventricular ejection power and central arterial stiffness/compliance.
Cardiac ejection elasticity index (**EEI**)	(P/T) x (-*b*/*a*)	An indicator of left ventricular ejection power and central arterial stiffness/compliance.
Dicrotic index (**DI**)	C/PH	An indicator of peripheral vascular resistance/constriction.
Dicrotic dilatation index (**DDI**)	(PH-C)/PH = 1-DI	An indicator of peripheral vasodilation.
Dicrotic elasticity index (**DEI**)	(D-D´)/C	An indicator of arteriolar dilatation/constriction.
b* /*a	*b*/*a*	The acceleration of blood flow from S to P (early systolic phase), an indicator of left ventricular power or central arterial compliance.
c* /*a	*c*/*a*	Positive wave in late systole, related to arterial stiffness and age.
d* /*a	*d*/*a*	The intensity of the reflected wave and thus the augmentation of aortal pressure, indicating afterload and likely the effects of vasoactive agents.
e* /*a	*e*/*a*	Positive wave in early diastole, related to age.
Aging index (**AI**)	$\frac{(b-c-d-e)}{a}$	A composite index of global arterial stiffness or vascular “age”.
a*-*b ***a*-*c*** ***a*-*d*** ***a*-*e***		Time intervals from peak to peak.

After an initial period of a minimum of 10 minutes rest in the supine position, two consecutive DPA recordings from the index or middle finger were performed. Participants were asked to restrain from alcohol for at least twelve hours, and coffee, tea, large meals and nicotine for at least three hours, prior to the planned visit. If PH was insufficient, or poor recognition with no numerical values for variables were obtained, a repeated measurement was performed after attempts to obtain a registration of better quality. Reasons for low pulse wave recognition can be cold hands, i.e. peripheral vasoconstriction, or nail polish. Participants were asked not to wear nail polish and when needed, the hand was warmed by a heating pad or rubber glove filled with warm water. For pregnant women, this was seldom necessary.

The mean value of two recordings were used in further analyses in all studies below. Based on the results in paper I and existing previous research on variables, analyses were restricted to DPA variables EEI and *b*/*a* for vascular assessment of large arteries, DI and *d*/*a* for peripheral arteries, and AI as a global arterial stiffness index, in the following papers (in paper II only *b*/*a*, *d*/*a* and AI).

Paper I

Study population and setting

The study was performed in collaboration between the Department of Obstetrics and Gynecology, and the Medical Research Unit of the Department of Internal Medicine, both at Skåne University Hospital in Malmö. Study participants of both genders above the age of 64 were recruited from an on-going screening study at the Medical Research Unit, and pregnant and non-pregnant women under the age of 64 were recruited as volunteers from the Department of Obstetrics and Gynecology. Medical background, life style factors and medical treatment were disregarded. Participants were instructed to take medications as prescribed.

Arterial stiffness measurements by applanation tonometry

Carotid-femoral PWV and pulse wave analysis (PWA) derived AIx were measured by applanation tonometry of the carotid, femoral and radial artery respectively, using a SphygmoCor™ device (CvMS version 9, AtCor Medical, West Ryde, NSW, Australia). For PWV calculations, the vascular distances are approximated by standardized measurements of the lengths between the carotid recording point and the suprasternal notch, the suprasternal notch and the umbilicus, and the umbilicus and the femoral recording point. Using the distances and ECG synchronization, the velocity of the pulse wave can be assessed. The distances were measured with a vernier caliper for correct assessment despite of a protruding uterus or abdominal obesity. Using a transfer function, the SphygmoCor™ calculates the central AIx from the radial pulse pressure wave and automatically adjusts the AIx to a HR of 75.

Study protocol

Participants were measured for weight and height and then placed in the supine position and connected to the SphygmoCor™ ECG and Meridian DPA™. Women in late pregnancy were tilted slightly to the left by a towel under the right hip in order to decompress the vena cava. The temperature in the examination room was 22±1^{o}C. The locations of the strongest pulse at the carotid and femoral level were marked and distances measured as above. Brachial blood pressure was measured twice with a one-minute interval using an automatic device (Omron Intellisense model M8 RC, Omron Healthcare, Hoofdorp, The Netherlands) after 5 minutes of rest. Three PWV and PWA recordings and two DPA recordings were performed in series simultaneously. Only recordings of high quality according to the devices' built-in quality control systems were accepted.

Paper II

Study population and setting

Pregnant women were asked to participate in the study upon their first visit at four different maternal health care service units in or in the vicinity of Malmö. Exclusion criteria were multiple pregnancy, cardiovascular disease, renal disease, or diabetes at enrollment, or on-going medical therapy with vasoactive medications. Normotensive women with a history of preeclampsia were accepted in the study.

Study protocol

The study was performed in connection with the routine visits at the health care unit according to the Swedish maternity care program at around 12-15, 20-24, 30, 37 and 41 gestational weeks and postpartum. Due to the extent of data, it was decided that the postpartum control would be analyzed and published separately at a later time. Relevant clinical data was registered upon first visit. At each visit, brachial blood pressure was measured using the units own equipment, and two DPA recordings performed after 10 minutes of rest in the supine position slightly tilted to the left. Pregnancy complications or deviations from the study protocol were noted.

Paper III

Study population and setting

The study was performed at Nordic IVF in Malmö which is a private clinic offering in vitro fertilization (IVF) treatment. Inclusion criteria were women planned for IVF for various infertility reasons. Exclusion criteria were hypertension or other cardiovascular disease, renal disease or diabetes diagnosed previous to enrollment. Women using medications with an established cardiovascular effect were also not asked to participate.

Controlled ovarian hyperstimulation and in vitro fertilization

In outline, IVF is when fertilization of the oocyte is performed in an artificial environment outside the human body. In order for this to be accomplished, the ovaries have to be stimulated to produce multiple mature follicles from which oocytes can be harvested. This is achieved by administration of gonadotropins. Premature ovulation is prevented through administration of a gonadotropin releasing hormone (GnRH) agonist or antagonist. The dose-response to gonadotropins is individual why follicle growth is monitored by ultrasound examinations, and when optimal follicle maturation is achieved, ovulation is induced. Oovum pick up (OPU) is performed transvaginally using ultrasound

imaging. The fertilization of the oocyte is performed, cell division initiates, and an embryo is then placed in the uterine cavity.

In the study, COH and IVF were performed according to clinical routine, and regimen was decided by the attending clinician. Two types of protocols were used depending on if ovarian down regulation was achieved with GnRH-agonist (long protocol) or GnRH-antagonist (short protocol). In the long protocol, ovarian stimulation was preceded by GnRH-agonist treatment, and in the short protocol, GnRH-antagonist treatment was started on stimulation day 5.

Follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) were used for ovarian stimulation, and response monitored by serial transvaginal ultrasound examinations starting on stimulation day 7-8. When leading follicles were of optimal size (18-20 mm), ovulation was induced with a single dose of human chorionic gonadotropin (hCG). After 36 hours OPU was performed transvaginally, and embryo transfer (ET) 2, 3 or 5 days after OPU depending on embryo development. Vaginal progesterone for luteal support was given until time for pregnancy test 20 days after OPU. In women who became pregnant, ultrasound examinations were performed in gestational week 7-8.

Since the individual response to gonadotropins routinely is monitored by ultrasound assessment of follicle growth at the clinic, and not normally by testing of hormone levels, the ovarian sensitivity index (OSI) defined by Huber et al. [56], was used in the study to evaluate ovarian response. The OSI was developed to standardize the definition of ovarian response, and is related to clinical outcome variables of IVF treatment.

Study protocol

Baseline DPA measurements were performed upon the first visit to the clinic, before treatment was initiated. Demographic characteristics, factor for infertility and medical history were noted. DPA measurements were then repeated at the first scheduled visit for ultrasound examination during the ovarian stimulation and after ET, as well as at the visit in gestational week 7-8 for women who became pregnant. All measurements were performed in a secluded room after 10 minutes of rest in the supine position, and preceded by brachial blood pressure measurement (Omron M3, Omron Healthcare Europe, Hoofddorp, The Netherlands).

Papers IV and V

Study populations and settings

The studies were performed at the Department of Obstetrics and Gynecology in Malmö. Women pregnant in the second or third trimesters referred for Doppler blood flow velocimetry on clinical indications were asked to participate. As a separate cohort, women presenting with UAS 3 or 4 were recruited for a pilot intervention study with flavonoid-rich chocolate (paper V) and the Doppler ultrasound examination repeated three days later. Indications for examinations were noted as well as on-going medical treatment.

Uterine artery ultrasound Doppler blood flow velocimetry

The technique uses the fact that sound waves reflected on moving objects shift in frequency, called the Doppler effect, and can be used to calculate the maximum velocity of movement. In the UtAs, with moving erythrocytes, the Doppler technique allows for calculation of the maximum blood flow velocity at the measuring point and generates a blood flow velocity curve for every heartbeat. Quantitative measurements as well as the shape of the UtA Doppler curve can be analyzed to assess resistance to blood flow distal of the measuring point. As an estimate of resistance to blood flow, the indices S/D ratio (peak systolic/diastolic velocity), resistance index (RI = (peak systolic – end diastolic velocity)/ peak systolic velocity) and pulsatility index (PI = (peak systolic – end diastolic velocity)/ mean velocity) are commonly used [129]. PI has the advantage of by definition allowing interpretation of the entire waveform, also in cases with absent diastolic flow, and is thus used in the Department of Obstetrics at Skåne University Hospital. UtA PI is inversely related to maternal HR which must be considered for PI interpretation when maternal pulse frequencies are extreme [161].

In addition to UtA PI, the shape of the waveform can be analyzed. In non-pregnant individuals, the uterine arteries show a diastolic "notch", i.e. an accelerative flow velocity phase occurring during diastole, creating a biphasic shape of the decelerative diastolic velocity curve (Figure 7). In normal pregnancy, diastolic flow becomes continuous, and UtA notching disappears around mid-second trimester as compliance of the uterine artery increases [128].

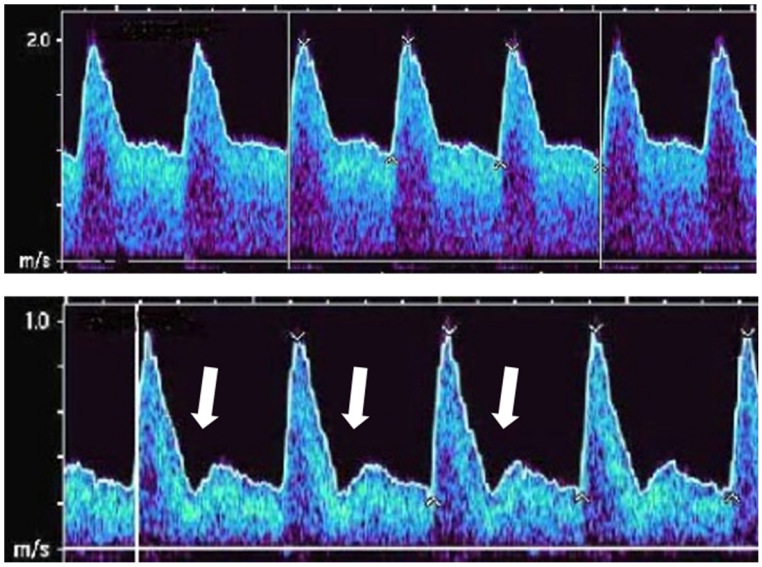

Figure 7.
Authentic uterine artery Doppler velocimetry waveforms without (upper panel) and with (lower panel) early diastolic notching.

Persistent diastolic notching and increased PI are associated with histopathological changes of the placenta [162] and increased risk of PE and IUGR [129]. There is also a direct relationship between UtA blood flow velocity changes and severity of adverse pregnancy outcome [163]. A mean PI > 1.2 from both UtAs have shown to be of better predictive value for perinatal adverse events than PI from the placental side alone [164]. The uterine artery score (UAS) is a scoring system to quantify UtA blood flow changes taking both the PI and diastolic notching in to account, where PI > 1.2 and presence of a diastolic notch gives one point each in each UtA, and thus a minimum of 0 and maximum of 4 points [165] (Table 2).

Table 2.
Uterine artery score classes.

Uterine artery score	
0	Bilateral normal blood velocity waveforms in the uterine arteries
1	One abnormal parameter present (unilateral high PI or notch)
2	Two abnormal parameters present
3	Three abnormal parameters present
4	Four abnormal parameters present (bilateral notch and high PI)

Doppler blood flow velocimetry in the studies was performed according to Gosling et al. [166] using a GE Voluson 730 Expert (GE Medical Systems, Tiefenbach, Austria) ultrasound scanner with an AB 2–7 transducer. UAS, UtA PI and the presence or

absence of an early diastolic notch was noted for both sides. All measurements were performed by highly experienced biomedical scientists.

Study protocol

Study IV was a cross-sectional study. After the Doppler ultrasound examination, still in supine position, brachial blood pressure was measured using an automatic device (Omron M8 Comfort, Omron Healthcare, Hoofddorp, Netherlands) and two DPA recordings were performed. Notching was defined as presence of a notch in the blood flow waveform in the UtAs, uni- or bilaterally, and increased resistance was defined as PI>1.2 as a mean value from the right and left UtA.

Study V was interventional pilot study. After the blood flow examination, eight Xoçai X Power Square™ (MXI Corp, Reno, Nevada, USA) of 6 g and a total of 672mg of flavonoids each [167], were given to the women with the instruction to consume them for three consecutive days: the first and second day three times daily, and two times the third day, with the last piece two hours before scheduled repeated Doppler blood flow examination and DPA measurements.

Statistical methods

Statistical calculations were performed with the StatView version 5.0.1 (SAS Institute, Cary, NC), IBM SPSS Statistics version 23.0 (IBM Corp, Armonk, NY), MedCalc (MedCalc Software, Mariakerke, Belgium), and Gauss (GaussTM, Aptec Systems Inc., Maple Valley, WA, USA) computer softwares.

Non-parametric testing was predominantly used in the thesis based on either skewed distribution of data or small sample sizes, and values were then presented as median (range or percentiles). Sample sizes were well within thresholds for using non-parametric tests according to Mundry and Fischer [168]. When relevant, or if normal distribution of data could not be determined with certainty despite a large study group (as in paper I), the results of both parametric and non-parametric testing, and values as mean (SD) as well as median (range or percentiles), were presented. Non-parametric tests are more robust than their parametric equivalents, but when the assumptions of parametric tests are fulfilled, they have more power to find true associations or differences. The significance level was set to a two-sided *p*-value of < 0.05 throughout.

In paper I, associations between continuous variables were tested with Spearman's rank correlation, and linear correlations with simple or second grade polynominal linear regression (Pearson's).

In order to investigate the repeatability of the DPA variables provided by the Meridian DPA™ system, *agreement* (i.e. a quantification of the difference of two

measurements on the same subject under identical conditions) and *reliability* (i.e. the magnitude of measurement error) were calculated for the two repeated DPA measurements. Agreement was investigated by Bland-Altman analysis which plots the relationship of the difference (delta values) and the mean of two measurements. Upper and lower limits of agreement (LoA, difference between measurements ±1.96 SD), as well as the coefficient of repeatability (CoR, upper LoA minus mean difference between measurements) were calculated. The CoR has the advantage of being expressed in the same units as the tested measurement tool, and accounts for the value below which, with 95% probability, the true absolute differences between two measurements are.

Reliability was investigated by calculation of the intraclass correlation coefficient (ICC) for DPA variables, which is the correlation between two measurements made on the same subject and estimates the measurement error (1-ICC). The ICC is influenced by the heterogeneity in the population as it is determined by the between-subject standard deviations. Hence, a more homogenous population will result in lower ICCs. The ICC of DPA variables were calculated with a statistics tool box online (http://department.obg.cuhk.edu.hk/index.asp?scr=1920, accessed June 2015).

The correlation coefficient of simple linear regression analysis, e.g. Pearson's *r*, is commonly used in research to describe the reliability of a measurement method. The *r* expresses how strong the linear relationship between two measurements are, but not the slope of the line, and therefore not necessarily the absolute agreement, why *r* is not an optimal estimate of test-retest reliability.

For comprehensive description of the repeatability of the DPA variables, delta and mean values, LoA, CoR, ICC and *r*, were presented in paper I.

In paper II, changes over gestation of repeated longitudinal DPA measurements were tested with mixed effect models. The model allows for both within and between subject variations and estimates the mean longitudinal association of all entered data, and is therefore superior to tests that only compare means between measurement points. The distribution of the investigated variables for the first measurement obtained in gestational weeks 14-24 was presented as percentiles and means respectively. Tests for normal distribution of the data were not significant, but mean and median values were centered, class mean values not skewed, and sample size large enough, why the requirements for ANOVA and ANCOVA (parametric tests) were stilled fulfilled, and thus used for testing associations between maternal age and parity and DPA variables. ANOVA tests for differences in means between separate class variables, whereas ANCOVA is used when one variable is continuous. In study II, associations were tested with maternal age entered both as a class and continuous variable respectively.

In papers III-V, associations between continuous variables were tested with Kendall's tau rank correlation, and linear simple and multiple regression for variable

dependency. The Mann–Whitney U test (2 groups) and Kruskal–Wallis test (>2 groups) were used for the comparison of continuous variables between groups, and Wilcoxon matched-pairs signed-rank test when variables were paired. Categorical variables before and after intervention (paired) were compared with the McNemar test (paper V).

To illustrate differences in median values, box plots depicting the median, intra quartile range (IQR) and total range, and hence suitable for skewed data or small sample sizes, were used in paper III.

In papers III and IV, calculations of the confidence intervals (CI) around the median were calculated according to J. Björk [169] as:

$$\frac{n}{2} - \frac{1.96 \times \sqrt{n}}{2} = \text{C} = \text{rank n for lower CI (95\%) limit}$$

$$n + 1 - C = \text{rank n for upper CI (95\%) limit}$$

Ethical considerations

Photoplethysmography is a non-invasive method and entails no discomfort or increased risk for the pregnant woman or her fetus. Ultrasound examinations were only performed on clinical indications except for follow up in paper V. When performed by expert examiners according to guidelines for ultrasound safety [170], exposure to ultrasound is not considered to give serious cause for concern for fetal adverse effects. No medical treatment was given for study reasons. Flavonoid-rich chocolate as a dietary supplement, as studied in paper V, seems to be associated with more beneficial than harmful effects, and was considered minimal risk with the given dose in the study. The study was not performed during the period of organogenesis, and the fetal circulation was monitored as part of the study protocol.

All participants were able to assimilate given information, and gave their oral and written informed consent. Studies were approved in advance by the Regional Research Ethics committee in Lund (Dnr. 532/2006, 110/2012, 2014/648).

Results and comments

Paper I

We recruited a heterogeneous study population of 112 individuals consisting of both men (n=25), and pregnant (n=14) and non-pregnant (n=73) women of different ages (range 21-84 years). Morbidities, smoking and medical treatment in the study group were disregarded. We decided to investigate all DPA indices except pulse height (PH), which is highly influenced by environmental and conditional factors.

Agreement and reliability of DPA variables

For agreement, Bland-Altman plots were performed and LoA and CoR calculated (plots shown for AI, *b*/*a* and *d*/*a* in Figure 8, outliers included). There was no systematic skewness for any DPA variable. The difference of mean values of two repeated measurements were close to zero for most DPA variables, however for some variables, CoR were high and LoA wide (Table 3). Since outlying values (e.g. due to technical problems or artefacts) have large impact on LoAs and CoRs and may be omitted according to Bland and Altman [171], data were also presented with three outliers excluded. Indeed, outlying values had large impact on the CoRs for especially DEI, AI, *c*/*a* and *d*/*a*.

Whether the degree of agreement of two measurements is good or not must be evaluated in the clinical context, why conclusions on the clinical value of the DPA method are precarious based solely on study I. In an ideal setting, acceptable limits of agreement are set in advance based on clinical significance, and sample sizes are adjusted accordingly. No references of such data were however available for the investigated variables.

Reliability of the DPA method was investigated by calculation of ICCs for the DPA variables (Table 3). As for all correlations, grading of correlations coefficients are arbitrary, but we considered our findings of reliability for variables DI, DDI, EEI, AI, *b*/*a* and *e*/*a* (ICC 0.80-0.87) to be good, the reliability for variables EI, *c*/*a*, *d*/*a*, *a*-*b* and *a*-*e* (ICC 0.53-0.72) to be moderate, and the reliability for variables ETc, DEI, *a*-*c* and *a*-*d* (ICC 0.26-0.46) to be poor. As the study population was heterogeneous, ICCs cannot be automatically applied to a different maybe more homogenous population, which must be considered when interpreting the results.

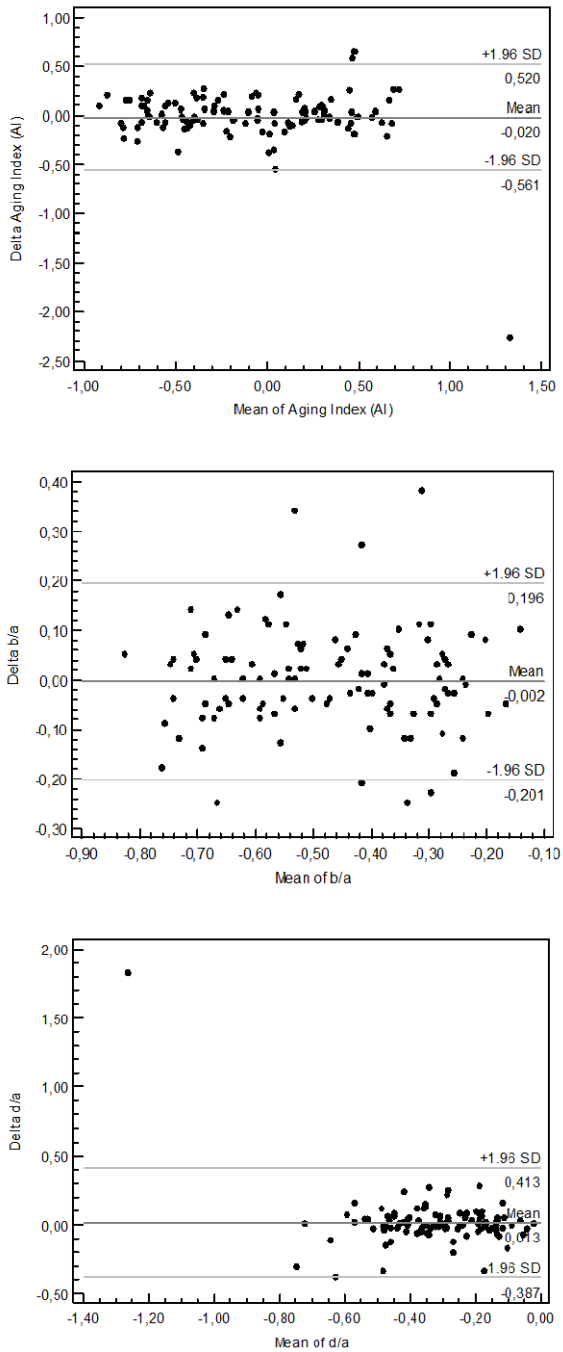

Figure 8.
Bland Altman plots for aging index (AI), *b*/*a* and *d*/*a*.

Table 3.
Measurements of agreement and reliability between two repeated measurements of digital pulse analysis (DPA) variables in 112 individuals.

DPA variable	1st measurement		2nd measurement		Mean of measurements	1st vs. 2nd measurement, simple linear regression analysis		Agreement: Bland-Altman analysis						Reliability
	Mean	SD	Mean	SD		*p*	r	-1.96 SD[a]	Mean ΔDPA	+1.96 SD[b]	CoR	Mean ΔDPA[c]	CoR[c]	ICC
ETc	341.86	36.41	342.92	30.21	344.73	< 0.0001	0.47	-69.54	-1.29	66.97	68.25	-2.4	67.1	0.46
EI	0.97	0.26	0.96	0.25	0.97	< 0.0001	0.72	-0.39	0.015	0.42	0.38	0.00	0.35	0.72
DI	0.74	0.15	0.75	0.13	0.74	< 0.0001	0.80	-0.18	-0.010	0.16	0.17	0.002	0.14	0.80
DDI	0.26	0.15	0.25	0.13	0.26	< 0.0001	0.81	-0.15	0.015	0.18	0.17	0.008	0.14	0.81
EEI	0.49	0.29	0.49	0.29	0.50	< 0.0001	0.82	-0.34	0.004	0.35	0.35	0.00	0.28	0.82
DEI	0.08	0.23	0.06	0.09	0.060	< 0.0001	0.42	-0.39	0.030	0.45	0.41	0.01	0.17	0.27
AI	-0.10	0.45	-0.07	0.51	-0.093	< 0.0001	0.84	-0.56	-0.020	0.52	0.54	0.00	0.34	0.84
b/a	-0.48	0.17	-0.48	0.18	-0.48	< 0.0001	0.83	-0.20	0.002	0.20	0.20	-0.01	0.18	0.84
c/a	-0.19	0.14	-0.20	0.15	-0.20	< 0.0001	0.63	-0.23	0.008	0.25	0.24	0.00	0.15	0.63
d/a	-0.31	0.17	-0.33	0.24	-0.31	< 0.0001	0.53	-0.39	0.013	0.41	0.40	-0.01	0.21	0.53
e/a	0.12	0.09	0.12	0.08	0.12	< 0.0001	0.88	-0.088	-0.003	0.082	0.084	0.00	0.073	0.87
a-b	94.36	27.79	93.88	26.25	94.70	< 0.0001	0.54	-50.39	0.64	51.67	51.48	0.60	51.7	0.55
a-c	173.72	28.76	177.20	29.80	176.66	0.0082	0.25	-73.24	-3.30	66.64	70.20	-3.2	69.8	0.26
a-d	233.50	35.15	238.22	28.68	236.82	< 0.0001	0.40	-72.53	-3.90	64.73	69.19	-4.9	67.0	0.40
a-e	331.23	37.09	331.82	40.54	334.12	< 0.0001	0.71	-59.24	-0.87	57.50	58.44	-2.1	53.1	0.72

ΔDPA, difference between measurements; CoR, coefficient of repeatability; ICC, intraclass correlation coefficient

[a] 95% lower limit of agreement (-1.96 × SD of ΔDPA).

[b] 95% upper limit of agreement (+1.96 × SD of ΔDPA).

[c] After exclusion of three outliers with ΔDPA values ≥ ± 4SD of mean ΔDPA (old age in two, low heart rate in two, and low pulse height in one).

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In general, repeatability was lower for variables representing late systole (*c* or *d* waves) or diastole (*e* wave), as well as for variables that represent measurements along the time axis (*a*-*b*, *a*-*c*, *a*-*d*, *a*-*e*) which showed the poorest reliability of all. The *c*, *d* and *e* waves may be less salient and thus difficult to calculate accurately [172], but variables calculated as quotas containing these inflection points without measurements on the time axis, reached moderate reliability (*d*/*a* ICC 0.53).

Associations between DPA variables and heart rate and body height

HR and body height were found not associated (Spearman's rank correlation, p=0.56; simple linear regression, p=0.98). Associations between DPA variables and HR and height were examined separately, as well as associations between HR-adjusted DPA variables and height if the DPA variable was dependent on HR. All DPA variables but *a*-*c* (p=0.058) were significantly correlated to HR and associations were linear for all variables (for *d*/*a* only at second order polynomial regression). No variable except for *e*/*a* showed significant correlations to height (Spearman's rank correlation), but in linear regression analyses also DEI. DPA variables adjusted to HR 75 (variable@75) showed significant correlations to height for *e*/*a*@75 and *a*-*e*@75, and at linear regression analysis also for DEI@75. These

variables were then also adjusted to a height of 170 cm (variable@75@170). DPA variable adjustments were performed according to DPA@75 = DPA_{HR} ± C x (75-HR), and DPA@170 = DPA_{height} ± C x (170-height), respectively, were C denotes the slope constant obtained from linear regression analyses (Table 4).

Table 4.
Linear regression equations for relationships between DPA variables and HR and height used to adjust DPA variables if $p<0.05$, or $p<0.10$ if Spearman's rank $p<0.05$.

DPA variables	Equation used to adjust variable (Y) for heart rate (X)	Equation used to adjust variable (Y) for height (X)	Equation used to adjust variable (Y) at HR@75 for height (X)
EI	Y = 0.174 + 0.012X	-	-
DI	Y = 1.215 – 0.007X	-	-
EEI	Y = -0.23 + 0.011 X	-	-
DDI	Y = -0.24 + 0.007 X	-	-
DEI	Y = -0.139 + 0.003 X	Y = -0.379 + 0.003X	Y = -0.374 + 0.003X
AI	Y = 0.376 – 0.007 X	-	-
b/*a*	Y = -0.105 – 0.006 X	-	-
c/*a*	Y = 0.035 – 0.004 X	-	-
d/*a*	Y= -1.214 + 0.024X – $0.0001575X^2$	-	-
e/*a*	Y = -0.067 + 0.003 X	Y = -0.412 + 0.003X	Y = -0.383 + 0.03X
a-*b*	Y = 129.561 – 0.528 X	-	-
a-*c*	Y = 219.262 – 0.645 X	-	-
a-*d*	Y = 331.187 – 1.428 X	-	-
a-*e*	Y = 465.463 – 1.988 X	-	Y = 452,135-0.807X

Although significant, the associations between DPA variables and HR were weak with rho or correlation coefficients ≤0.66 for all variables (AI, rho -0.22, *r* -0.19; EEI, rho 0.44, *r* 0.46; DI, rho -0.64, *r* -0.65; *b*/*a*, rho -0.39, *r* -0.39; *d*/*a*, rho 0.28). The finding that HR influence DPA variables must be considered in studies with major variations in HR.

Associations between DPA variables and pulse wave velocity and augmentation index

Associations between DPA variables and applanation tonometry variables (PWV and AIx) were highly significant for all variables except time measures containing the *c*, *d* and *e* waves (Table 5). Both crude and adjusted DPA variables were tested. Associations with adjusted variables were generally equally significant but slightly stronger, but the Pearson correlation coefficients increased in no case with more than 0.05. The strongest correlations to AIx were found for EEI@75, *b*/*a* and AI (*r* 0.75-0.78). *b*/*a* dominates in the calculation of the AI, and is also a factor in the calculation of EEI, thus they are dependent of each other and cannot be viewed as

entirely separate entities. For PWV, the strongest associations were with EEI@75, *d*/*a* and AI (*r* 0.61-0.65). Overall, the linear associations with AIx were stronger than with PWV. This is not surprising since DPA variables and AIx, unlike PWV, are derived from the pulse wave contour and thus is influenced not only by PWV, but also by wave reflections.

Table 5.
Correlations between applanation tonometry and DPA variables in 112 individuals.

DPA variables	Pulse wave velocity								Augmentation index @ heart rate 75							
	Crude				Adjusted				Crude				Adjusted			
	Spearman's rank correlation		Simple linear regression		Spearman's rank correlation		Simple linear regression		Spearman's rank correlation		Simple linear regression		Spearman's rank correlation		Simple linear regression	
	p	*rho*	*p*	*r*	*p*	*rho*	*p*	*r*	*p*	*rho*	*p*	*r*	*p*	*rho*	*p*	*r*
EI	< 0.0001	-0.55	< 0.0001	-0.48	< 0.0001	-0.57	< 0.0001	-0.53	< 0.0001	-0.63	< 0.0001	-0.62	< 0.0001	-0.64	< 0.0001	-0.66
DI	0.0002	0.36	<0.0001	0.37	0.0002	0.36	< 0.0001	0.42	0.0003	0.35	0.0002	0.35	0.001	0.32	0.0002	0.35
DDI	0.0004	-0.35	<0.0001	-0.36	0.0001	-0.37	<0.0001	-0.41	0.0003	-0.35	0.0002	-0.35	0.0002	-0.36	< 0.0001	-0.38
EEI	< 0.0001	-0.63	<0.0001	-0.56	< 0.0001	-0.66	<0.0001	-0.61	< 0.0001	-0.75	< 0.0001	-0.71	< 0.0001	-0.77	< 0.0001	-0.76
DEI	< 0.0001	-0.72	<0.0001	-0.49	< 0.0001[a]	-0.70	<0.0001[a]	-0.50[a]	< 0.0001	-0.69	< 0.0001	-0.58	< 0.0001[a]	-0.59[a]	< 0.0001[a]	-0.56[a]
AI	< 0.0001	0.72	<0.0001	0.65	< 0.0001	0.70	<0.0001	0.64	< 0.0001	0.79	< 0.0001	0.78	< 0.0001	0.78	< 0.0001	0.77
b*/*a	< 0.0001	0.62	<0.0001	0.55	< 0.0001	0.63	<0.0001	0.56	< 0.0001	0.76	< 0.0001	0.75	< 0.0001	0.77	< 0.0001	0.75
c*/*a	< 0.0001	-0.47	<0.0001	-0.43	< 0.0001	-0.54	<0.0001	-0.48	< 0.0001	-0.47	< 0.0001	-0.45	< 0.0001	-0.54	< 0.0001	-0.52
d*/*a	< 0.0001	-0.68	<0.0001	-0.60	< 0.0001	-0.68	<0.0001	-0.60	< 0.0001	-0.72	< 0.0001	-0.68	< 0.0001	-0.72	< 0.0001	-0.68
e*/*a	< 0.0001	-0.60	<0.0001	-0.50	< 0.0001[a]	-0.58	<0.0001[a]	-0.54[a]	< 0.0001	-0.67	< 0.0001	-0.63	< 0.0001[a]	-0.60[a]	< 0.0001[a]	-0.59[a]
a*-*b	0.0014	0.31	0.025	0.21	0.0027	0.29	0.034	0.20	0.046	0.19	0.0030	0.28	0.062	0.18	0.0065	0.26
a*-*c	0.13	-	0.025	-0.21	0.035	-0.20	0.0098	-0.25	0.0016	-0.30	0.0004	-0.33	0.0003	-0.35	< 0.0001	-0.38
a*-*d	0.90	-	0.41	-	0.21	-	0.11	-	0.58	-	0.85	-	0.51	-	0.46	-
a*-*e	0.31	-	0.80	-	0.50[a]	-	0.34[a]	-	0.015	-	0.084	-	1.0[a]	-	0.52[a]	-

[a] DPA variable adjusted for both heart rate and body height.

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von Wowern et al., PLoS ONE 2015;10(8):e0135659.

Conclusions of Study I

Our findings support that the DPA technique can be used to assess the arterial system in individuals with different vascular status. Although not to be used interchangeably, DPA variables reflect the vascular condition similarly to AIx. DPA variables are dependent on HR which must be considered when used, but the influence of HR when interpreting the physiological or pathophysiological impact seems to be small. The reliability was good for several variables, but agreement harder to interpret due to lack of clinical context. Considering repeatability and association to applanation tonometry variables, EEI, *b*/*a* and AI were the best DPA variables for vascular assessment.

Paper II

149 women were recruited but ten were later withdrawn: five due to development of hypertensive complications and one of IUGR, three women due to multiple pregnancy, and one woman because of technical problems at the first measurement and later miscarriage. 139 women then remained for analyses of which the majority had the first DPA measurement performed in gestational week 14-16.

Associations between DPA variables and gestational age

All investigated DPA variables (AI, *b*/*a*, *d*/*a*) showed significant associations with gestational age with linear and quadratic mixed effect models, with best fit for the quadratic models ($p<0.001$, Figure 9). The results were in congruence with TVR [71] and AIx [80, 83] as well as PPG derived stiffness parameter [84] changes in normal pregnancy. Since the greatest change in arterial compliance occur in the first trimester [66] it would have been preferred to obtain the first measurements even earlier in pregnancy, but the study was performed within the regular maternal health program and measurements were adherent to routine clinical visits, commonly starting in the early second trimester.

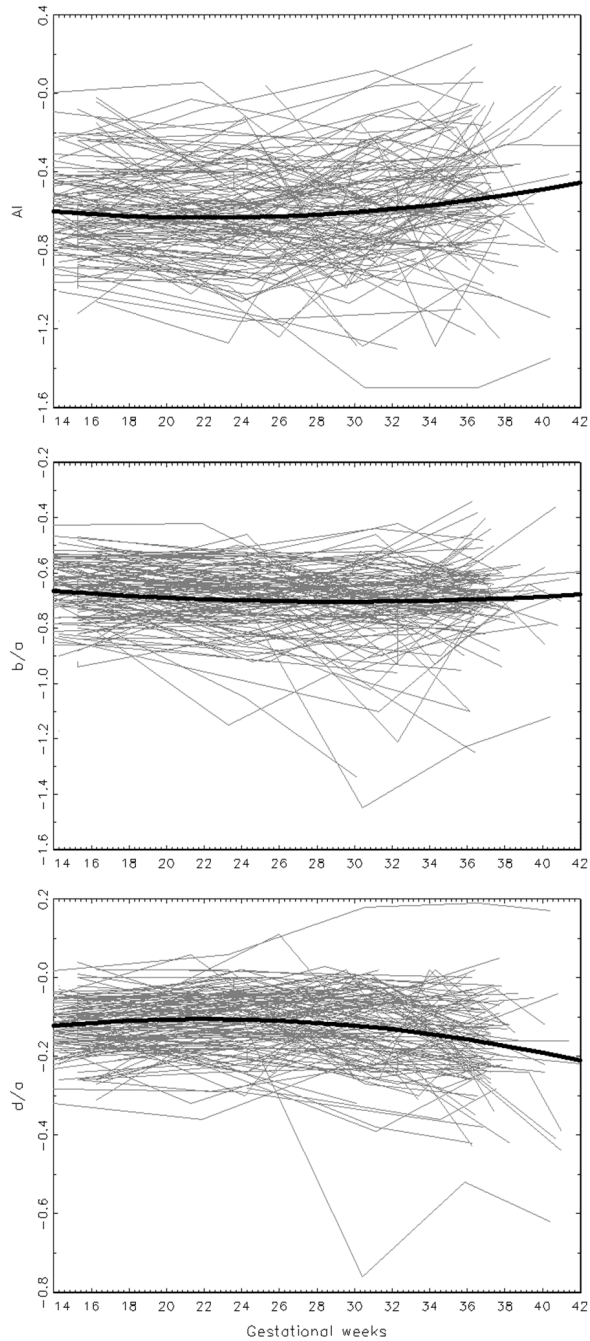

Figure 9.
Associations between maternal aging index (AI), *b*/*a* and *d*/*a*, and gestational age in 139 uncomplicated pregnancies. The associations were significant (p<0.001) when calculated with quadratic mixed-effects models.

Distributions of DPA variables in the cohort

Age and parity had independently of each other significant influences on DPA variables, so that arterial stiffness was increased in older women as well as parous compared with nulliparous women. However, age and parity did not significantly change the longitudinal associations to gestational age (shape ≥0.60). There were no major changes in DPA variables up to 24 gestational weeks, after when all DPA variables indicated a slight increase in arterial stiffness as pregnancy proceeded. The overall variations of DPA variables were large relative to the change with gestational age. Based on these findings, calculations of reference values were restricted to the first obtained measurements in gestational week 14-24 (if two measurements were performed during the time period the mean value was used), which would be the most relevant time period for potential screening with DPA for PE and IUGR. Table 6 shows the distributions of DPA variable values in the time period relative to age and parity, and Table 7 the independent impact of age and parity on DPA variables. Maternal age was independent of parity significantly associated with a reduction in *d*/*a*, and increase in AI (when age was entered as a continuous variable), indicating increased arterial stiffness with age. AI and *b*/*a*, but not *d*/*a*, were significantly increased in parous compared with nulliparous women independent of age, indicating increased arterial stiffness or reduced cardiac left ventricular performance in parous women. This finding seems to contradict results from previous studies, where aortal compliance was increased [173], and MAP and TVR decreased [77, 79], in parous versus nulliparous women. However, those other studies used different modalities, and we found no previous studies of arterial stiffness relative to parity to help interpret the results. The independent influence of parity on arterial stiffness is an interesting subject to be investigated in future research.

HR changes over gestation, and as found in paper I, DPA variables show weak correlations to HR. However, the predominant change in HR occurs in the first trimester [71], prior to when DPA measurements started in study II, and only with a 2 bpm increase after 20 weeks [174]. Therefore, DPA variables were not adjusted for HR in study II.

Table 6.
Distributions of aging index (AI), *b*/*a*, *d*/*a*, and mean arterial blood pressure among 139 women monitored between gestational weeks 14 and 24 (mean 17.5 weeks, median 15 weeks, inter quartile range 14-23.5).

					Percentiles					Mean	(95%CI)
		n	2.5	5	25	50	75	95	97.5		
AI											
	Total	139	-1.16	-1.06	-0.77	-0.62	-0.45	-0.14	-0.04	-0.62	(-0.66, -0.57)
	Maternal age (years)										
	<25	13			-0.92	-0.66	-0.57			-0.72	(-0.84, -0.60)
	25-29	40			-0.80	-0.72	-0.56			-0.67	(-0.75, -0.60)
	30-34	50			-0.78	-0.60	-0.42			-0.61	(-0.69, -0.52)
	35-39	32			-0.64	-0.54	-0.40			-0.54	(-0.64, -0.44)
	40+	4			-0.81	-0.36	-0.28			-0.43	(-0.79, -0.08)
	Parity										
	Nulliparae	51	-1.15	-1.08	-0.86	-0.72	-0.57	-0.31	-0.27	-0.71	(-0.77, -0.65)
	Multiparae	82	-1.23	-1.04	-0.73	-0.59	-0.40	-0.05	0.03	-0.55	(-0.61, -0.50)
b*/*a											
	Total	139	-0.93		-0.76	-0.66	-0.59		-0.48	-0.68	(-0.70, -0.66)
	Maternal age (years)										
	<25	13			-0.77	-0.71	-0.58			-0.70	(-0.77, -0.63)
	25-29	40			-0.78	-0.70	-0.60			-0.70	(-0.73, -0.66)
	30-34	50			-0.76	-0.64	-0.56			-0.67	(-0.71, -0.63)
	35-39	32			-0.74	-0.66	-0.56			-0.67	(-0.72, -0.62)
	40+	4			-0.72	-0.56	-0.52			-0.60	(-0.79, -0.42)
	Parity										
	Nulliparae	51	-0.91	-0.90	-0.80	-0.72	-0.56	-0.54	-0.52	-0.72	(-0.75, -0.68)
	Multiparae	82	-1.13	-0.86	-0.73	-0.64	-0.56	-0.52	-0.44	-0.65	(-0.68, -0.63)
d*/*a											
	Total	139	-0.29	-0.26	-0.16	-0.11	-0.06	0.02	0.04	-0.11	(-0.13, -0.10)
	Maternal age (years)										
	<25	13			-0.10	-0.08	-0.06			-0.07	(-0.11, -0.02)
	25-29	40			-0.12	-0.09	-0.05			-0.10	(-0.12, -0.08)
	30-34	50			-0.16	-0.11	-0.06			-0.11	(-0.13, -0.09)
	35-39	32			-0.20	-0.15	-0.08			-0.15	(-0.18, -0.12)
	40+	4			-0.26	-0.14	-0.11			-0.19	(-0.32, -0.05)
	Parity										
	Nulliparae	51	-0.22	-0.21	-0.13	-0.10	-0.06	0.04	0.06	-0.10	(-0.12, -0.08)
	Multiparae	82	-0.32	-0.29	-0.18	-0.11	-0.07	0.04	0.00	-0.13	(-0.15, -0.11)
Mean arterial pressure (mmHg)											
	Total	133	70.0	71.4	76.7	82.0	86.7	95.0	97.2	82.3	(81.1-83.6)
	Maternal age (years)										
	<25	13			72.5	75.0	82.5			78.4	(73.5, 83.3)
	25-29	38			76.7	83.3	86.7			82.3	(80.2, 84.5)
	30-34	48			77.1	83.3	90.0			82.9	(80.9, 84.9)
	35-39	30			76.7	82.0	85.7			82.2	(79.1, 85.3)
	40+	4			83.3	91.7	94.2			88.8	(77.8, 99.7)
	Parity										
	Nulliparae	49	66.5	70.8	76.7	83.3	87.0	95.0	98.8	82.5	(80.3, 84.6)
	Multiparae	79	70.0	70.7	76.7	81.3	86.7	95.0	97.3	82.2	(80.6, 83.9)

CI, confidence interval. Digital pulse wave analysis values are indices without quantitative measures.

Table 7.
The impact of maternal age and parity on values of aging index (AI), *b*/*a*, and *d*/*a*, respectively, at measurements in gestational weeks 14 to 24 (n=139).

	AI				***b*/*a***				***d*/*a***		
Results from ANOVA	**Contrast**	**95%CI**	***p***		**Contrast**	**95%CI**	***p***		**Contrast**	**95%CI**	***p***
Maternal age (years)											
<25	0	Reference	0.127		0	Reference	0.670			Reference	0.01
25-29	0.03	-0.12, 0.18			0.00	-0.08, 0.08			-0.03	-0.08, 0.02	
30-34	0.08	-0.08, 0.24			0.02	-0.06, 0.10			-0.04	-0.09 , 0.01	
35-39	0.14	-0.03, 0.32			0.02	-0.07, 0.11			-0.08	-0.13, -0.02	
40 +	0.28	-0.01, 0.56			0.10	-0.05, 0.24			-0.12	-0.20, -0.03	
Parity											
Multiparae	0	Reference	0.006		0	Reference	0.016		0	Reference	0.222
Nulliparae	-0.13	-0.22, -0.04			-0.06	-0.10, -0.01			0.02	-0.01, 0.05	
Results from ANCOVA	**Beta**	**95%CI**	***p***		**Beta**	**95%CI**	***p***		**Beta**	**95%CI**	***p***
Increase per five year maternal age increment	0.06	0.01, 0.11	0.014		0.02	-0.01, 0.04	0.136		-0.03	-0.04, -0.01	0.001
Nulliparae vs. multiparae	-0.12	-0.22, -0.04	0.007		-0.05	-0.10, -0.01	0.026		0.02	-0.01, 0.04	0.284

ANOVA, analysis of variance (maternal age entered as class variable); ANCOVA, analysis of covariance (maternal age entered as continuous variable).

Conclusions of Study II

Arterial stiffness, as assessed by DPA, changed significantly with progress of pregnancy. The changes were in accordance with the normal hemodynamic adaption to pregnancy, with arterial compliance peaking in the late second trimester. Reference values for AI, *b*/*a* and *d*/*a* were established.

Paper III

93 women were enrolled in the study. Drop-outs due to incomplete data or interrupted IVF treatment resulted in 68 women available for analysis at baseline, 59 at stimulation and 52 at ET. DPA measurements were performed on average on day 7 (mean 7, median 7, IQR 7-8) of FSH stimulation. 25 women became pregnant of whom 6 were lost to follow-up (miscarriages or early pregnancy visit at another clinic), thus DPA measurements were obtained in 19 women in 7+0 to 7+6 gestational weeks (in two women in 6+, one woman 9+ and one woman 10+ gestational weeks, respectively). Among the 68 women, 9 women were poor ovarian responders, 49 normal and 10 women high, when graded according to OSI cut-off values [56]. Demographic data at baseline of the participating women is shown in Table 8.

Table 8.
Demographic data of the study population.

	All (n = 68)	
	Median (range)	Mean (SD)
Age (years)	36 (26-44)	36 (5)
BMI (kg/m^2)	24 (18-35)	24 (4)
MAP (mmHg)	86 (68-134)	87 (10)
HR (bpm)	68 (42-87)	67 (10)
Parity (n)	1 (0-4)	-
FSH (IU)	1875 (822-5850)	2114 (989)
Follicles (n)	12 (2-28)	13 (6)
Oocytes (n)	10 (2-23)	10 (6)
OSI (nx1000/IU)	4.8 (0.67-21.9)	5.9 (4.4)
Infertility factor Male Anovulation Tubal Endometriosis Unidentified	*(n)* 22 6 4 4 32	
IVF protocol Agonist Antagonist	*(n)* 11 57	

SD, standard deviation; BMI, body mass index; MAP, mean arterial pressure; HR, heart rate; FSH, follicle stimulating hormone; OSI, ovarian sensitivity index (number of retrieved oocytes x 1000 divided by the cumulative FSH dose); IVF, in vitro fertilization.

Baseline measurements

DPA variables EEI and DI were significantly associated with HR ($p \leq 0.045$). HR differed significantly between measurement points, hence EEI and DI were adjusted to a fixed HR of 75 (denoted DPA@75) for comparisons. All DPA variables were significantly associated with age ($p \leq 0.016$) in the direction of increasing arterial stiffness with older age, which is in congruence with previous studies (see Introduction). LogOSI was significantly associated with age ($p < 0.0001$, tau -0.35), confirming a decreasing ovarian sensitivity with older age. LogOSI was also significantly associated with DPA variables EEI, *b*/*a*, AI and *d*/*a*, and independently of age with AI (p 0.032) and *b*/*a* (p 0.008), indicating that women with poorer ovarian sensitivity had higher arterial stiffness. There were no significant differences for any DPA variable at baseline between women treated with the antagonist or agonist GnRH-protocol ($p \geq 0.21$)

Comparisons at interventions

Results at interventions are shown in Table 9 and Figure 10. Compared with baseline values, there were no significant changes in any DPA variable at the stimulation measurement or in early pregnancy. However, at ET, there were modest but significant changes towards increased arterial stiffness in variables representing central (EEI, *b*/*a*) and peripheral (DI, but not *d*/*a*) vessels, as well as the composite variable AI, indicating global arterial stiffness. The changes in DPA variables were paralleled with a discrete increase in MAP. HR was significantly increased at all measurement points. Since female sex hormones are potential vasodilators, we had expected changes towards reduced arterial stiffness. Previous studies of arterial stiffness changes in the normal menstrual cycle showing decreased arterial elasticity in the early luteal phase support the finding of increased arterial stiffness at ET [47, 50, 51, 175], although previous research have not been unanimous. Hormonal effects can depend on concentrations and bioactivity, and it has been suggested that an increased arterial stiffness in the luteal phase could be secondary to actions of progesterone or other hormones counteracting the vasodilatory effect of estrogen, e.g. activation of the RAAS. Indeed, increased levels of renin, angiotensin and aldosterone have been found in women undergoing IVF, peaking a few days after ovulation induction [176, 177]. Manau et al. have in two studies of IVF patients confirmed catecholamine and RAAS activation in the luteal phase, as well as hemodynamic changes towards vasodilation [60, 61], which contradict the results in our study. However, in the study by Manau et al. the study group was small and a relatively large proportion of patients had the complication of OHSS [61], which could implicate different hemodynamic alterations. We have found no previous studies of arterial stiffness parameters in IVF treatment to enlighten us.

Another explanation for increased arterial stiffness in the luteal phase could be an increased plasma volume, a known effect of female sex hormones [178]. The DPA

method cannot separate arterial stiffness caused by plasma volume expansion from stiffness caused by vasoconstriction.

We could not convincingly show any association between the magnitude of change in DPA variables and ovarian response as estimated by OSI. This could be attributed to the inadequacy of OSI as a surrogate variable for hormonal response, but it could also be that other factors caused the change in arterial stiffness at ET.

The stimulation measurements were for practical reasons obtained at the first visit for ultrasound monitoring when hormone levels are expected to have increased well above normal levels after a week of FSH administration. Measurement of hormone plasma levels lacks clinical importance and is not a clinical routine in the standard IVF treatment at the unit, and was thus not included in the study protocol. Full standardization of pharmacological treatment could unfortunately not be achieved due to individualized clinical patient care. At ET, the changes in DPA variables from baseline were not significantly different depending on GnRH-protocol (for *d*/*a* p=0.066, for others $p \geq 0.27$). Excluding the 11 women with agonist protocol from the analysis did not change the results. A weakness is that baseline measurements were obtained without relation to the menstrual cycle. It is however reasonable to believe that normal physiologic alterations in arterial elasticity in the menstrual cycle would not influence the results of arterial elasticity changes in the COH setting with supraphysiologic hormone levels.

There were no significant changes in DPA variables from baseline to measurements performed at approximately seven gestational weeks in the women who became pregnant. This could possibly be explained by the small study group (type II error), or it was too early in pregnancy to see an effect. However, Mahendru et al. [179] found reduced AIx already in gestational week 6 compared to pre-conceptional values in spontaneous pregnancy.

Table 9.
Hemodynamic effects of oocyte stimulation and pregnancy, compared with basal measurements before conception. Digital pulse wave analysis values are indices without quantitative measures.

	Basal n=68	**Stimulation n=59**			**Embryo transfer n=52**			**Pregnancy n=19**		
	Median (95% CI)	**Median (95% CI)**	***p***	**Effect[a]**	**Median (95% CI)**	***p***	**Effect[a]**	**Median (95% CI)**	***p***	**Effect[a]**
MAP (mmHg)	86 (84, 90)	84 (83, 86)	0.57	-	89 (85, 93)	0.007	Increase	85 (80, 90)	0.91	-
HR (bpm)	68 (65, 71)	72 (68, 75)	0.0003	Increase	75 (72, 79)	<0.0001	Increase	77 (73, 85)	0.003	Increase
EEI@75	0.630 (0.562, 0.691)	0.630 (0.535, 0.692)	0.66	-	0.533 (0.459, 0.688)	0.021	Increased large artery stiffness, decreased LV power	0.501 (0.477, 0.590)	0.75	-
b/a	-0.600 (-0.630, -0.550)	-0.585 (-0.650, -0.535)	0.86	-	-0.563 (-0.625, -0.490)	0.035	Increased large artery stiffness, decreased LV power	-0.530 (-0.600, -0.505)	0.15	-
DI@75	0.691 (0.652, 0.715)	0.692 (0.642, 0.702)	0.77	-	0.710 (0.676, 0.737)	0.003	Peripheral vasoconstriction	0.705 (0.592, 0.773)	0.16	-
d/a	-0.170 (-0.190, -0.145)	-0.165 (-0.190, -0.145)	0.65	-	-0.198 (-0.235, -0.140)	0.15	-	-0.195 (-0.265, -0.110)	0.30	-
AI	-0.455 (-0.565, -0.360)	-0.460 (-0.545, -0.375)	0.37	-	-0.355 (-0.480, -0.205)	0.008	Increased global arterial stiffness	-0.350 (-0.470, -0.230)	0.14	-

HR, heart rate; MAP, mean arterial pressure; EEI@75 ejection elasticity index corrected to heart rate 75 bpm; DI@75, dicrotic index corrected to heart rate 75 bpm; AI, aging index; LV, left cardiac ventricle.

[a]) Effect compared to basal measurement, statistics performed with Wilcoxon matched-pairs signed-rank test.

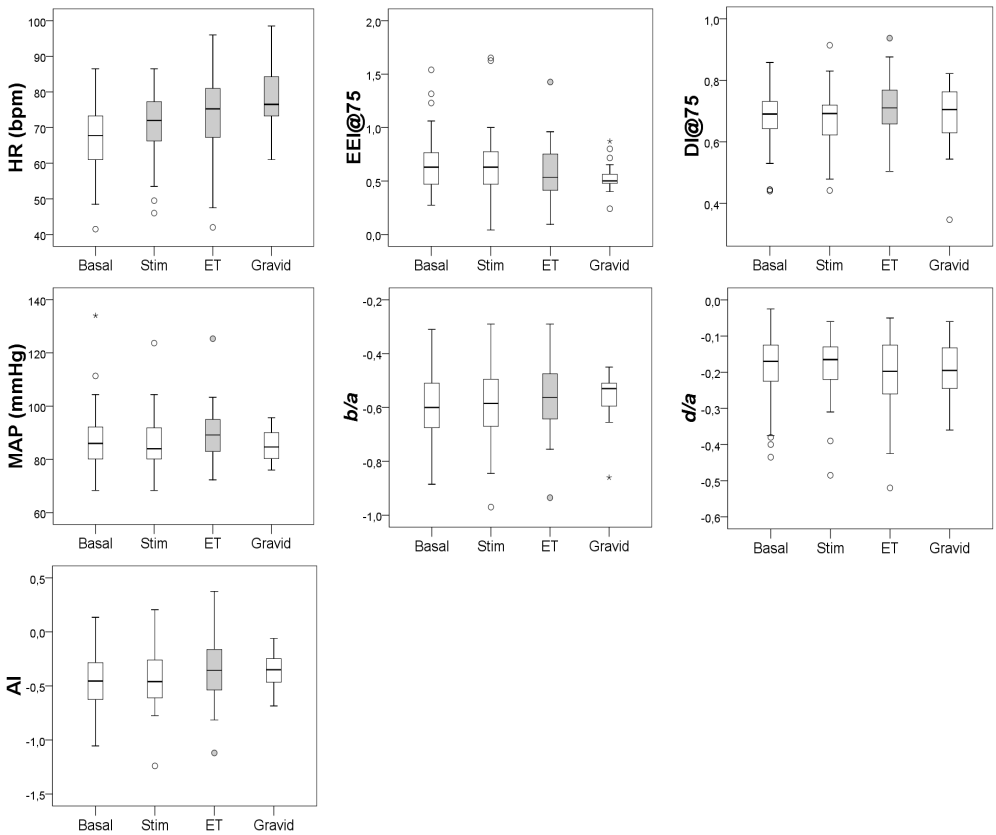

Figure 10.
Boxplots showing cardiovascular changes after administration of follicle-stimulation hormone (Stim), at embryo transfer (ET) and in early pregnancy (Gravid) compared to baseline values (Basal). Gray boxes indicate significant changes (p<0.05) compared with basal measurements, and star symbols denote values outside the panel boxes. HR, heart rate; MAP, mean arterial pressure; AI, aging index; EEI@75, cardiac ejection elasticity index adjusted to heart rate 75; DI@75, dicrotic index adjusted to heart rate 75.

Conclusions of Study III

During COH for IVF, arterial stiffness was increased in the early luteal phase (ET), but no significant changes were found in the follicular phase or in early pregnancy, as detected by DPA. The effect at ET appeared modest, however. An absent vasodilation could possibly be due to a hemodynamic activation of unknown origin counteracting the vasodilatory effect of estrogen.

Paper IV

A total of 196 women were included in the study. In 23 women, values were missing for one or several DPA measurements, why the recordings were excluded since the quality of the measurement could not be assured. Thus, 173 women remained for statistical analyses. Demographic and Doppler velocimetry data are shown in Table 10. UtA blood flow changes were present in 43 women of whom all had UtA notching, and nine also had PI >1.2. Only two women had an UAS 4, why UAS 3 and 4 were merged for statistical analyses. All investigated DPA variables (EEI, *b*/*a*, DI, *d*/*a* and AI) showed independent associations with maternal age and HR, except for *d*/*a* and AI, which only were associated with age, and DI for which the HR independent association with age was just above significance level. There were no significant differences in maternal age or HR between women with or without UtA notching, or normal or high mean UtA PI, why no DPA variables were adjusted in further analyses.

Table 10.
Demographic and Doppler data of 173 women examined with Doppler ultrasound velocimetry and digital pulse wave analysis. Values are median (10-90 percentile) or numbers.

	UAS 0	UAS 1	UAS 2	UAS 3	UAS 4	Notch	Mean PI > 1.20	All
	n = 130	***n =16***	***n = 19***	***n = 6***	***n = 2***	***n = 43***	***n = 9***	***n = 173***
Age (years)	32 (25-39)	28 (24-38)	30 (25-39)	33 (25-38)	31, 32	30 (25-38)	33 (25-40)	31 (25-39)
GA (weeks)	33 (24-39)	34 (29-39)	33 (27-38)	32 (24-35)	28, 27	32 (27-38)	28 (23-35)	33 (24-39)
SBP (mmHg)	112 (100-132)	113 (104-135)	110 (96-146)	110 (97-155)	113, 132	113 (98-144)	120 (106-146)	112 (99-134)
DBP (mmHg)	74 (64-85)	77 (64-92)	76 (66-92)	72 (62-114)	78, 85	77 (64-93)	83 (70-93)	75 (64-86)
MAP (mmHg)	88 (80-106)	88 (80-106)	89 (75-110)	85 (74-128)	90,100	89 (77-109)	95 (82-110)	88 (80-106)
HR (bpm)	85 (70-99)	84 (63-104)	88 (64-97)	81(67-102)	76, 74	85 (65-99)	88 (70-94)	85 (68-99)
	n	*n*	*n*	*n*	*n*	*n*	*n*	*n*
Duplex	8	-	-	-	-	-	-	8
Smokers	10	-	2	-	1	3	2	13
BFC > 0*	3	-	2	-	1	2	1	6†
Indication for referral								
Diabetes	15	-	3	-	-	3	-	18
Complicated obstetric history	29	5	6	1	1	14	4	42
Decreased FM	15	2	3	-	-	5	-	20
IUGR/SGA	36	8	5	2	-	15	2	51
Hypertension/PE	5	-	2	1	-	2	1	8
Fetal arrhythmia	4	-	-	-	-	-	-	4
Intercurrent maternal disease	14	1	-	-	-	2	1	15
Other	12	-	-	2	1	2	1	15

UAS, uterine artery score; PI, pulsatility index; GA, gestational age; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate; FM, fetal movements; BFC, blood flow class; IUGR/SGA, intrauterine growth restriction/small for gestational age; PE, preeclampsia

* BFC 0 = umbilical artery pulsatility index (PI) ≤ 2 SD

† four cases with BFC 1 (umbilical artery PI > 2 SD ≤ 3 SD), two with BFC 2 (umbilical artery PI > 3 SD and forward diastolic blood flow)

Comparisons of groups with and without Doppler blood flow changes

Results of comparisons between groups of women with and without UtA blood flow changes are shown in Table 11.

DI was significantly higher in the group with UtA notching (p 0.044), indicating peripheral arterial stiffness or vasoconstriction in the notching group. There was no significant difference between women with or without notching for other DPA variables. When women who in addition to notching had high PI were excluded from analysis, the tendency towards increased DI remained (p 0.060).

All DPA variables (EEI, b/a, d/a, AI) except DI were significantly changed towards increased arterial stiffness in the group with mean UtA PI>1.2 ($p \leq 0.040$). Since all women with high PI also had notching, groups of blood flow abnormalities could not be analyzed separately.

A significant but weak association was found between UAS and DI (p 0.025, tau 0.12), but not with other DPA variables, indicating a relationship between the severity of blood flow changes and increased peripheral arterial stiffness.

Table 11.
Digital pulse wave analysis (DPA) variables versus presence or absence of uterine artery diastolic notching and normal or abnormal uterine artery pulsatility index (cut off 1.20) obtained at Doppler velocimetry, and correlation between DPA variables and uterine artery score (UAS). Values are indices without quantitative measures and the figures represent median (95% confidence interval).

DPA	Diastolic notching			Pulsatility index			UAS
	Present *n = 43*	Absent *n = 130*	*p**	> 1.20 *n = 9*	≤ 1.20 *n = 164*	*p**	*p*†
EEI	0.730 (0.630, 0.800)	0.790 (0.750, 0.830)	0.11	0.603 (0.480, 0.730)	0.782 (0.750, 0.830)	0.0045	0.071
b/*a*	-0.630 (-0.675, -0.570)	-0.640 (-0.665, -0.615)	0.37	-0.552 (-0.645, -0.520)	-0.640 (-0.660, -0.620)	0.014	0.31
DI	0.640 (0.595, 0.665)	0.585 (0.555, 0.625)	0.044‡	0.608 (0.525, 0.725)	0.595 (0.575, 0.630)	0.49	0.025 (tau 0.12)
d/*a*	-0.180 (-0.235, -0.135)	-0.170 (-0.185, -0.155)	0.56	-0.225 (-0.380, -0.185)	-0.170 (-0.180, -0.150)	0.023	0.41
AI	-0.475 (-0.575, -0.265)	-0.490 (-0.540, -0.430)	0.56	-0.292 (-0.575, -0.005)	-0.500 (-0.540, -0.430)	0.040	0.60

* Mann-Whitney U test

† Kendall rank correlation (tau)

‡ When individuals with PI >1.20 excluded, $p = 0.060$

PE is associated with defective placentation and increased resistance in the uteroplacental bed, as well as maternal endothelial dysfunction and increased arterial stiffness. A previous study found an association between UtA PI and AIx, as well as between AIx and subsequent development of PE, IUGR and fetal birth weight [132]. The findings of increased arterial stiffness by DPA in women with UtA blood flow changes in study IV are in congruence with these previous findings, and support the hypothesis that the maternal cardiovascular condition influences the development of preeclampsia and IUGR. Whether vascular assessment with DPA could have clinical value in risk evaluation or diagnosis of pregnancy complications remains to be investigated.

The results of study IV indicate that the pathophysiological background of UtA high PI and notching differ. Computer models have shown that changes of the UtA Doppler velocimetry waveform are determined by both increased resistance to blood flow and reduced vessel diameter [180], and that increased PI is related to increased transcotelydonary resistance, and notches to wave reflections determined by uterine artery tone or compliance [181, 182].

Contrary to the other DPA variables, DI showed no difference between women with normal and elevated PI, but was significantly increased in the notching group. The explanation could of course be a Type I error, but also the different mathematical and physiological background of calculating DI. DI was the only investigated variable solely derived from the PPG waveform, i.e. the crude volume pulse waveform, while the other DPA variables are derived from the APG waveform, or PPG and APG waveforms combined (see Methods). While DI represent the incisura that marks the transition from systole to diastole, *d*/*a* is a late systolic wave determined by the intensity of wave reflection from the periphery. Hence, DI and *d*/*a* reflect the physiological condition differently. DI is, as shown in paper I, not as strongly correlated with PWV and AIx as *d*/*a* and the other investigated DPA variables are.

Conclusions of Study IV

UtA Doppler blood flow changes were related to increased arterial stiffness as determined with DPA, indicative of a systemic vascular affection or dysfunction also outside the uteroplacental unit. High UtA PI was associated with an increased generalized arterial stiffness, while UtA diastolic notching was associated with increased arterial stiffness in peripheral arteries only, suggesting different pathophysiological backgrounds of the two UtA blood flow velocimetry abnormalities investigated in the study.

Paper V

30 women with AUS 3-4 were enrolled in this pilot study. Five women were later excluded (three due to UAS<3 at baseline measurements, one woman due to initiation of steroid treatment and one woman due to increased dose of pindolol between measurements), and 25 women then remained for statistical analyses. The median age of participating women was 32 years (range 19-41) and the gestational age 32 weeks (range 24-38).

Comparisons before and after chocolate consumption

After three days of flavonoid-rich chocolate intake, there were no significant changes in DPA variables (Table 12), indicating either that there was no effect of the flavonoid-rich chocolate intake on arterial compliance as detected by DPA, or that the effect was too small to find. UtA blood flow changes have been associated with the severity of endothelial dysfunction [110], and endothelial dysfunction is present in women destined to develop PE even prior to onset [108]. As women in this study were high-risk for hypertensive complications, we cannot exclude that they had an impaired ability for NO-mediated vasodilation.

Table 12.
Results chocolate. Hemodynamic and Doppler blood flow velocimetry data in 25 women before and after flavonoid-rich chocolate ingestion.

Variable	DPA interpretation	Significance of difference (*p*) and direction of change	Change before/after	
			Median (range)	Mean (±SD)
MAP	-	0.034 ↑	99 (75-116) / 99 (76-124)	97 (12) / 100 (13)
PP	-	0.19	-	-
HR	-	0.33	-	-
EEI	Cardiac left ventricular power, elasticity/vascular tone in central arteries	0.17	-	-
b/*a*	Cardiac left ventricular power, elasticity/vascular tone in central arteries	0.19	-	-
d/*a*	Intensity of wave reflection, elasticity/vascular tone in peripheral arteries	0.95	-	-
DI	Elasticity/vascular tone in peripheral circulation	0.54	-	-
AI	Global arterial elasticity/vascular tone, "vascular age"	0.24	-	-
Mean UtA PI	-	0.049 ↓	1.25 (0.99-1.95) / 1.18 (0.96-1.75)	1.29 (0.24) / 1.23 (0.21)
UmbA PI*	-	0.23	-	-
UAS	-	0.025 ↓	3 (3-4) / 3 (2-4)	3.16 (0.37) / 2.96 (0.61)

DPA, digital pulse wave analysis; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; EEI, ejection elasticity index; DI, dicrotic index; AI, aging index; UtA PI, uterine artery pulsatility index; UmbA PI, umbilical artery pulsatility index; UAS, uterine artery score.

* adjusted for gestational age

UtA PI and UAS were significantly lower after chocolate consumption (Table 12). The majority of enrolled women had bilateral UtA notching at both examinations. One woman showed an impairment from unilateral to bilateral notching, and four women improvements from bilateral to unilateral notching, but the differences were not significant. The finding of decreased UtA PI was in accordance with a previous study showing a beneficial effect of flavonoid-rich chocolate on uteroplacental blood flow resistance, but the effect seen in that study could however not be convincingly associated with the flavonoid or theobromine chocolate content [157]. The potential beneficial effects of flavonoids and other components of chocolate on the vasculature and endothelium in pregnancy remain to be elucidated, but this pilot study awakes the interest for further research on the subject.

Conclusions of Study V

In women with impaired uteroplacental blood flow, hemodynamic effects on the systemic circulation by a high-flavonoid chocolate regimen could not be demonstrated, but potentially beneficial effects were found in the uteroplacental circulation.

Overall conclusions and future perspectives

The findings of the studies indicate that DPA reflects vascular status in different populations and in pregnancy. Standardization of measurements are important, as for all assessments of organ functions under autonomic control with rapid shifts depending on physiological and environmental factors. When good signal quality is achieved, DPA seems to be a reliable and easy manageable method. Maternal cardiovascular function as assessed by DPA is associated with uteroplacental blood flow changes, which further emphasizes the role of vascular and endothelial dysfunction in hypertensive pregnancy complications. This raises the question whether maternal cardiovascular assessment with pulse wave analysis could contribute to improved risk prediction in clinical practice, or monitoring of therapeutic interventions in pathological conditions. The results of this thesis should be viewed as a foundation for future studies with the DPA technique with those objectives. Further studies for validating and increasing the knowledge of the physiological background of DPA indices under various circumstances, as well as their potential additional value to standard care in obstetrics or cardiovascular medicine, would be desired.

Methodological considerations

Photoplethysmographic DPA is not a regular reference method for vascular assessment at present, why this thesis has partly focused on the feasibility of the method, and partly on its possible value as a tool for hemodynamic assessment in pregnant women. Although there is good evidence in the literature for the physiological analysis and interpretation of the digital volume pulse wave contour, the full understanding of particularly the "acceleration photoplethysmogram", APG, and its composition is lacking. Inevitably, registrations of the pulse wave at more peripheral sites will be more prone to damping than measurements from central arteries, and inflection points may then possibly be identified with less accuracy.

Takazawa et al. [32] investigated the APG with comparisons to invasive measurements, but studies using invasive techniques are scarce and the findings have later not been confirmed. Consistencies between the PPG and radial pressure pulse wave, as well as between APG indices and cardiovascular pathology and morbidity, support the accuracy of the method.

Another consideration is that most innovative studies have been performed in Japanese populations, which might not fully translate to Western populations. However, it is reasonable to believe that basic physiological mechanisms do not differ. DPA, being easy, fast and operator-independent, has motivated research with this method. As technical progress advance, the golden standard method or other techniques might develop to make arterial stiffness assessment more manageable, and then suitable for in-office evaluations or screening. Indeed, in the years since the work with this thesis started, modern updates of the SphygmoCor™-system have reduced the examination time and increased feasibility of the device, but not yet to the simplicity and swiftness of the DPA method. However, it should be emphasized that DPA indices cannot be viewed as surrogate measures of applanation tonometry parameters. The only DPA variable directly comparable to an applanation tonometry equivalent was ETc, corresponding to the SphygmoCor™-variable LV ejection duration (denoted LVET in paper I), measuring the left ventricular ejection time in milliseconds. The association between the variables was weak however, possibly due to low reliability of ETc (paper I).

The Meridian DPA™ (Salcor AB, Uppsala, Sweden) used in this thesis, is a FDA approved system for photoplethysmographic DPA. However, in the literature DPA has been performed with various apparatuses, in engineering papers not seldom with

self-built systems. It is therefore not possible to state with certainty that values of DPA variables are quantitatively or qualitatively comparable between different systems. DPA variables based on quotas, such as the APG variables, were predominately used in this thesis, which theoretically would be more transferable when using other systems.

The PPG signal could be disturbed and distorted by electrical noise, and wave contour mapping is based on mathematical algorithms filtering background noise. This might lead to distortion of particularly the APG. There are no standard algorithms for PPG signal processing, and algorithms differ between equipments [183]. In addition, the use of different indices and nomenclature of the PPG and APG in previous research is further challenging. With this in mind, terminology as recommended by Elgendi [37] was used in this thesis.

As for all methods for vascular assessment, the DPA should be performed under standardized conditions due to the rapid changes in the arterial system in response to autonomic nervous system control. In 2017, the International Working Group on Maternal Hemodynamics published recommendations for studies of maternal vascular function [184]. The studies for this thesis were however planned and executed years prior to this publication, but were to a great extent performed according to the recommended standards. In all protocols for included studies, DPA measurements were to be performed after rest in the same (supine) position. Women were asked to avoid large meals, caffeine and nicotine before the visits. Room temperature was only controlled for in study I, whereas in studies III-V examinations were performed in rooms with minimal disturbances and changes. In study II, measurements were performed at four different maternal health care units, why a full environmental standardization was not possible.

Based on the findings of the DPA variable repeatability and associations with applanation tonometry parameters in paper I, as well as in regard to existing literature references, we decided to restrict the number of DPA variables in subsequent studies.

Multiple statistical testing increases the risk of type I error, why significance levels sometimes are tightened (e.g. Bonferroni correction of *p*-values). Corrected *p*-values increase the risk of type II errors however, and when multiple tests that are heavily statistically dependent on each other are performed at the same time, *p*-value correction should be avoided [169].The variables were not all independent of each other, and also believed to reflect in part the same physiologic background. Consequently, we used crude *p* values. The results in this thesis have been interpreted considering the risk of statistical errors, and conclusions made on solitary findings have been avoided.

Populärvetenskaplig sammanfattning på svenska

Artärerna, som för syrerikt blod från hjärtat ut i kroppen, är elastiska i sin struktur för att bibehålla ett jämnt kontinuerligt blodflöde och minska tryckskillnaderna mellan hjärtats pulsslag. Elasticiteten är som störst i stora kroppspulsådern, *aorta*, och minskar sedan successivt längre ut i kärlträdet. Med ökad ålder minskar elasticiteten i artärerna vilket innebär att hjärtat måste arbeta hårdare. Hur snabbt detta sker påverkas av ärftliga faktorer, men också av sjukdomar i kärlväggen såsom t.ex. åderförkalkning och diabetes. Kärlstelhet har visats sig vara en oberoende och viktig riskfaktor för hjärt-kärlsjukdom.

När en kvinna blir gravid sker stora förändringar i hennes hjärt-kärlsystem för att kunna försörja det växande fostret och förbereda kroppen för förlossning. En onormal fysiologisk omställning har visats sig ha samband med graviditetskomplikationer såsom havandeskapsförgiftning och tillväxthämning av fostret. Kvinnor som drabbats av havandeskapsförgiftning har också ökad risk för hjärt-kärlsjukdomar senare i livet. Artärstelheten är ökad före, under, och år efter att en kvinna insjuknat i havandeskapsförgiftning, vilket gör artärstelhet intressant att studera. Eventuellt skulle artärstelhet kunna användas för att förutspå vem som kommer drabbas av havandeskapsförgiftning, eller hur tillståndet bäst behandlas.

De vanligaste metoderna att mäta artärstelhet är i regel omständliga och tidskrävande varför det är svårt att undersöka stora populationer. En enkel och snabb metod är digital pulsvågsanalys (DPA) som lätt mäts med en klämma på fingret. Denna metod är mindre utforskad än andra metoder, och nästan inte alls studerad på gravida kvinnor.

Syftet med denna avhandling var att undersöka artärstelhet under normal och komplicerad graviditet genom att använda DPA.

I första arbetet undersökte vi artärstelheten hos män och gravida samt icke gravida kvinnor i olika åldrar med både DPA och den mest etablerade referens-metoden. Studien visade att DPA var relativt tillförlitlig, och att det fanns tydliga samband mellan de olika metoderna, varför slutsatsen drogs att DPA kan användas för att undersöka artärstelhet.

I det andra arbetet undersökte vi friska kvinnor upprepade gånger under en normal graviditet med DPA för att se om metoden återspeglade den fysiologiska omställningen i kärlen, och beskriva normalvärden för DPA mätningar under graviditet. DPA förändringen under graviditet såg ut att visa den förändrade eftergivlighet i kärlen som konstaterats med andra metoder.

I det tredje arbetet undersöktes om artärstelhet påverkas av hormonstimulering inför provrörsbefruktning och tidig graviditet. Kvinnliga könshormoner har avslappnande effekt på kärlen varför hypotesen var att kärlstelheten skulle minska. Vi fann dock att kärlstelheten istället ökade något hos de undersökta kvinnorna i samband med att embryot återfördes i livmodern veckan efter ägglossningen. Hos de kvinnor som blev gravida sågs inga skillnader i artärstelhet i tidig graviditet. Fynden skulle kunna bero på en aktivering av andra hormonsystem än könshormonerna.

I de sista arbetena undersöktes gravida kvinnor som genomgick blodflödesundersökning av livmoderartärerna med ultraljud. Ett ökat blodflödesmotstånd i livmoderartärerna innebär ökad risk för utveckling av havandeskapsförgiftning och tillväxthämning av fostret. Deltagarna undersöktes med DPA i anslutning till ultraljudsundersökningen.

I det fjärde arbetet undersökte vi om ökat motstånd i livmoderartärerna hade samband med ökad artärstelhet vilket vi också fann.

I det femte arbetet studerade vi om en speciell ”hälsochoklad” hade några effekter på artärstelhet eller blodflödet i livmoderartärerna hos gravida kvinnor med konstaterade blodflödesförändringar. Kakao har vistats ha gynnsamma avslappnande effekter på kärlen. Vi fann inga skillnader i artärstelhet mätt med DPA före och efter chokladintaget, däremot förbättrades blodflödet i livmoderartärerna något.

Sammanfattningsvis är slutsatserna av avhandlingen i stort att DPA verkar kunna användas för att undersöka artärstelhet och är enkel och snabb att använda. Det förefaller också som att hormonstimulering kan ha en påverkan på artärstelhet, och att blodflödesförändringar i livmoderartärerna har ett samband med förändringar i kvinnans övriga kärl. Om DPA kan vara en värdefull metod för att hitta eller bedöma kvinnor som har, eller har ökad risk att utveckla, graviditetskomplikationer, återstår att se.

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