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Left ventricular and endothelial functions with references to pre-pregnancy physical activity

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Norwegian University of
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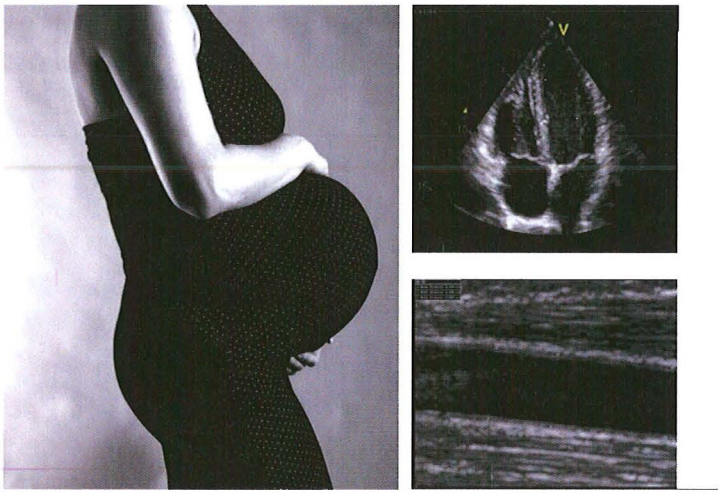

Thesis for the degree of philosophiae doctor

Trondheim, January 2011

Norwegian University of Science and Technology
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Department of Circulation and Medical Imaging

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ISBN 978-82-471-2602-8 (printed ver.)
ISBN 978-82-471-2603-5 (electronic ver.)
ISSN 1503-8181

Doctoral Theses at NTNU, 2011:40

Printed by Tapir Uttrykk

Hjarte-/karfunksjon ved svangerskapsforgifting – med fokus på venstre ventrikkelfunksjon, endotelfunksjon og fysisk aktivitet før svangerskapet

Endotelet, det innerste cellelaget i blodårene, hjartekamra og lymfeårene, spelar ei viktig fysiologisk rolle gjennom mellom anna å vere med på å regulere blodårediameteren. For å måle endotelfunksjonen er det utvikla ein ultralydbasert metode, men denne metoden er forbunden med ein del variabilitet. Gjennom å undersøke kva som var den viktigaste kjelda til variabiliteten, fann vi at sjølve analyseprosessen bidrog mest. Ved å analysere ultralydbileta tre gongar vil variabiliteten reduserast med 30%.

Endoteldysfunksjon er sentral i sjukdomsutviklinga både ved svangerskapsforgifting og ved hjarte-/karsjukdom. Kvinner som har hatt svangerskapsforgifting, har også auka risiko for hjarte-/karsjukdom seinare i livet, og det kan tenkast at denne auka risikoen er knytta til endoteldysfunksjon. Vi undersøkte om hjarte- og endotelfunksjon var redusert hos kvinner med svangerskapsforgifting, både under svangerskapet og tre månader etter fødsel. Hjartefunksjonen var redusert under svangerskapet, men hadde normalisert seg tre månader etter fødsel. Vi fann ingen statistisk signifikant reduksjon i endotelfunksjon under svangerskapsforgifting, men tre månader etter fødsel var endotelfunksjonen signifikant redusert i svangerskapsforgiftingsgruppa. Funna våre viser at hjartefunksjonen truleg er meir påvirka under svangerskapet ved svangerskapsforgifting enn tidlegare antatt, men resultata styrker ikkje hypotesen om at det er ei direkte kopling mellom endoteldysfunksjon og svekka hjartefunksjon ved svangerskapsforgifting.

Endoteldysfunskjon kan betrast ved trening, og trening reduserer risikoen for hjarte-/karsjukdom. Det kan difor tenkast at trening før svangerskapet reduserer risikoen for svangerskapsforgifting. Vi undersøkte risikoen for utvikling av svangerskapsforgifting i forhold til nivået av fysisk aktivitet før svangerskapet hos kvinner som deltok i den fyrste Helseundersøkelsen i Nord-Trøndelag (HUNT-1), og fann ingen samanheng mellom fysisk aktivitet før svangerskapet og risiko for svangerskapsforgifting.

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Finansieringskjelder: NTNU og Helse Midt-Norge

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden philosophiae doctor i klinisk medisin.
Disputas finner sted i auditoriet Gråkallen, Øya helsehus (2. et.), mandag 14. februar kl. 12.15.

Contents

1 Acknowledgements

The work on which this thesis is based was carried out at the Department of Circulation and Medical Imaging at the Norwegian University of Science and Technology, and was financed from a research fellowship by the Norwegian University of Science and Technology and the Central Norway Regional Health Authority.

I would like to thank my supervisor Professor Stig Arild Slørdahl for giving me the opportunity to go into medical research and for the guidance given along the way. Your contagious optimism has been very important to me through these years.

I would like to thank Associate Professor Eirik Skogvoll, who has been of great importance to the first paper included in my thesis. Your impressive statistical knowledge and your general enthusiasm are highly appreciated. Erik Madssen, who performed the parallel analyses of the data in that same paper, deserves special thanks. By your eagerness and prompt replies you made our project go like clockwork.

Sincere thanks are offered to Associate Professor Asbjørn Støylen who has been highly engaged in the second paper. You are a true oracle within the field of cardiology and ultrasound technology. Thank you for generously sharing your knowledge with me.

This thesis would not have been about preeclampsia if it had not been for Professor Bjørn Backe. Your lecture on preeclampsia during medical school was so interesting that I just *had* to learn more about this fascinating subject. Thank you! You rendered the second paper possible by offering your obstetric expertise, and by making the inclusion of preeclamptic women possible.

The contribution of Professor Pål Richard Romundstad has been invaluable to the third paper. I genuinely admire you for your thorough statistical and epidemiological knowledge. Your crystal clear answers and suggested solutions have enlightened my work.

Special thanks go to Charlotte Björk Ingul, one of the most energetic women I have ever met. At the time when we shared an office you taught me a lot about both ultrasound and sugar! Thank you for your proof-reading and for your illustration (Figure 2). Your contribution is highly valued.

My gratitude is also directed towards the midwives at the Obstetrics department, especially Lillian Andersskog Ekle, and the midwives at the maternal antenatal care centres in Trondheim, especially Carina Svensson, who helped me to include women in the clinical study. I would also like to thank the research nurses Anne-Lise Antonsen and Eli Granviken, who provided great assistance in getting the ultrasound scanner ready for the clinical study. Kari Slørdahl also deserves thanks for helping me with blood samples when my skills were insufficient.

Moreover, I would like to thank Øivind Rognmo, Aud Hiller and Ragna Elise Støre Govatsmark, my first research group at the Department of Circulation and Medical

Imaging. You made me feel very welcome from the first day. Thank you to all my office colleagues throughout the years at the department, Trine Tegdan Moholdt, Anne Berit Johnsen, Dorthe Berg Stensvold, Anne Marie Ormbostad, Guri Kaurstad and Morten André Høydal. Thank you also to my colleagues in echocardiography research Brage Amundsen, Anders Thorstensen, Håvard Dalen, Siri Ann Nyrnes and Harald Edvard Mølmen Hansen, for your generous help and company. My gratitude is also directed towards all involved in the 'Exercise in Medicine'-group, especially professor Ulrik Wisløff, Anja Bye, Eli-Anne Skaug, Ragnhild Røsbjørgen, Arnt-Erik Tjønna, and Tomas Ottemo Stølen for always including me although I have only touched into exercise physiology research. Thank you, Rigmor Austgulen, for including me in your preeclampsia research group. I am also truly grateful for the technical support given by Vidar Lundberg, Tor Arne Grindberg, and Jørgen Mæhle of GE Vingmed Ultrasound AS.

I would further like to thank my dear friend Sara Maria Wörlund, who was enrolled in the Research Program together with me in 2002. We have shared ups and downs, both in research and in life. I really appreciate your friendship.

During my period as a PhD-student, we moved to Lillehammer, and the writing of this thesis would not have been easy if I had not had access to an office outside our home. A workplace was first kindly provided at Glommens og Laagens Brukseierforening by the managing director Are Mobæk, and later by Innlandet Hospital Trust, Division Lillehammer by the division manager Randi Mølmen. Thank you to my new friends and future colleagues in Lillehammer, especially to my office-mates Elin Amrud, Geir V. Berg and Jacob Grundt, and to my coffee-break fellows Inger-Johanne Rosenvinge Smidesang, Janne Kværnø, Gro Anita Sæbø, Gunvor Øfsti, and Stine Kristiansen.

Howard Medland, Trans-Consult, deserves thanks for correcting my English. I am very grateful for the great effort you have put into this work.

Thank you, Lovisa og Tora Kristine, both born during the time of my fellowship. You bring so much joy and laughter to my life, serious thoughts and wise considerations. I love you so much! And, Klaus, my dear husband, my patient boy-friend, the best father that the girls could ever have – thank you for all your support every single day. I love the life together with you.

But most of all, I would like to thank you, mum. You made this thesis possible by coming to Lillehammer to help me out. You ended up staying a month and a half, reading my drafts, cooking dinner, cleaning our house and clothes, collecting the girls from day nursery, and by being a wonderful grandma. You are so brave and strong, so generous and honest. Thank you, mum!

2 Papers included

Paper I

Tyldum EV, Madssen E, Skogvoll E, Slørdahl SA. Repeated image analyses improve accuracy in assessing arterial flow-mediated dilatation. Scandinavian Cardiovascular Journal 2008; 42: 310-315.

Paper II

Tyldum EV, Backe B, Støylen A, Slørdahl SA. Maternal left ventricular and endothelial functions in preeclampsia. *Submitted.*

Paper III

Tyldum EV, Romundstad PR, Slørdahl SA. Pre-pregnancy physical activity and preeclampsia risk: a prospective population-based cohort study. Acta Obstetricia et Gynecologica Scandinavica 2010; 89: 315-320.

3 Background

Women with a history of preeclampsia are at increased risk of cardiovascular disease later in life. A central feature of both preeclampsia and cardiovascular disease is endothelial dysfunction. Endothelial dysfunction can be improved by physical activity, and physical activity also reduces the risk of cardiovascular disease. These interwoven relationships constitute the foundation on which my thesis is based.

3.1 Endothelial function

The *endothelium* is the single layer of cells forming the innermost lining of the blood vessels, cardiac chambers (the *endocardium)* and lymphatic vessels. The endothelial cells are the only cells of the blood vessel wall which, under normal circumstances, are in direct contact with the circulating blood. Endothelial cells are the main components of the tunica intima (L. *tunic*, coat or covering; L. *intimus*, innermost), one of the three layers of the vessel wall. The only components separating the endothelial cells from the circumferentially arranged vascular smooth muscle cells of the nearby tunica media is a thin subendothelial layer of loose connective tissue and a delicate internal elastic lamina. The vascular smooth muscle cells are also surrounded by an external elastic lamina, which separates them from the loose connective tissue of fibroblasts and associated collagen fibers of the tunica adventitia.

Even though its structure seems simple – a single layer of a single cell type – the endothelium is responsible for balancing a number of important functional processes in the body. In addition to regulating the passage of fluids, substances and cells from the blood to the interstitium, the endothelium promotes dilatation, has anti-inflammatory and anti-thrombotic properties, and prevents growth and migration of vascular smooth muscle cells. However, under pathological influence, the endothelial cells may be transformed into their own counterparts. Under such circumstances, constriction and

inflammation are promoted, a thrombogenic surface is provided, and growth and migration of vascular smooth muscle cells are stimulated.

The endothelium causes vasodilatation mainly through its synthesis of the vasodilatators nitric oxide (NO) (1-4) and prostacyclin PGI_2 (5, 6), substances which also possess anti-inflammatory and anti-growth abilities (5-9). The endothelium further induces dilatation through pathways which are independent of both nitric oxide and prostacyclin, but rather mediated through pathways causing hyperpolarising of the vascular smooth muscle cells, a discovery which introduced the term 'endothelial-derived hyperpolarising factor' (EDHF) (10).

3.2 Assessment of endothelial function

The term endothelial dysfunction is often used when referring to impaired endothelial vasomotor function, i.e. an impaired ability of the endothelium to induce vasodilatation. *Endothelial activation* usually refers to the pathological state when the functions of the endothelial cells have been transformed, so that they work in a pro-inflammatory, pro-thrombotic, pro-growth and pro-migration manner (11).

A widely used method to assess endothelial function is to measure the flow-mediated dilatation of a peripheral conduit artery, such as the brachial, radial or femoral artery, by high-resolution ultrasound. (In the context of measuring flow-mediated dilatation, high-resolution ultrasound is currently considered when the transmitted ultrasound has a wave-frequency exceeding 10 MHz (12).) By this method, increased shear stress is produced, which stimulates the endothelium to synthesise vasodilatators, where nitric oxide is considered to be the most important in healthy subjects (13). In subjects with essential hypertension, prostacyclin is thought to contribute substantially more – probably in an indirect manner also involving nitric oxide (14-16).

Laminar shear stress acts on endothelial mechanoreceptors, thought to be either ion-channels, G-proteins, integrins, or caveolae (17). The mechanoreceptors transform the mechanical signals from shear stress into biochemical signals, where elevated intracellular calcium concentrations are one of the signals proposed. Biochemical signals activate the enzymes catalysing the synthesis of the endothelial-derived vasodilatators: cyclooxygenase (COX) for the production of prostacyclin (18), and endothelial nitric oxide synthase (eNOS) for the production of nitric oxide (13). Nitric oxide diffuses into the underlying vascular smooth muscle cells where it activates guanylate cyclase, an enzyme which converts guanylate triphosphate (GTP) into cyclic guanylate monophosphate (cGMP). Prostacyclin, on the other hand, acts through a G-protein-coupled receptor, named the IP-receptor, present in the cell membrane of the vascular smooth muscle cells. The IP-receptor activates the enzyme adenylate cyclase, a transmembrane protein in smooth muscle cells, which converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (19). Both cyclic guanylate monophosphate and cyclic adenosine monophospate lower the levels of intracellular calcium in the vascular smooth muscle cells, and thereby prevent contraction and induce relaxation and dilatation of the artery (20).

In the method of assessing flow-mediated dilatation, increased shear stress is produced by inducing a reactive hyperaemia in the artery. By inflating a sphygmanometer cuff positioned around the limb in question to suprasystolic pressures for a few minutes, blood flow through the artery is occluded. The ischemia stimulates vasodilatation in the tissues distal to the site of occlusion, which causes reduced resistance in the artery. When the occluding cuff is deflated, the resulting flow in the artery reaches higher levels than the baseline flow, due to the increased pressure drop along the artery. A *reactive hyperaemia* is created, defined as the transient increase in organ blood flow that occurs following a brief period of ischemia.

The vessel diameter is measured before (at baseline diameter) and after the increase in shear stress. Flow-mediated dilatation is in general presented as the dilatation relative to the baseline diameter, and is given in percentage values. Impaired brachial artery flow-mediated dilatation detected by this method has been shown to be an independent predictor of future cardiac events (21-26).

To check for vascular smooth muscle dysfunction, the assessment of endothelial-dependent dilatation (i.e. flow-mediated dilatation) is recommended to be followed by the assessment of endothelial-independent dilatation, usually produced by the administration of a single dose of nitroglyserin/glyseryl trinitrate sublingually (27-30).

3.2.1 Flow-mediated dilatation – an endothelial function?

The important role of the endothelium as a regulator of vessel dimension was discovered by Furchgott and Zawadzki in 1980 (31). They deduced that acetylcholine acted as a vasodilatator through its action on the endothelial cells, which responded by releasing one or more substances causing relaxation of the vascular smooth muscle cells (31). The endothelial-derived relaxant factor produced in response to acetylcholine-stimulation was later found, by Fuchgott, Ignarro and Murad with their respective colleagues, to be nitric oxide (1-4), a discovery for which they were awarded the Nobel Prize in Medicine in 1998.

In 1986, Pohl et al. showed that also flow-mediated dilatation was endothelium-dependent. They produced increased flow in the femoral artery of anesthetised dogs before and after the mechanical removal of the endothelial cells by means of a balloon catheter (32). Removal of the endothelial cells abolished the flow-mediated dilatation, leading to the suggestion that flow-mediated dilatation, like acetylcholine-mediated dilatation, was mediated by the endothelium (32).

Anderson et al. described flow-mediated dilatation of the brachial artery in humans in 1989 (33). Reactive hyperaemia was created by the release of an occluding cuff placed on the forearm, distal to the site of measurement, and the artery diameter change was measured by a dual-crystal pulsed Doppler system (33). Celermajer et al. applied this method with the use of conventional two-dimensional ultrasound. They showed that flow-mediated dilatation was reduced or absent in smokers, in children with familial hypercholesterolaemia, and in adults with coronary artery disease (34).

3.3 Preeclampsia

Endothelial dysfunction and activation are considered to be central features in the pathogenesis of preeclampsia (35-39). Preeclampsia is a multisystem syndrome affecting virtually any organ system (40), particularly evident from its complications including stroke, disseminated intravascular coagulation, pulmonary oedema, hepatic rupture, and placental abruption. Preeclampsia is a heterogeneous condition, which may be present as asymptomatic hypertension and proteinuria in one woman, and as multi-organ failure in another.

The leading hypothesis of the pathogenesis of preeclampsia is that a deficient vascular invasion of placental trophoblasts due to a maternal-foetal immunological imbalance leads to placental underperfusion (41-44). The underperfused placenta is thought to release substances into the maternal blood which cause a general maternal endothelial dysfunction, endothelial activation, and systemic inflammation (39).

Preeclampsia is defined as new-onset hypertension and proteinuria arising after the 20^{th} week of pregnancy (45). It affects 2-8% of pregnancies worldwide (46), and affected 4.5% of all births in Norway in 2002 (47). Preeclampsia is classified as one of *the hypertensive disorders of pregnancy*, including preeclampsia/eclampsia, preeclampsia superimposed on chronic hypertension, gestational hypertension, and chronic hypertension (45). The

hypertensive disorders in pregnancy were the second most common causes of maternal death in the UK 2003-2005 (48). Preeclampsia is also a major cause of foetal intrauterine growth restriction, and is responsible for 15% of all preterm births, both spontaneous and induced (49).

Preeclampsia is more common in the first pregnancy (nulliparity) (50, 51), and in the first pregnancy with the actual partner (primipaternity) (52-56). This has been suggested to be due to a maternal immunological response to new paternal genes present in the woman's circulation (57). However, as increasing maternal age (58) and large interval between pregnancies (59, 60) also increase the risk of preeclampsia, these factors may also be responsible for the increased risk related to primipaternity (59), since a change of partner is also linked to longer intervals between births. Multiple pregnancy is another factor which increases the risk of preeclampsia (61), which has been suggested to be due to increased foetal demand exceeding the placental supply and rendering the placental function inadequate (62). Santema et al., however, argued that the increased risk of hypertension in multiple pregnancy might be due to higher cardiac output and circulating plasma volume in such cases, together with a failure to decrease peripheral resistance further than the demands of a singleton pregnancy (63).

A history of previous preeclampsia (53) increases the risk of preeclampsia, which may suggest that maternal constitutional factors are of importance to the pathogenesis. Women with a family history of preeclampsia are at increased risk (53, 64, 65), and there is increasing evidence of genetic components involved in the pathogenesis (66-72). Chronic hypertension (73), obesity (50, 51, 61), gestational diabetes (74), and diabetes type-I (61) are all risk factors that preeclampsia has in common with cardiovascular disease. Smoking, however, does not fit into this picture. Whereas smoking is associated with a number of adverse pregnancy outcomes, like growth restriction, preterm birth, stillbirth, and spontaneous abortion (75, 76), it has

been shown to have a protective effect on the risk of the development of preeclampsia (51, 76-80). However, when preeclampsia does occur in a smoking woman, her outcome is worse than in non-smokers (79, 81, 82). It has not been determined whether smoking 'protects' against preeclampsia, or only 'masks' the symptoms and diagnosis of preeclampsia (83).

Early-onset preeclampsia may have an underlying pathology differing from that of late-onset preeclampsia (84). Early-onset preeclampsia is associated with greater maternal morbidity (85) and mortality (86) than late-onset preeclampsia, and involves a worse outcome for the foetus and the newborn, primarily due to more severe prematurity (87). Placental lesions are more common in placentae of women with preeclampsia of early onset as compared to preeclampsia of late onset (88, 89), and also when compared to preterm deliveries of other causes (90). Intrauterine growth restriction (IUGR) is more strongly linked to early-onset preeclampsia (85, 91, 92). A u-shaped distribution of birthweight characterizes late-onset preeclampsia (85), where the mean birth weight is generally normal (91, 93-95), but the proportions of babies both small (85, 94) and large for gestational age (85, 91, 92) are increased. The risk of cardiovascular disease and the metabolic syndrome later in life is increased after early-onset preeclampsia (96, 97) as compared to late-onset preeclampsia.

3.3.1 Endothelial dysfunction and activation in preeclampsia

Endothelial dysfunction in preeclampsia is supported by in vitro studies (98-100), where attenuated endothelial-mediated dilatation in myometrial vessels and omental vessels from women with preeclampsia in response to stimulation by bradykinin (99) and acetylcholine (98) has been reported. Interestingly, endothelial-mediated dilatation of myometrial vessels from women with normal pregnancy is also attenuated when incubated in plasma from preeclamptic women (101), even when the plasma is taken before the onset of the clinical syndrome (102). Cultured endothelial cells have also shown

altered function after the exposure to plasma or serum from preeclamptic women, with increased permeability and increased nitric oxide production (103, 104). These findings support the hypothesis that circulating factors exerting a negative impact on the endothelial cells are present in preeclampsia.

An imbalance in the endothelial production of vasodilatators and vasoconstrictors has been found in preeclampsia. Although it has not been clearly established whether the nitric oxide production is impaired, as both lower (105-107), and increased (108-111) levels of nitrite/nitrate concentrations have been reported, the serum levels of the vasoconstrictor endothelin-1 (ET-1) are elevated (108, 112). An imbalance in the ratio of the production of the vasodilatator prostacyclin to the vasoconstrictor thromboxane A_2 has also been reported (113). Although the main synthesisis of thromboxane A_2 occurs in platelets, it is also synthesised in endothelial cells (114, 115), and in trophoblasts (116).

Pro-inflammatoric endothelial activation in preeclampsia is suggested by elevated plasma levels of the soluble forms of the endothelial cell adhesion molecules (sE-selectin, sVCAM-1, s-ICAM-1) (117-120) and interleukins (IL-6 and IL-8) (105, 121-124). A pro-throbotic activation of the endothelial cells, with increased levels of von Willebrand factor and an imbalance in plasminogen activator inhibitor activity, probably contributes to the hypercoagulable state in preeclampsia (123, 125, 126). The imbalance in the ratio of prostacyclin to thromboxane A_2 found in preeclampsia (113) also acts pro-thrombotic. Acetylsalicylic acid restores this balance, and has been shown to reduce the risk of preeclampsia and intrauterine growth restriction in high-risk patients when treatment is initiated early in pregnancy (127, 128).

Soluble fms-like tyrosine kinase-1 is present at elevated levels in preeclampsia (129, 130), and is elevated even prior to the onset of the clinical syndrome, especially prior to preeclampsia of early onset (131, 132). Soluble fms-like

tyrosine kinase-1 binds to and inactivates vascular endothelial growth factor (133, 134), a factor which is important to angiogenesis and essential for endothelial integrity. Soluble fms-like tyrosine kinase-1 has been suggested to be responsible for the clinical manifestations of preeclampsia, including endothelial function. Interestingly, a vascular endothelial growth factor inhibitor, *bevacizumab*, prescribed in cases of renal carcinoma, produces the adverse effects of hypertension and proteinuria (135), the clinical hallmarks of preeclampsia.

3.3.2 Flow-mediated dilatation in preeclampsia

Flow-mediated dilatation is increased in normal pregnancy, from as early as 10-14 weeks of gestation (136, 137). In preeclampsia, flow-mediated dilatation has been reported to be lower when compared to normal pregnancy, both in the brachial (138, 139) and in the radial arteries (140-142). Lower levels of flow-mediated dilatation have also been reported to precede the development of preeclampsia in high-risk patients (143-146). In vitro studies, measuring flow-mediated dilatation in isolated myometrial arteries and small subcutaneous arteries, have also shown attenuated flow-mediated dilatation in arteries from women with preeclampsia (147, 148).

Lower levels of flow-mediated dilatation are also found in women with a *history* of preeclampsia (149-151). The attenuation of flow-mediated dilatation in these women has been found not to be due to changes in antiphospholipid antibodies, uric acid, L-arginine, asymmetrical dimethylarginine, vascular endothelial growth factor, or soluble fms-like tyrosine kinase-1, factors which have been suggested to be responsible of endothelial dysfunction in preeclampsia (37, 129, 145). Only in one study were nitrates found to be present at lower levels in women with a history of severe preeclampsia, suggestive of a defect in endothelial nitric oxide synthesis (149). However, nitric oxide is not only produced by endothelial cells, and reduced nitric oxide levels are not specific to endothelial dysfunction (152).

Endothelial-*in*dependent vasodilatation is generally not measured during pregnancy. Nitroglycerine may cause hypotension and directly affects the pregnant uterus with its relaxant effects (153, 154), and as a general rule, all unnecessary drugs should be avoided during pregnancy. Endothelial-independent vasodilatation has, however, been measured in women with a history of preeclampsia, and both reduced (149, 150, 155) and similar (151, 156, 157) endothelial-independent dilatation have been reported from these studies. Reduced endothelial-independent dilatation in women with a history of preeclampsia suggests that also other factors, like smooth muscular dysfunction and atherosclerosis, may contribute to the reduced endothelial-mediated dilatation present at least in a subgroup of these women

In a study by Chambers et al., brachial artery resting diameter was found to be increased in women with a history of preeclampsia, compared to women with a history of normal pregnancy, and to be even further increased in women with a history of recurrent preeclampsia. However, after adjusting for body surface area, no difference in artery diameter was found between the groups (151). In a study excluding women with other risk factors of cardiovascular disease, like hypertension, obesity/overweight, the metabolic syndrome, and smoking, no difference in brachial artery diameter was found in women with a history of preeclampsia compared to women with a history of normal pregnancy (149). These studies suggest that the larger brachial artery in women with a history of preeclampsia may be linked to obesity, increased body surface area or other cardiovascular risk factors. Large brachial artery diameter has previously been reported in postmenopausal women with the metabolic syndrome (158, 159), and has been found to be correlated with cardiovascular risk factors and prevalent cardiovascular disease (21, 160, 161). Moreover, larger brachial artery diameter has been found to be independently associated with coronary artery disease in women (161), and to be an independent predictor of

cardiovascular events in both women and men (21). The mechanisms behind this link are not clear.

3.3.3 Preeclampsia and cardiovascular risk

Women with a history of preeclampsia are at increased risk of cardiovascular disease later in life (97, 162-169). The risk of death from cardiovascular causes is greater after early-onset preeclampsia (97, 168) and after preeclampsia with complications such as intrauterine growth restriction and stillbirth (170). The underlying link between preeclampsia and cardiovascular disease is not known, but endothelial dysfunction is considered to be a central feature of both conditions (171-174). The fact that preeclampsia and cardiovascular disease share the same risk factors, including dyslipidemia, obesity, insulin resistance, diabetes mellitus, thrombophilia, and a family history of heart disease or stroke (78, 175-177), suggests that these two conditions may have related pathophysiological mechanisms. Smoking, however, which reduces the risk of preeclampsia (51, 76-80) does not fit into this picture.

3.3.4 Left ventricular function in preeclampsia

The maternal cardiovascular adaptation to pregnancy begins early in gestation. In normal pregnancy, plasma volume increases by 45% during the first two trimesters, and stays elevated until term (178). In spite of this, the mean blood pressure falls slightly (5 mmHg) and reaches its nadir in mid-pregnancy (179), whereafter it rises to pre-pregnancy or slightly higher levels (179, 180). Stroke volume is increased by approximately 30% at its maximum in the 30th week. This is caused by the increase in preload and the reduction in afterload, resulting from the rise in plasma volume and the fall in blood pressure, respectively. In addition to this, an increase in cardiac contractility has been reported, also contributing to the rise in stroke volume (181, 182). Due to a concurrent slight, but steady rise in heart rate throughout gestation (183, 184), the cardiac output is increased even further than stroke volume, and reaches its maximum level approximately 50% above non-pregnant levels in the 30th

week of pregnancy (183). The higher work load, caused by the increased blood volume and cardiac output, is suggested to be responsible for the myocardial hypertrophy of pregnancy (182, 184, 185), which has been taken to resemble the changes found in long-distance runners (184, 185).

The diastolic function indices are also influenced by the altered loading conditions in normal pregnancy. The increased blood volume causes increased mitral inflow velocities (cf. p. 29 f.) already from the first trimester. However, between the second and the third trimesters, the early diastolic mitral inflow velocity is reduced. This has been attributed to the myocardial hypertrophy causing reduced compliance of the left ventricle (182). A larger atrial contribution to left ventricular filling in late pregnancy is suggested to be responsible for the reduction in the ratio of the early and late diastolic mitral inflow velocities in the third trimester (182, 184). The left ventricular early diastolic mitral annular velocity was by Fok et al. found to be unchanged during pregnancy, although a tendency towards lower levels was found towards term (182). The ratio of early diastolic mitral inflow velocity and early diastolic mitral annular velocity, an index which has been suggested to be a marker of left ventricular filling pressure (cf. p. 31), was generally found to be unchanged in early pregnancy and only slightly reduced in the third trimester (182).

In women with preeclampsia, the plasma volume does not increase to a similar extent as in normal pregnant women (178, 186). In the study by Silver et al., a 16% less plasma volume was found in preeclamptic women as compared to women with normal pregnancy (186). An increase in the total body/peripheral hematocrit ratio was further found, suggesting a central redistribution of blood and a vasoconstricted condition (186).

Higher blood pressure (although within normal values) is evident already from 11-14 weeks' gestation in women subsequently developing preeclampsia or

gestational hypertension (179, 187). In contrast to normal pregnancy, the blood pressure does not decrease towards mid-pregnancy, and after mid-pregnancy it further increases with a steeper slope (179). The blood pressure has been found to be higher even before pregnancy in women subsequently developing preeclampsia (78), which has been suggested to represent one of the unfavourable constitutional factors in women at risk of preeclampsia.

Cardiac output has been found to vary extensively in preeclampsia (e.g. 3.9-13.2 L/min) (188-190), underlining the heterogeneousity of the condition. Several reports suggest that different subgroups of preeclampsia can be characterised by either low or high cardiac output, at least in the preclinical phase of preeclampsia. The preclinical phases of early-onset preeclampsia (191) and preeclampsia associated with intrauterine growth restriction (187) are associated with low cardiac output, whereas the preclinical phases of late-onset (93) and mild preeclampsia (187), are associated with high cardiac output. Valensise et al. found that one year after delivery the cardiac output was still higher in women who had experienced late-onset preeclampsia, whereas the cardiac output had normalised in the group with a history of early-onset preeclampsia (93). They suggested that the two subgroups of preeclampsia, with early and late onsets, may develop from two separate hemodynamic conditions.

The left ventricular mass is higher in preeclampsia as compared to normal pregnancy (185, 192-194), and left ventricular end-diastolic diameter is larger (192, 193). The hypertrophy has been suggested to be due to the increased workload caused by the systemic hypertension in these women.

It has been suggested that the left ventricular performance in patients with preeclampsia reflects a mechanically appropriate response to the increased afterload (185, 193). The ejection fraction is shown to be preserved (193), and fractional shortening to be preserved by some (185, 193), but not all

investigators (192). The ratio of left ventricular end-systolic stress (ESS) and mean velocity of circumferential fiberthickening (V_{CFC}), an index described as a load-independent measure of contractility, has been reported to be unchanged in preeclampsia (185, 195). Systolic function measured by tissue Doppler imaging (cf. p. 30 f.), a more recent method of evaluating left ventricular function, also regarded to be relatively load-independent (196, 197), has not previously been reported in preeclampsia.

In a study using tissue Doppler imaging to assess *diastolic* function in preeclampsia, reduced diastolic function was suggested (192). An increased ratio of early diastolic mitral inflow velocity and early diastolic mitral annular velocity (E/e'), was proposed to indicate increased left ventricular filling pressure as compared to normal pregnancy (192). The early diastolic inflow velocity (E) was shown to be similar (192), and the late diastolic mitral inflow velocity (A) to be increased in preeclampsia (192). Lower ratios of early and late diastolic mitral inflow velocities (E/A) have been reported, both during pregnancy (192, 193) and after delivery (93, 192), supporting the suggested diastolic dysfunction. Both longer (192) and similar (185) isovolumetric relaxation time (IVRT) have been reported, while the deceleration time of the early diastolic mitral inflow (E-DT) has been found to be similar in preeclampsia as compared to normal pregnancy (192).

In early-onset preeclampsia, pulmonary capillary wedge pressure has been found to be non-statistically increased compared to normal pregnancy, although the difference found was small (mean 7 mmHg (range-1-20 mmHg) versus 5 mmHg (range 1-8 mmHg)) (198). Similar, but slightly higher pulmonary capillary wedge pressure in severe preeclampsia has been reported by others, with means ranging from 8.3 mmHg to 10 mmHg, with a markedly further increase in preeclamptic women with pulmonary edema (mean 18±1 mmHg) (190). However, these latter studies did not include women with normal pregnancy for comparison (190, 199).

3.3.5 Preeclampsia and physical activity

Physical activity is known to reduce blood pressure (200) and dyslipidaemia (201, 202), and to improve insulin resistance (203-206) in non-pregnant women, factors which all increase the risk of preeclampsia (78, 207-209). Physical activity improves endothelial function (210-213), which is considered central in the pathogenesis of preeclampsia (35-39). Physical activity also reduces the risk of cardiovascular disease (214-217), which is increased in women with a history of preeclampsia. In theory, physical activity before pregnancy may therefore prevent preeclampsia, by reducing endothelial dysfunction and other risk factors of preeclampsia.

A reduced risk of preeclampsia with pre-pregnancy physical activity versus 'none' was strongly suggested in a previous retrospective case-control study by Rudra et al. and Sorensen et al. (218, 219). There is, however, a risk of recall bias in retrospective case-control studies. Reduced risk of preeclampsia with physical activity one year prior to pregnancy has also been reported in a previous cohort study (220), while two other cohort studies reported no significant link (50, 221). In these previous cohort studies, the women were asked about their pre-pregnancy physical activity while they were pregnant (interviewed/filled out questionnaire before 16-22 weeks of pregnancy). Although the clinical syndrome of preeclampsia is not diagnosed before 20 weeks, it is generally appreciated that the disease process has begun already in the first trimester (35, 222). Many functional changes are also found this early in pregnancy, e.g. lower flow-mediated dilatation, higher cardiac output, and higher blood pressure (136, 137, 179, 187, 223, 224), and it cannot be ruled out that these early changes may affect the way physical activity is perceived by the women who are to develop preeclampsia, and that they may influence the women's responses regarding their pre-pregnancy physical activity.

The results from studies on physical activity *during* pregnancy are also conflicting. A Cochrane review on randomised clinical trials concluded that

there is insufficient evidence for reliable conclusions to be drawn on the effect of physical activity during pregnancy on preeclampsia risk (only two studies, n=45, combined) (225). Case-control studies which have investigated the link between physical activity during pregnancy and preeclampsia risk generally show a reduced risk of preeclampsia with physical activity (219, 226), but again, there is a risk of recall bias in retrospective studies. A large Norwegian cohort study (n=59,573) found that physical activity in early pregnancy reduced the risk of preeclampsia by 20%, but no protective effect was found in women with a body mass index exceeding 30 kg/m^2 (227). Three other large-scale cohort studies (n=85,139 (228), n=3,679 (229) and n=2,241 (220)) found no link between physical activity in early pregnancy and preeclampsia. The data from one of them rather indicated that high levels of physical activity (>270 min/week) in the first trimester might well increase the risk of severe preeclampsia (228).

4 Aims of the study

4.1 Overall aim

To gain knowledge about the cardiovascular changes in preeclampsia

4.2 Specific aims

1. To investigate the variability of the ultrasound-based assessment of flow-mediated dilatation in the brachial artery, and to determine the relative contribution from the defined sources of variability, i.e. *interday* variability, *interpatient* variability, *interobserver* variability and *intraobserver* variability.
2. To investigate whether diastolic and systolic left ventricular functions, examined by tissue Doppler imaging, are reduced during pregnancy in preeclampsia, and to investigate whether endothelial function, measured by the method of flow-mediated dilatation, is reduced during pregnancy in preeclampsia. Furthermore, to study whether left ventricular and endothelial functions are reduced after delivery.
3. To investigate whether pre-pregnancy physical activity prevents preeclampsia.

5 Study subjects

5.1 Paper I

Twenty-two healthy adults (seven females, 15 males) with a mean age of 27 years (range 23 to 54) were recruited among university students and staff. Exclusion criteria were smoking, diabetes, known cardiovascular disease and pregnancy.

5.2 Paper II

Twenty-three women with preeclampsia were enrolled at the time of admission for preeclampsia to the Department of Obstetrics, St. Olav's Hospital, Trondheim, Norway. These were matched with 23 normotensive pregnant women, in terms of gestational age (± one week) and parity (nulliparous versus parous), from a cohort provided by community midwives at the maternal health centres of the City of Trondheim, Norway. Three women had to be retrospectively excluded in accordance with our study protocol, since we learnt, by checking the included women's medical charts, that they had received antihypertensive medication before our first examination. Diabetes, hypertension, known vascular disease, and multiple gestations were defined as exclusion criteria.

5.3 Paper III

3,656 women were included in our study. These were women who answered the physical activity questionnaire in the population-based health study HUNT-1 (cf. p. 33 f.), and who gave birth after their participation in the health study. Only women with singleton live births at a gestational age of more than 22 weeks, or birth weight above 500 grams, at least nine months after their participation in the HUNT-1, qualified for inclusion. The women pregnant at the time of participation in the HUNT-1 were excluded.

6 Methods and methodological considerations

6.1 Study designs

The three studies included in the current thesis are of three different study designs; one methodological, one clinical observational, and one epidemiologic prospective cohort study.

6.1.1 Methodological study (Paper I)

The study in Paper I was a methodological study, where the variability of the ultrasound-based measurement of flow-mediated dilatation in the brachial artery was explored. *Variability* is in statistical terms defined as the spread of the distribution of a variable from the sample mean. Both the reproducibility and the repeatability of the method were examined in our study. *Reproducibility* is the variability of the measurements caused by operator behaviour, in our study termed *interobserver variability*, and *repeatability* is the variability of measurements obtained by one person measuring the same item repeatedly, which in our study was termed *intraobserver variability*. In addition to these purely methodological considerations, also the biological variability between subjects (*interpatient variability*) and the variability between days (in the same subjects) (*interday variability*) were examined. The proportional contributions of these four sources of variability were calculated.

6.1.2 Clinical observational study (Paper II)

The study in Paper II was an *observational study*, since no intervention was made, and a clinical *comparison study*, where the cardiovascular function in two groups was compared.

The inclusion of women was performed in a case-control manner. In case-control studies, the study subjects are selected by their disease status (case group) and compared with subjects not affected by the disease (control group). The control group should ideally be selected from the same population which produced the cases, and the factors differing between the cases and the

controls should ideally all be linked to the disease studied. A possible confounding factor in our study is a selection towards fitter women in the normal pregnancy group. The women with preeclampsia were already in hospital when they were asked if they wanted to participate, and there may therefore have been a lesser degree of selection in this group.

As our measurements were performed after the disease onset we could not infer a temporal sequence between the cardiovascular findings and the development of preeclampsia. Such information was, however, sought by a follow-up examination three months post partum. If an impairment persisted after delivery, no assumptions could be proposed as to pre-pregnancy function. On the other hand, if an impairment found during pregnancy was absent after delivery, it was unlikely that it had been present before pregnancy.

6.1.3 Epidemiological prospective cohort study (Paper III)

The study in Paper III was an epidemiological prospective cohort study where the population investigated was the inhabitants of Nord-Trøndelag county in Norway.

Generally, the incidence rate and the risk ratio are calculated in cohort studies. The incidence rate is the proportion of subjects who develop the disease under study within a specified time period, and the relative risk is the ratio of the incidence rate of the subjects exposed, to that of the subjects who were not exposed. In our study we calculated odds ratios rather than risk ratios. The reason for this is that odds ratios are more available for covariate adjustment through logistic regression, which can be calculated in SPSS, the statistical software applied in our group (SPSS Inc., an IBM Company, Chicago, Illinois, USA). When the incidence of the outcome of interest in the study population is low (<10%), the odds ratios will approximate the risk ratios (230). This applies to our study, where the incidence of preeclampsia was 4.6%. The more frequent the outcome becomes, the more the odds ratio will overestimate the

risk ratio when the odds ratio is >1, and underestimate the risk ratio when the odds ratio is <1 (230).

6.2 Flow-mediated dilatation

In the studies in Papers I and II, endothelial function was measured by the assessment of flow-mediated dilatation as described in the guidelines by Corretti et al. (231). The right brachial artery was longitudinally scanned in two-dimensional mode (*B-mode*, brightness mode) with a Vivid 7 ultrasound scanner (GE Vingmed Ultrasound AS, Horten, Norway), and a handheld linear array transducer (M12L) with a frequency of 14 MHz.

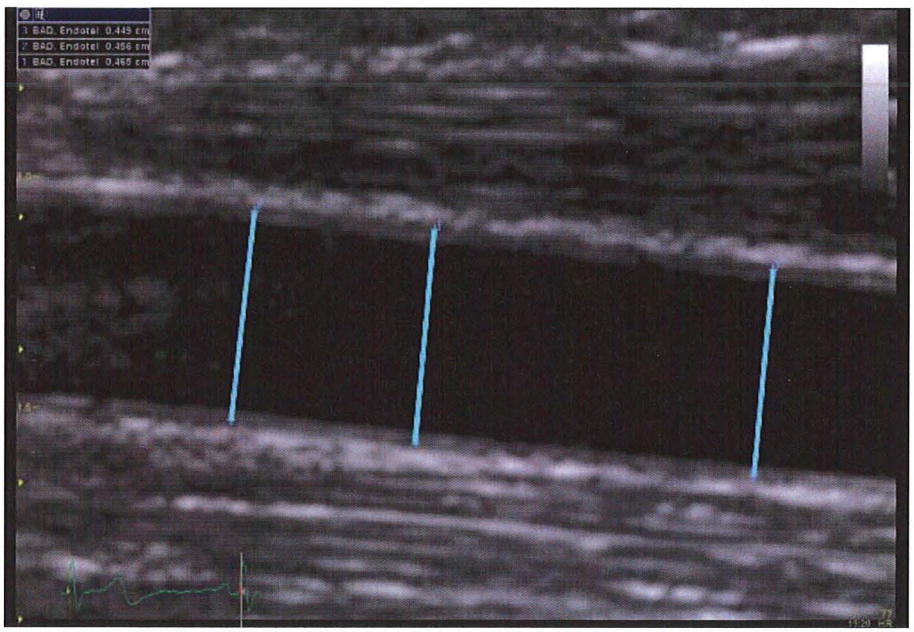

Figure 1. Example of an ultrasound image of a brachial artery in the dilatated state, with three measurements performed along the vessel with electronic callipers.

Images of the brachial artery at baseline were recorded and stored as cine loops of five heartbeats. A pneumatic blood pressure cuff, positioned around the upper arm proximal to the site of imaging, was thereafter inflated to 250 mmHg for five minutes, inducing reactive hypaeremia. Images of the brachial artery were again stored at pre-determined points in time during the first five

minutes after cuff release, i.e. after 0.5, 1, 1.5, 2, 3, 4, and 5 minutes (Figure 1).

Image analysis was performed using the software analysis program EchoPAC PC (GE Vingmed Ultrasound AS, Horten, Norway) on a personal computer, with a caliper resolution of 0.1 mm. The vessel diameter was measured at baseline and at the given points in time after cuff release. We measured the vessel from the anterior to the posterior media-adventitia interface (the '*m-lines*') at the R-wave of the QRS-complex of the electrocardiogram (end-diastole), or as close to the R-wave as allowed by the temporal resolution of the ultrasound recordings, to avoid systolic variation due to compliance (232). In the study in Paper I, three measurements from a single cardiac cycle were performed, whereas in the study in Paper II, three measurements from three cardiac cycles were performed. These were averaged in further calculations.

6.3 Echocardiography

The echocardiography in the study of Paper II was performed according to the recommendations of the American Society of Echocardiography (233). The same ultrasound scanner was used as in the assessment of flow-mediated dilatation (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway), but with a 2.5 MHz phased-array matrix transducer (M3S). The women rested in the left lateral decubitus position during the examinations. Images were stored digitally and analysed off-line, using the analysing program EchoPacPC (GE Vingmed Ultrasound AS, Horten, Norway) on a personal computer.

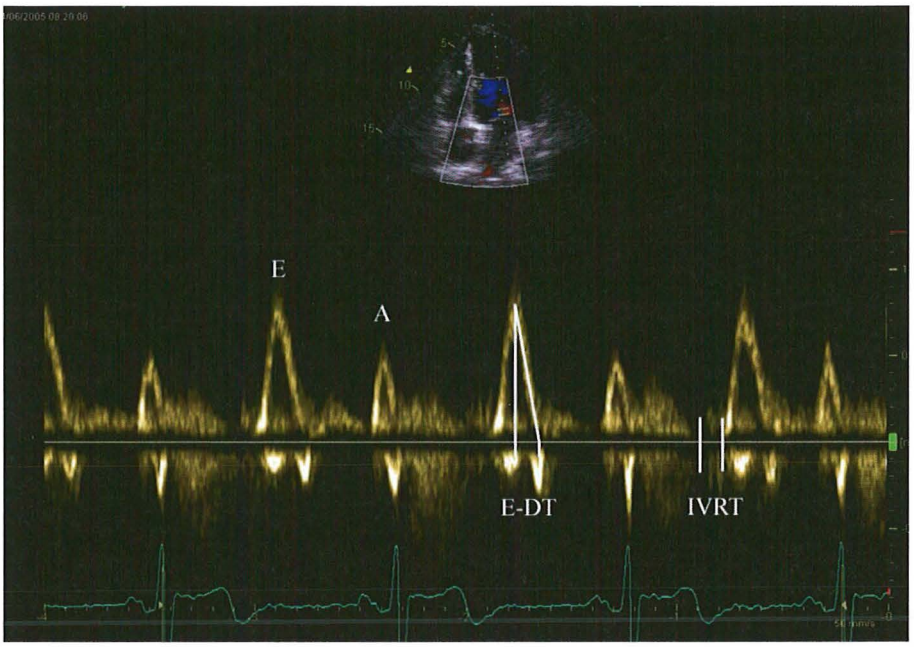

Figure 2. The mitral annular blood flow velocity-curve. *E*, early diastolic mitral inflow velocity; *A*, late diastolic mitral inflow velocity; E-DT, duration time of the deceleration of the early diastiolic mitral inflow velocity; IVRT, iso-volumetric relaxation time. (Image: Charlotte Björk Ingul).

The velocity of blood flow into the left ventricle through the mitral leaflets during diastole was measured by pulsed-wave Doppler. The assessment of the mitral inflow velocity pattern is the traditional way to assess diastolic function. The mitral inflow velocity curve has two peaks, an early diastolic peak (E) and a late diastolic peak (A) (Figure 2). Normally, most of the filling of the left ventricle occurs immediately after the opening of the mitral valve, and only a small contribution of the atrial contraction is needed for adequate filling. In this situation, the early diastolic mitral inflow velocity exceeds the late diastolic inflow velocity, and the ratio of E/A is >1. In pathological conditions the E/A ratio may be reduced to levels <1, and both the isovolumetric relaxation time (IVRT), the time between aortic valve closure and mitral valve opening, and the E-deceleration time (E-DT), the duration of the deceleration-slope of the E-wave, increase. Such characteristics of the mitral inflow velocity are termed a *restrictive pattern*. Further function reduction may cause the left atrial pressure to rise. The early diastolic mitral inflow velocity is

mainy dependent on the pressure differences between the left atrium and the left ventricle. Hence, when the atrial pressure rise, the early diastolic mitral inflow velocity will increase as well, and the E/A will be >1 again. The isovolumetric relaxation time and the deceleration time of the early diastolic mitral inflow velocity will also normalise in this situation. The resulting normal mitral inflow velocity pattern – despite the diastolic dysfunction – is called a *pseudonormal pattern*. In such circumstances, the underlying dysfunctional condition cannot be detected by examining the mitral inflow velocity curve. The interpretation of the clinical condition in such circumstances can, however, be aided by the assessment of pulmonary vein velocities and by tissue Doppler imaging.

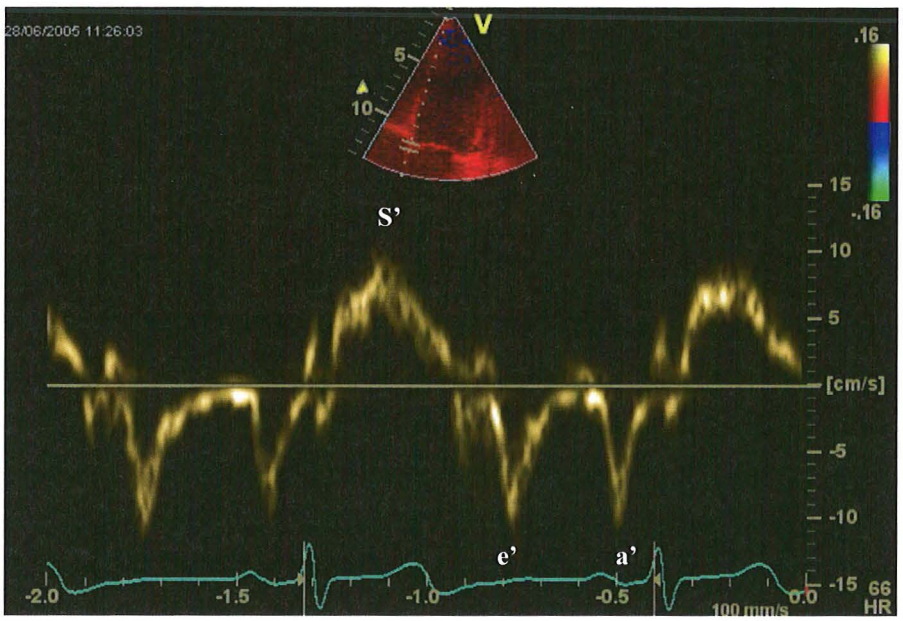

Figure 3. Mitral annular tissue Doppler curve in one of the preeclamptic women measured at the septal position of the mitral annulus. *S'*, peak systolic mitral annular velocity; *e'*, early diastolic mitral annular velocity; *a'*, late diastolic mitral annular velocity.

Tissue Doppler imaging allows the measurement of the velocities of the cardiac muscle. The tissue Doppler curve of the mitral annular velocity has two peaks during diastole, one early peak (e') and one late peak (a'). In contrast to the mitral inflow velocity curve, it also has a systolic peak, denoted

S' (Figure 3). We measured the tissue velocity of the mitral annulus with pulsed-wave tissue Doppler in four positions of the mitral annular ring (septal, lateral, anterior, and posterior). The values from these four positions were averaged in our further analysis, since this has been shown to yield higher test-retest reproducibility as compared to values from single positions (234). An angle deviation of 30% of the transmitted ultrasound wave from the direction of the cardiac movement was accepted.

Tissue Doppler-derived function indices have been found to be less load-dependent than the mitral inflow velocities (235), and early diastolic mitral annular velocity has been found to be a more sensitive measure of diastolic function than the traditionally measured mitral inflow velocities, and to be able to determine subclinical disease, e.g. subclinical cardiac disease in diabetics and hypertensives (236-238). Moreover, the early diastolic mitral annular velocity does not pseudonormalise in the same way as the early diastolic mitral inflow velocity. The ratio of the early diastolic mitral inflow velocity and the early diastolic mitral annular velocity (E/e') can therefore aid the determination of a pseudonormal filling pattern, since E/e' is often elevated in such cases (8-15 cm/s or greater) (239). Ommen et al. compared the E/septal-e' with the time constant of relaxation (τ) obtained by pressure measurements in the left ventricle by catheterisation, and found that a E/septal-e'<8 was indicative of normal filling pressures, and a septal E/e'>15 was indicative of pathologically-elevated filling pressures (45).

The left ventricular ejection fraction (EF) is the most widely used index of left ventricular systolic function (EF = end-diastolic volume - end-systolic volume/end-diastolic volume x 100%), but the index is highly dependent on high image quality, as the endocardial border must be clearly defined in the entire ventricle. The peak systolic mitral annular velocity (S') has been found to be a more sensitive (240) and accurate measure of global left ventricular contractility than the ejection fraction (241). We therefore chose to use peak

systolic mitral annular velocity as the main outcome variable for systolic function in our study.

Left ventricular volumes were calculated by the biplane method with the modified Simpson's rule. The Simpson's rule is an algorithm where the left ventricle is divided into a number of discs by parallel planes perpendicular to its long axis, a procedure which is automatically performed by the analysing software after the endocardial borders have been manually traced. The volumes of the discs are calculated and summarised. In the biplane method, the discs are considered to be ellipsoid, with their two axes deduced from the apical four-chamber view and the apical two-chamber view, respectively. In the single plane method, each disc is considered to be circular. Both the biplane and the single plane volumes were calculated in our study.

The stroke volume (SV) was both obtained from Doppler recordings, and from the left ventricular volumes from the two-dimensional images. The velocity of the blood in the left ventricular outlet tract during systole was measured by pulsed-wave Doppler, and the velocity time integral of the velocity curve (the area under the velocity curve) was automatically obtained by the analysing software after its borders had been manually traced. The velocity time integral, also termed the *stroke distance*, can be considered as the linear distance that the blood travels during a single heartbeat. By multiplying the velocity time integral with the area of the left ventricular outlet tract, the stroke volume was acquired ($SV=VTI_{LVOT}xLVOT_{area}$). The stroke volume derived from the two-dimensional images was calculated by subtracting the end-systolic volume from the end-diastolic volume (SV = end-diastolic volume-end-systolic volume). Doppler-derived stroke volumes are considered to be more reliable than those calculated by the two-dimensional method (242). The Doppler-derived stroke volume is, however, highly dependent on correct measurements of the left ventricular outlet diameter, since the radius of the left ventricular

outlet tract is squared in the calculation of the stroke volume. Cardiac output was calculated by multiplying the stroke volume by heart rate.

6.4 HUNT-1 and the Medical Birth Registry of Norway

The Health Study of Nord-Trøndelag-1 (HUNT-1), the first of three population-based health studies performed in Nord-Trøndelag county in Norway, took place between 1984 and 1986. All inhabitants aged 20 years or more were invited to participate (n=85,100) and 43,602 of those invited were women. Amongst women in the age group relevant to our study (20-49 years), the participation rate in the baseline study (both responders and non-responders to the physical activity questionnaire) was 83% of the total population (16,966 of 19,586).

The HUNT-1 study consisted of two questionnaires and a physical examination. The first questionnaire was mailed to the participants together with the study invitation. The second questionnaire, requesting information on physical activity, was handed out during the physical examination and asked to be returned by mail. This questionnaire is shown in Paper III. Through the physical activity questionnaire the participants were asked how many times per week on average they were physically active. Those active once a week or more were further asked about the average intensity and duration per session.

The data from HUNT-1 was linked to data in the Medical Birth Registry of Norway, which provided information on the women's first birth after their participation in the HUNT-1 study.

6.5 Methodological considerations

6.5.1 Flow-mediated dilatation

6.5.1.1 Coding

Prior to analysis of flow-mediated dilatation in the studies in Papers I and II, each brachial artery cine loop was coded. Coding of data, also called

reversible anonymisation, or pseudonymisation, involves separating personally identifying data but maintaining a link between them through an arbitrary code, or key (243, 244). This key is stored separately and securely, but allows the data to be re-identified under certain circumstances.

In the studies in Papers I and II, each single image cine loop was extracted from its examination file and given such an arbitrary code as its file name, a five-digit number created by the random-function in Microsoft Excel (Microsoft Corporation Inc., Redmond, USA). The code was kept for re-identification after analysis. This procedure ensured that the observers were unaware of subject identity, day of acquisition, and whether it was a recording at baseline or in the dilated state. The image loops were also randomly intermingled prior to analysis, which ensured that the analysis of one image loop would not be affected by the analysis of the preceding loop.

6.5.1.2 Cuff occlusion position

In the guidelines by Corretti et al. (231), the positioning of the occluding cuff was suggested to be either proximal or distal to the ultrasound transducer. In our studies, the proximal occlusion position was chosen. The character of the dilatation response differs after proximal and distal occlusions. After proximal occlusion, the dilatation response is greater (245-248), the peak dilatation occurs later (245, 247), and the dilatation is more prolonged (245, 247, 249).

A proximal cuff occlusion position is no longer recommended, as it was shown that the dilatation response after proximal occlusion was only partly nitric oxide dependent. After inhibiting the nitric oxide synthesis, the dilatation response after proximal occlusion was reduced by 50%, whereas the dilatation response after distal occlusion was completely abolished (246).

Impaired flow-mediated dilatation after both proximal and distal occlusion has, however, been shown to be linked to cardiovascular risk factors to a similar degree (250), and flow-mediated dilatation has been reported to be an

independent predictor of long-term cardiovascular events, both when assessed by proximal occlusion (24, 25) and by distal occlusion (22, 23, 26). Thus, it seems as if both the occlusion positions yield results of equal clinical relevance. However, since the distal approach is now regarded to be the method of choice, and since the mediator-mechanisms responsible for the dilatation following distal occlusion are better described, it would certainly have been advantageous to have applied the distal occlusion approach in our study.

6.5.1.3 Adjusting for shear stress

Another more recent update regarding the method of assessing flow-mediated dilatation involves an adjustment for the magnitude of the shear stimulus (shear rate) (12). Shear rate is calculated by eight times the mean blood velocity/inner diameter if a large, centred sample volume is used to measure blood velocity with Doppler ultrasound, and by four times the mean blood velocity/inner diameter if a small centred sample volume is used (12). The two different equations are due to the parabolic velocity profiles of the blood flow in the artery, with lower velocities against the vessel wall and higher velocities in the centre of the artery, and reflect a source of inaccuracy in the calculation of shear stress.

Another problem encountered when measuring blood velocities in the brachial artery for the calculation of shear stress, is the insonation angle of the ultrasound wave. Since the brachial artery runs parallel to the skin at a 90 degree angle to the propagation direction of the ultrasound wave, the artery is optimal for good quality two-dimensional images, as the ultrasound wave will be directly reflected by the vessel walls. However, a 90 degree angle of the artery represent the least favorable angle for velocity recordings. The blood velocity is calculated from the Doppler shift, which is the change in frequency of the received ultrasound wave as compared to the transmitted wave. The Doppler shift is proportional to the vector component of the blood velocity parallel to the propagation direction of the ultrasound wave. Hence, to find the

blood velocity, the Doppler shift is multiplied by the cosine of the angle between the blood velocity vector and the propagation direction of the ultrasound wave, which is zero when this angle is of 90 degrees. Small changes in the insonation angle will produce large changes in the measured velocity, especially when the angle exceeds 60 degrees (60-120 degrees) (12).

By steering the angle of the ultrasound wave used for Doppler shift recordings in the brachial artery, an angle of 60 degrees can be achieved. A 60 degree angle is, however, only considered to be the 'best of the worst' angles (12).

6.5.2 Echocardiography

An angle deviation of 30 degrees between the ultrasound beam and the plane of the cardiac movement was allowed in our analysis of tissue Doppler images, higher than the maximum angle of 20 degrees recommended by the American Society of Echocardiography (251). An angle deviation of 30 degrees would lead to a 13% underestimation of the cardiac velocity, compared to the 6% underestimation of a 20 degree angle deviation. We considered the higher level of acceptance of angle deviation appropriate, as in our study we were not interested in individual values, but rather in group means, and as we have no reason to believe that the angle deviation was different between the groups The standard deviations of our tissue Doppler indices approximated the standard deviations of the reference values in a healthy population of women <40 years (234), which shows that our level of acceptance of 30 degrees did not lead to higher variability in our data.

We did not measure the pulmonary vein blood flow velocity, which, if measured, probably would have added information regarding the filling pressure of the left ventricle in our study. However, the pulmonary vein blood flow velocities, like the mitral inflow velocities, are of the highest value when the ejection fraction is reduced (252), which was not the case in the women included in our study.

As the uterus enlarges during pregnancy, the diaphragm becomes elevated. This causes the heart to be displaced upward and somewhat to the left with rotation on its long axis. The cardiac displacement during pregnancy may have affected the echocardiographic indices in our study. We have no reason to believe that the displacement was different in the women with preeclampsia as compared to the women with normal pregnancy. However, the displacement should be taken into account when interpreting the within-group differences between the values obtained during pregnancy and after delivery.

6.5.3 HUNT-1 and the Medical Birth Registry of Norway

6.5.3.1 Self-reported physical activity

In the study in Paper III, self-reported physical activity was linked to the risk of preeclampsia. The level of self-reported physical activity does not necessarily correlate with the physiological changes of physical activity, e.g. endothelial function. Self-reported physical activity is, however, a cost-effective means of measuring physical activity, and is widely used in epidemiological studies.

The physical activity questionnaire in HUNT-1 was validated by Kurtze et al. (253), who found a moderate, significant correlation between the responses and the maximal oxygen uptake (r=0.48). The validation study was performed only one week after the participation in HUNT-1, and it is a moot point whether the results were still valid years after HUNT-1. Kurtze et al. did not include women in their study. Wisløff et al., however, performed another validation study of the physical activity questionnaire from HUNT-1, including women (215), and found no differences by sex in the reported physical activity intensity relative to the maximal oxygen uptake (215).

6.5.3.2 Time interval between the participation in HUNT-1 and the index birth

One of the inclusion criteria in our study was that the index birth should occur at least nine months after the participation in HUNT-1, to ensure that women pregnant at the time of participation in the baseline study were excluded. Only inhabitants of twenty years or more were invited to HUNT-1. A maximum maternal age of 40-45 years was expected, and the largest interval from the baseline study to the index birth would therefore be of 20-25 years. However, the mean interval was expected to be shorter, since the highest frequency of births in Nord-Trøndelag 1984-1988 and 1990-1998 occured in the maternal age-group 25-29 years (254). The interval between the women's participation in HUNT-1 and the index birth represents a limitation in our study, since physical activity habits may change over time.

6.5.3.3 Misclassification

All births occurring in Norway are registered in the Medical Birth Registry of Norway, by the mandatory reports from the maternity institutions. We did not check the medical charts of the women included, and misclassification of preeclampsia may have occurred.

6.6 Statistics

6.6.1 REML

The method of restricted (or residual) maximum likelihood estimation (REML), a technique for estimating variance components in multi-classified data, was used in the study of Paper I. The classes defined in our study were *observer* (different persons analysing the ultrasound images), *day*, and *subject*. These were considered to be independent random factors contributing to the total variance of the outcome variables. The resulting variance component model can be summarised as

$$\text{var}(y) = \sigma^2_{subj} + \sigma^2_{day} + \sigma^2_{obs} + \sigma^2_{res}$$

where y is the outcome variable of interest, *subj* is subject, *day* is day and *obs* is observer. The final component, *res*, denotes the residual or intraobserver

variance, and represented the variance that could not be accounted for otherwise. The variance components were estimated hierarchically, as observer within day within patient, by the method of REML. A figure showing the study design is given in Paper I.

6.6.2 Coefficient of variation

The coefficient of variation (CV) was calculated in Paper I. The standard deviations of the differences between the measurements by the same observer, the different observers, the different days, and the different patients were found and divided by the respective means and multiplied by 100%.

$$CV = (\text{standard deviation} / \text{mean}) \times 100\%$$

6.6.3 Paired t-test

In the study in Paper II, the paired Student's t-test was used in our statistical calculations. In the paired Student's t-test, the difference between the observations is calculated for each pair, and the null-hypothesis tested is that the difference between each pair is zero. The paired test was chosen in accordance with our inclusion design, where each woman with preeclampsia was matched with a woman with normal pregnancy of similar gestational age. A significance level (α) of 0.05 and a two-sided test were chosen.

6.6.4 Odds ratio and logistic regression

In the study in Paper III, the outcome variable was a dichotomous categorical variable, as it had only two possible values, 'preeclampsia' (coded 1) and 'not preeclampsia' (coded 0). Linear regression would in this case be inadequate, since by this method the predicted variable could obtain values below 0, decimal values, or values exceeding 1, which in our case would be uninterpretable. Logistic regression analysis can handle categorical outcome variables, since the outcome variable is not modelled directly, but instead, the probability of a specific *Y*-value is estimated. Since our outcome variable was dichotomous, we used binary logistic regression. Logistic regression is also available for covariate adjustments.

6.6.5 Sample size

Sample size is estimated to ensure adequate statistical power of a study, i.e. the probability that the test will reject a false null hypothesis. The estimation of the sample size in the study in Paper II was based on estimates of the outcome variables, i.e. the mitral annular tissue velocities (e' and S'), the ratio of early diastolic mitral inflow velocity and early diastolic tissue Doppler (E/e'), and flow-mediated dilatation. With a two-sided test with a significance level (α) of 0.05 and a power (1-β) of 0.8, the equation used to estimate sample size was:

$$n = 16\frac{sd^2}{(\mu_1 - \mu_2)^2} + 0.96$$

where n is the number needed in each group, sd is the estimated standard deviation, μ_1-μ_2 is the estimated difference between the group means (255).

When we planned our study, to our knowledge no other studies had estimated left ventricular function in preeclampsia by tissue Doppler imaging. In our estimations of sample size we therefore applied the standard deviations of e' and S' reported by Alam et al. in healthy subjects < 40 years of age, which were 2.4 cm/s and 1.4 cm/s, respectively (256), and the standard deviation of E/e' reported by Mungala et al. in healthy subjects 40-49 years of age, which ranged from 1.38 in the lateral position of the mitral annular ring, to 2.14 in the septal position (257). The estimated difference between the groups was suggested to be 3 cm/s for e' and S' and 3 for E/e'. To calculate the sample size needed to yield statistical power adequate enough to find statistical differences in flow-mediated dilatation between the groups, we applied the standard deviation of flow-mediated dilatation which we found in the study in Paper I. The difference in mean between the groups was estimated by averaging the mean differences reported in four previous studies (138-141), resulting in an average difference of 6.8%. Our calculations yielded an estimated number of women needed in each group ranging from five to 17 across the outcome variables, when rounded off upwards to the nearest whole

number (Table 1). Twenty-three women were finally included in our study, to ensure adequate statistical power in case of drop-outs.

Table 1. Sample size estimation in the study in Paper II.

	sd	μ_1-μ_2	n (each group)
e'	2.4	3	12
E/e'	1.38-2.14	3	5-10
S'	1.4	3	17
FMD	6.8	6.8	17

As we applied the paired t-test in our analyses, the mean difference between the matched pairs should have been used in our estimation of sample size, instead of the difference in group means; and the standard deviation of the differences instead of the standard deviations of group means. Since these values were difficult to estimate, estimators based on group means were applied as an approximation. The paired t-test generally has higher statistical power than the independent t-test, implying that this modification would lead to a slight overestimation of sample size in our study.

6.7 Ethical considerations

The studies were performed in accordance with *the World Medical Association's Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects*, and approved by *the Regional Committee for Medical Research Ethics, Mid-Norway*. Written informed consent was obtained from the study participants at the study entry in the studies in Paper I and Paper II. In the study in Paper III we used data from the HUNT-1 study, which was collected between 1984 and 1986, at a time before written informed consent was generally required in medical research in Norway (the Regional Committees for Medical Research Ethics, Norway, were officially appointed in 1985). In the HUNT-1 study, the study subjects were informed about the study through the invitation which was sent out by mail, and the act of showing up at the study centres was regarded as an indication of the study subject's consent to participate.

The method of measuring flow-mediated dilatation involves some discomfort during the five minutes of cuff occlusion when blood flow to the forearm and the hand is arrested. The study subjects were informed about this discomfort prior to study entry, and were notified about their right to withdraw from the study at any time, also during the examination.

The women with preeclampsia were included after their admission to hospital, which may have rendered it difficult for them to refuse to join our study. We tried to balance this by underlining the fact that participation in our study was entirely voluntary, and not a part of their medical treatment. We also made it clear that whether they chose to participate or not would not affect the medical treatment or care which they would be given. Those preeclamptic women who were interested in participating were given at least an hour to read the information letter and to consider their decision, before they were asked whether they wanted to be included in our study. The women with normal pregnancy interested in participating received an information letter from their midwife and were contacted by us after a couple of days.

In the study in Paper II, the data from the HUNT-1 study were correlated with data in the Medical Birth Registry of Norway. The linkage was performed by the HUNT administration, and only un-identifiable data were present in the file that we were given in hand.

7 Summary of results

7.1 Paper I

Repeated image analyses improve accuracy in assessing arterial flow-mediated dilatation

The relative proportional contributions to the total variance (defined as 1.00) from the different variance components *intraobserver, interobserver, interpatient* and *interday* were as follows: 0.41 from *intraobserver* variance, 0.18 from *interobserver* variance, 0.25 from *interpatient* variance and 0.15 from *interday* variance. Hence, the major source of variability in the assessment of flow-mediated dilatation of the brachial artery in our study was *intraobserver* variability. This implies that the total variance will be substantially reduced by repeating image analysis.

7.2 Paper II

Left ventricular and endothelial functions in preeclampsia

Our results suggest reduced diastolic function, with pseudonormalisation and increased filling pressures in preeclampsia. We found a lower early diastolic mitral annular velocity, e', and a higher ratio of early diastolic mitral inflow velocity and early diastolic mitral annular velocity (E/e'). Early diastolic mitral inflow velocity (E) was similar between the groups, whereas late diastolic mitral inflow velocity (A) was higher in the preeclampsia group during pregnancy. The ratio of early and late mitral inflow velocities (E/A), the isovolumetric relaxation time and the E-deceleration time were similar between the groups. Systolic function was found to be relatively impaired in the preeclampsia group, since a pregnancy-related increase in peak contraction velocity (S') found in normal pregnancy was not present in preeclampsia. Both diastolic and systolic functions normalised three months after delivery.

The flow-mediated dilatation was similar in the preeclampsia group and the normal pregnancy group during pregnancy, although a tendency towards lower

values in the preeclampsia group was found. Three months after delivery, the flow-mediated dilatation was significantly impaired in the preeclampsia group as compared to the normal pregnancy group. The preeclampsia group had larger brachial arterial diameter than the normal pregnancy group during pregnancy, but there were no differences in artery size between the groups after delivery.

7.3 Paper III

Pre-pregnancy physical activity and preeclampsia risk: a prospective population-based cohort study

Overall, we found no link between preeclampsia and pre-pregnancy physical activity. Only among the women active 120 min per week or more a non-significant tendency of reduced risk was found.

8 Results and discussion

8.1 Variability in the method of assessing flow-mediated dilatation

The greatest contributor to the variability of the method of assessing flow-mediated dilatation in the brachial artery by high-resolution ultrasound was found to be the intraobserver variability, accounting for a proportion of 0.41 of the total variance (total variance defined as 1.00). The simplest way to reduce intraobserver variability effectively is for the observer to average the results from repeated image analyses. If three repetitions of image analyses are performed, the total variance will be reduced by 30%.

Automatic edge-detection software may render lower variability than the manual placement of electronic callipers. Woodman et al. found substantially lower intraobserver variability when using automatic edge-detection software compared with the manual placement of callipers (coefficient of variation 6.7% versus 24.8%) (258). However, considerable intraobserver variability has also been reported in studies where automatic edge-detection software has been used (coefficient of variation 44.7% (259)), showing that this alone does not solve the problem of the high variability of the method. It has been suggested that automatic edge-detection software renders more objective diameter measurements (12), but the most commonly used software program, Vascular Research Tools 5 (Medical Imaging Application, LLC, Iowa City, IA, USA (12, 260)), is only semi-automatic, as both the location and the size of the region of interest (the region where the software program detects the vascular wall) are determined by the user. The suggested vessel borders must also be manually approved. An advantage of the automatic edge-detection software is that it renders a less time-consuming analysis process, which facilitates the implementation of the repeated image analyses. Furthermore, by edge-detection software, the vessel is measured multiple times along the

picture frame, presumably yielding a more representative value of the artery diameter. Another advantage of the edge-detection software is that continuous artery diameters can be measured over a time period after cuff release, provided that the artery is also depicted continuously. The true peak diameter can thereby be assessed, as well as the time to peak, which may be of clinical significance (261).

In our study, we provided objectivity of our diameter measurements by the coding of each single cine loop of the brachial artery images prior to analyses. We thereby ensured that the analyser was unaware of the vessel state during analyses, i.e. whether it was an image acquired at baseline or in the dilatated state, and of the study subject identity. Since the image loops were randomly intermingled we also ensured that the diameter measured at one point in time was unaffected by the preceding measurement within the same study subject. The process of coding was, however, time-consuming and is not a standard procedure.

Not surprisingly, the frequency of image acquisition seemed to be of importance to the variability of the method. High frequency yields short wave-length of the ultrasound wave ($c=f\lambda$; c, speed of sound transmission in tissue; f, frequency; λ, wavelength), which improves the radial resolution (i.e. resolution along the beam). We used a probe with a frequency of 14 MHz in our study. The use of high frequency probes (>10 MHz) characterizes the studies reporting intraobserver variability similar to or lower than ours (258).

Interobserver variability also contributed considerably to the total variability in our study, with a proportional contribution of 0.18. This implies that the analysis should preferably be performed by one single observer.

Unfortunately, we did not include a sufficient number of study subjects to allow for the assessment of gender-specific variability. This would have been

interesting, since it has been shown that women have greater flow-mediated dilatation than men of similar age (262-265). The larger dilatation response in women has been suggested by some investigators to reflect a positive effect of estrogen on endothelial function (265). Others have, however, suggested that the increased flow-mediated dilatation in women simply reflects smaller arteries (262-264), It has been argued that the larger dilatation in smaller arteries is due to the fact that smaller arteries are exposed to a greater shear stress stimulus by virtue of their size (266-268). However, as increased dilatation in smaller arteries has also been observed after the administration of glyceryl trinitrate (269), which is not mediated by shear stress, also other factors are probably involved in the larger dilatation in smaller arteries.

High overall variability of the method of assessing flow-mediated dilatation in the brachial artery was found in our study, with a coefficient of variation of 22% of the percentage dilatation measured in the same patient on two consecutive days (interday variability). This implies that the method is not an appropriate tool for the clinical setting. For research purposes, where groups are compared, it may, however, add valuable information.

The methodological study in Paper I was not performed in pregnant women, although this would have been valuable to us in the planning of the study in Paper II. The reason for this was that our results were intended to be applied in the planning of several other studies in our research group, the majority of which involve the endothelial response to physical exercise, where pregnant women are not in the target group. Nevertheless, according to our findings, improvements were made in our protocol of measuring flow-mediated dilatation in women with preeclampsia and normal pregnancy. Image analysis was performed by one single observer, and was repeated three times. The standard deviation found in the methodological study was also applied in our calculation of sample size in the study in Paper II.

8.2 Flow-mediated dilatation in preeclampsia

The endothelial function, measured by flow-mediated dilatation, was not significantly lower in the preeclampsia group as compared to the normal pregnancy group during pregnancy in our study, although a tendency towards lower levels was found (Figure 4(a)). This contrasts with previous reports, where lower flow-mediated dilatation in preeclampsia has been shown (138-141). Three months after delivery the flow-mediated dilatation was significantly lower in the preeclampsia group in our study (Figure 4(b)), in line with previous reports (149-151).

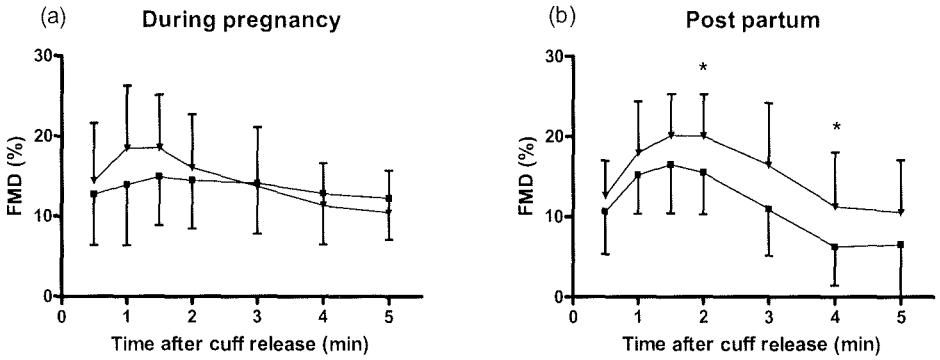

Figure 4 Flow-mediated dilatation (FMD) in women with normal pregnancy (▼) and in women with preeclampsia (■). (a), during pregnancy; (b), post partum. *, $p<0.05$.

As can be seen from Figure 4(a), the flow-mediated dilatation-time curve seemed to be less dynamic in the preeclampsia group during pregnancy, with no clear peak, but rather a prolonged maximum dilatation. After delivery, the shape of the curve had normalised, but the magnitude of the response was still lower (Figure 4(b)). The early peak in flow-mediated dilatation has been suggested to be mainly nitric oxide-mediated, whereas other mechanisms are thought to be recruited as the dilatation process is prolonged (268). The missing early peak in the preeclampsia group may thus be due to reduced nitric oxide production, or to reduced nitric oxide bioavailability. As mentioned above, there is some debate as to whether the nitric oxide production is reduced during preeclampsia (105-111).

Changes in flow-mediated dilatation dynamics have previously been reported in elderly (261) by Black et al., who speculated as to whether smooth muscle cell responsiveness, free radical production or changes in mechanical properties in the vascular wall could explain their findings (261). Disrupted vascular smooth muscle function in preeclampsia has previously been suggested by in vitro studies (270, 271). Van Wijk et al. showed that calcium sensitivity was significantly increased in vascular smooth muscle cells of subcutaneous vessels from women with preeclampsia (271), and Wismalasundera et al. reported a reduced rate of relaxation and a decline in intracellular calcium in resistance arteries from women with preeclampsia which was still present after the endothelium had been removed (270). The reduced rate of relaxation was suggested to be due to the decreased activity of the plasma membrane calcium ATP-ase (PMCA), responsible for mitochondrial uptake and cellular efflux of calcium (270). By measuring endothelial-independent dilatation, the confounding effect of vascular smooth muscle dysfunction on flow-mediated dilatation can be tested. However, endothelial-independent vasodilatation is generally not measured during pregnancy, and vascular dysfunction has not been controlled for in studies measuring flow-mediated dilatation in preeclampsia.

The lack of a statistically significant difference in flow-mediated dilatation between the groups during pregnancy in our study may have been caused by an inadequate sample size. In our calculation of sample size in the planning of our study, we applied the standard deviation of flow-mediated dilatation found in the methodological study in Paper I, of 6.8%, which turned out to be adequate, since the standard deviations of the group means in the study in Paper II ranged from 4.4% to 7.7% across the measurements performed at the different points in time after cuff deflation (0.5, 1, 1.5, 2, 3, 4, and 5 minutes). The difference between the group means, however, turned out to be smaller than estimated. In our a priori calculation of sample size we applied the

averaged difference between group means reported in four previous studies (6.8%). This turned out to be an overestimation, since the maximum difference in flow-mediated dilatation found between groups in our study was 4.5% during pregnancy and 5.5% after delivery. Approximately 38 women in each group would have been needed to yield adequate statistical power to prove such a difference.

Furthermore, in contrast to the studies referred to (138-141), we chose to explore our data by means of the paired t-test, due to the matched design of our study. The matched design was chosen as it was assumed that the haemodynamic changes within the groups throughout gestation would be greater than the differences found between the matched pairs of similar gestational age. This turned out not to be the case regarding flow-mediated dilation, where the mean standard deviation of the matched pairs was found to be larger than the standard deviation of the group means, resulting in less statistical power for the paired t-test compared to the independent t-test. In supplementary analyses by means of the independent t-test, a statistically significant lower flow-mediated dilation in the preeclampsia group during pregnancy was found also in our material, The results regarding the left ventricular function were similar both when the paired t-test and the independent t-test were applied, suggesting that the pregnancy-related changes in left ventricular function were more gestational age-dependent than the changes in the flow-mediated dilation.

Another issue which may have caused incongruous results in our study as compared to those previously reported, are differences in the study populations. While age and blood pressure were comparable in our study and in the previous ones, the ethnicity of the populations studied differed. In three of the studies reporting lower flow-mediated dilatation in preeclampsia, Japanese women were examined (139-141). Preeclampsia in Japanese women (272), but not in Indian (105), or American white and Hispanic women (273),

has been found to be associated with a gene variant with a polymorphism at position 298 (Glu298Asp) of the endothelial nitric oxide synthase gene. This gene variant has been reported to be associated with altered nitric oxide production (274), and has also been reported to be associated with lower flow-mediated dilatation in pregnancy (275). Thus, the association of flow-mediated dilatation and preeclampsia may differ between different ethnic groups.

The standard deviation of the mean flow-mediated dilatation in the preeclampsia group in our study was considerably higher than in the studies referred to (6.8% versus range 1.16% to 3.8%) (138-141). The reason for this may be the coding of the image loops, which we performed prior to analyses, and the fact that the image-loops from the study subjects were randomly intermingled during analysis. Even though the coding of the images resulted in higher variability in our study, we consider this process to be one of the strengths of our study, ensuring a more unbiased result. The proximal cuff occlusion position, the frequency applied, the handheld transducer and the manual placement of electronic callipers in our study cannot, however, explain the higher variability as compared to others, since these methodological approaches did not differ across the studies (138, 140, 141).

We found a larger brachial artery in the preeclampsia group than in the normal pregnancy group during pregnancy, in accordance with a previous report (140). Larger brachial artery diameter has been shown to be an independent predictor of cardiovascular events in both women and men (21), and it might be suspected that larger brachial artery diameter is part of a cardiovascular risk profile found in non-pregnant women, increasing their risk of both preeclampsia and cardiovascular disease. The brachial artery diameter was, however, similar between the groups three months after delivery in our study, and it seems unlikely that the larger diameter was present before pregnancy in the preeclampsia group. Rather, the diameter seems to have been affected by

the condition of preeclampsia. Savvidou et al. showed that the brachial artery diameter is increased after the 30th week in normal pregnancy as compared to non-pregnant women, and suggested that this may be due to increased blood flow in pregnancy, with following increased shear stress leading to a greater stimulus of the endothelial cells which causes the release of nitric oxide producing vasodilatation (137). Unfortunately, we did not measure blood flow velocity in the brachial artery and could not determine whether the flow was higher in the preeclampsia group as compared to the normal pregnancy group.

Lower flow-mediated dilatation is generally found in larger brachial arteries (269, 276). This may partly be due to the fact that the flow-mediated dilatation is calculated by dividing the dilatation in absolute values by the baseline brachial artery diameter. As discussed above, it has also been argued that the lower percentage dilatation in larger arteries is, at least partly, due to lesser shear stress in larger vessels (266-268), underlining the importance of adjusting for shear stress (12), especially when comparing study samples with differences in artery diameter between the groups. Since the blood flow velocity in the brachial artery was not measured in our study, we could not adjust our data for shear stress. However, adjusting for shear stress presumably would have resulted in higher flow-mediated dilatation in the preeclampsia group during pregnancy, and thereby a lesser difference between the groups, since the preeclampsia group had larger arteries than the normal pregnancy group during pregnancy.

The impairment in endothelial function post partum (Figure 4(b)) is supported by other studies, where lower flow-mediated dilatation has been reported years after preeclamptic pregnancies (149-151). The impaired flow-mediated dilatation in women with a history of preeclampsia shows that reduced flow-mediated dilatation is not only a marker of, or influenced by, preeclampsia. Reduced flow-mediated dilatation may have been present even before

pregnancy in women who develop preeclampsia. Prospective studies are needed to investigate this issue.

8.3 Left ventricular function in preeclampsia

Both diastolic and systolic left ventricular functions, measured by tissue Doppler imaging, were impaired in preeclampsia (Figure 5). Three months after delivery the left ventricular function had normalised.

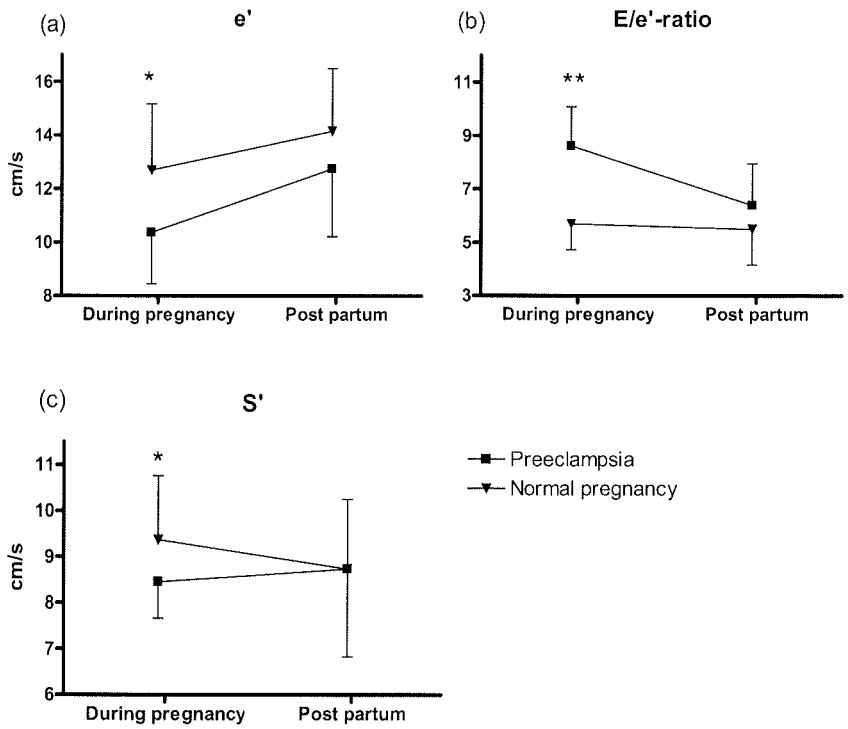

Figure 5. Left ventricular tissue Doppler-based function indices during pregnancy and post partum in women with preeclampsia (■) and in women with normal pregnancy (▼). The bars represent standard deviations. Graph (a) shows the early diastolic mitral annular velocity (e'), (b) the ratio of the early diastolic mitral inflow velocity and the early diastolic mitral annular velocity (E/e'), and (c) the peak systolic mitral annular velocity (S'). *, $p<0.05$; **, $p<0.005$.

A lower early diastolic mitral annular tissue Doppler (e') was found in the preeclampsia group than in the normal pregnancy group, which means that the relaxation of the left ventricle during early diastole was slower in preeclampsia. The normal range of e' in *non-pregnant* women <40 years of

age is 10.0-19.2 cm/s (234). The mean value of e' in the preeclampsia group was 10.4 cm/s, i.e. just within normal values of the non-pregnant. However, as many as eight out of 20 preeclamptic women had e'-values lower than 10 cm/s, whereas only one woman in the normal pregnancy group had an e'-value lower than 10 cm/s in our study.

A correlation between e' and S' was found in our material, both in the preeclampsia and in the normal pregnancy groups (Pearson's coefficient of correlation 0.74, $p<0.001$ and 0.63, $p=0.005$, respectively), showing the close relationship between diastolic and systolic function. We found no correlation between e' and systolic or diastolic blood pressure, or left ventricular wall thickness in our material.

Our study supports the view that preeclampsia is a condition of elevated filling pressures compared to normal pregnancy, as previously suggested by Rafik-Hamad et al. (192). The ratio of the early diastolic mitral inflow velocity and the early diastolic mitral annular tissue velocity (E/e') was found to be increased in preeclampsia as compared to normal pregnancy. This was mainly due to the increase in the early diastolic mitral annular tissue velocity (e'), since the increase in the early diastolic mitral inflow velocity (E) was only borderline significant. The mean ratio of the early diastolic and the late diastolic mitral inflow velocities (E/A) was similar between the groups, although there was a non-significant tendency towards lower values in preeclampsia. Since the isovolumetric relaxation time and the E-deceleration time were similar between the groups, pseudonormalisation was indicated. Unfortunately, we did not measure the pulmonary vein flow velocities, which might have added information regarding the filling pressure.

Although we observed significant differences in the E/e' between the groups, the increased E/septal-e' in the preeclampsia group of 9.8±2.1 was not within the range indicative of pathologically-elevated filling pressures suggested by

Ommen et al. (>15) (45). The study population examined by Ommen et al. was not pregnant, had a mean age of 61 years, and only 39% were women, and is therefore not directly comparable to our study population. (For comparison, the E/septal-e' in the normal pregnancy group was 6.8±1.9, and the difference between the groups was highly significant, p=0.001.)

A relative impairment in systolic function during preeclampsia was found in our study. In the normal pregnancy group, elevated peak systolic mitral annular velocity (S') was found during pregnancy as compared to the values after delivery, according to previous reports (182, 277). No such pregnancy-related increase in peak systolic mitral annular tissue Doppler was found in the preeclampsia group. Correspondingly, pregnancy-related increases were not found among the other systolic function indices measured, i.e. the ejection fraction and the mitral annular excursion. It has, however, been shown that the peak systolic mitral annular tissue Doppler provides a more accurate measure of global left ventricular contractility than the ejection fraction (241). No correlation was found between S' and flow-mediated dilatation, or systolic or diastolic blood pressures. Peak systolic mitral annular velocity was, however, found to be negatively correlated with brachial artery diameter in the preeclampsia group, but not in the normal pregnancy group (Pearson's coefficient of correlation, preeclampsia, -0.58, p = 0.02).

We found a tendency towards left ventricular hypertrophy (increased left ventricular wall thickness) during pregnancy in the preeclampsia group, although this was not statistically significant. Increased left ventricular wall thickness has been reported up to one year post partum after preeclampsia (93, 185) suggesting persistent hypertrophy. Our study did, however, not confirm this. In a within-group analysis, we found that the wall thickness had decreased significantly in the preeclampsia group post partum compared to the values during pregnancy, a finding which rather indicates reversible hypertrophy in preeclampsia.

The stroke volume and the cardiac output were similar in the preeclampsia group and the normal pregnancy group during pregnancy in our study. Both higher (185) and lower (193) stroke volume has previously been reported in preeclampsia, but in neither of these studies were the cases and the controls matched for gestational age. Higher cardiac output in the preclinical phase of preeclampsia has previously been reported in two large longitudinal cohort studies (188, 278). In one of these, a cross-over to lower cardiac output in the preeclamptic group was found in week 36 (188), as mentioned above. As the cardiac output was measured at the time of diagnosis in our study, our values may correspond to the time of cross-over.

The stroke volume and the cardiac output may differ among subgroups of preeclampsia. Lower stroke volume may be associated with foetal growth restriction (*IUGR*, intrauterine growth restriction) in normotensive women (279), also found in the preclinical phase of preeclampsia (187). Even though only two women in the preeclampsia group in our study delivered babies who were small for gestational age, a tendency towards lower stroke volume in these women compared to their normal pregnant controls was found in our material, whereas a tendency towards higher stroke volume as compared to normal pregnancy was found in the remaining preelamptic women.

The time of preeclampsia onset has also been suggested to be linked to differences in stroke volume and cardiac output. Valensise et al. reported larger stroke volume, larger cardiac output and higher heart rate in the preclinical phase of late-onset preeclampsia, whereas in the preclinical phase of early-onset preeclampsia, these indices were lower than in normal pregnancy (93). The same tendency towards larger stroke volume in late-onset preeclampsia and smaller stroke volume in early-onset preeclampsia were found in our material, although not statistically significant, due to inadequate

sample size for such sub-analyses, as only eight women had preeclampsia of early onset in our study.

The stroke volume was found to be higher three months after delivery in the preeclampsia group in our study, in accordance with a previous report (185). However, since the velocity time-integral of blood flow in the left ventricular outlet tract (LVOT VTI) was similar between the groups, this higher Doppler-derived stroke volume was simply due to the larger left ventricular outlet tract diameter in the preeclampsia group after delivery. The left ventricular outlet tract diameter was similar between the groups during pregnancy, and we suspect the larger diameter after delivery to represent a chance finding. The stroke volume based on the two-dimensional recordings, showed no difference between the groups after delivery.

Although endothelial and left ventricular functions are known to change with age (280-284), maternal age was not chosen as a matching variable in our study. Since preeclampsia is more common among women with higher maternal age, age-dependent matching might have been appropriate (58). When planning our study we considered it likely that the age-related changes in endothelial and left ventricular functions in women over 18 years of age (defined as inclusion criterion), but within the reproductive range, would not be of clinical significance.

We did neither match our cases and controls by body mass index. The accuracy of two-dimensional echocardiography has been shown to be reduced when body mass index exceeds 25 kg/m^2 (285), presumaby due to increased signal attenuation caused by a greater distance to the heart from the transducer, and by increased scatter due to inhomogeneous fat-containing tissue. The body mass index was higher in the preeclampsia group than in the control group during pregnancy, which may have affected our results. However, since a causal relationship between aspects associated with high

body mass index and preeclampsia cannot be disregarded (e.g. insulin resistance or other metabolic disturbances), we chose not to match our controls with the cases by body mass index.

A longer interval than three months after delivery in our post partum examination would have been advantageous, as the vascular changes probably had not resumed baseline levels at that time (180). The values of the tissue Doppler indices at our post partum examination were, however, similar to the normal values given in women <40 years in the publication by Dalen et al. (234).

In future studies it would be interesting to investigate whether impairments in endothelial function and left ventricular function are present prior to pregnancy in women who later develop preeclampsia. It would also be interesting to investigate whether the reduced flow-mediated dilatation and the changed flow-dynamics are related to the time of onset of preeclampsia or the growth of the foetus in a longitudinal study, with the first measurements performed before pregnancy.

8.4 Pre-pregnancy physical activity and preeclampsia

In contrast to what we expected, women physically active prior to pregnancy were not at reduced risk of developing preeclampsia. Overall, we found no link between physical activity and preeclampsia risk.

No significant dose-response relationship between physical activity and preeclampsia risk was found in our study, in accordance with previous reports (50, 220). Only among women with the highest duration of physical activity (>120 min a week), a non-significant tendency towards a lower risk of preeclampsia was found. Such tendency was also reported in a recent study by Hegaard et al. in their 'moderate-to-high' physical activity group (50). Rudra et al. reported a significant tendency towards a reduced risk of preeclampsia

with increasing perceived exertion (Borg scale), but not with increasing duration (220).

It is possible that the physical activity level given by the women in HUNT-1 might not still be valid at the time of their index birth years after the baseline study. The median time interval from the participation in HUNT-1 and the index birth was 3.2 years (range 0.75-20 years). However, our results were not affected when adjusted for the time that had elapsed between the baseline study and the index birth.

In the main analysis, we combined the group 'never' physically active with the group physically active 'less than once a week', due to the small number in the 'never' physically active group in our study (n=266). When analysing the risk of preeclampsia among those 'never' physically active versus the physically active, we surprisingly found a tendency towards a higher risk of preeclampsia among the physically active. This finding may be unrelated to physical activity and rather due to other factors common to the inactive, since this tendency was not supported in our remaining analyses, and since the 'never' active group was of such small number.

When adjusting for smoking the odds ratios were reduced in our study, i.e. the risk of preeclampsia was lowered among the physically active. A lower proportion of the women physically active (across all levels of physical activity, including those physically active 'less than once a week') were smoking, compared to those never physically active (40% versus 53%), As smoking has been shown to be associated with reduced risk of preeclampsia (75, 77, 78, 80), some of the increased risk of preeclampsia in the women physically active may be linked to there being a smaller proportion of smokers among these women.

Adjusting for body mass index had an almost negligible effect on our results; only with regard to the intensity of physical activity, a small increase in odds ratio was observed. A lack of modification by prepregnancy body mass index on the link between physical activity and preeclampsia risk has also been reported by others (50). In our study, a lower proportion of women with body mass index exceeding 30 kg/m^2 participated in high-intensity physical activity (1 % compared to 5% and 4% of women with body mass index 30+ kg/m^2, 25-29 kg/m^2 and <25 kg/m^2, respectively). However, it can be questioned whether an adjustment for body mass index should have been performed in our analyses, since physical activity might reduce the risk of preeclampsia through the reduction of body mass index.

In future studies, it would be interesting to investigate whether high levels of maximal oxygen uptake, and whether randomised pre-pregnancy physical activity reduce the risk of preeclampsia.

9 Conclusions

We found that the greatest contributor to variability in the method of assessing flow-mediated dilatation of the brachial artery was the intraobserver variability. This implies that the variability of the method can effectively be reduced by repeating image analysis.

The flow-mediated dilatation was found to be similar in the preeclampsia group and the normal pregnancy group during pregnancy. Three months after delivery, flow-mediated dilatation was lower in the preeclampsia group.

Both diastolic and systolic left ventricular functions were reduced during preeclampsia, but had normalised three months after delivery.

Physical activity prior to pregnancy was found not to reduce the risk of preeclampsia in our study.

10 References

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Paper I

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Scandinavian Cardiovascular Journal. 2008; 42: 310–315

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ORIGINAL ARTICLE

Repeated image analyses improves accuracy in assessing arterial flow-mediated dilatation

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Abstract

Objectives. A high degree of variability has been reported regarding the ultrasound-based assessment of flow-mediated dilatation. We wanted to investigate the variability and find out how it might be reduced most efficiently. *Design.* Brachial artery flow-mediated dilatation was measured by high-resolution ultrasound in 22 healthy adults on two consecutive days. Two observers analysed all images twice. The total variance was split into variance components and estimated hierarchically using the method of restricted maximum likelihood. *Results.* The relative proportional contributions from intraobserver (residual), interobserver, interpatient and interday variance components, with percentage dilatation as outcome variable, were 0.41, 0.18, 0.25, and 0.15, respectively. *Conclusions.* The major source of variability when assessing flow-mediated dilatation was found to be intraobserver variability. The simplest way to reduce total variability is for the observer to average results from repeated image analyses. We suggest that three repetitions are sufficient. This will reduce the total variance by 30%.

Key words: *Flow-mediated dilatation, variability, reproducibility, endothelial function, brachial artery, ultrasound, method*

Endothelial vasomotor function can be evaluated non-invasively by high-resolution ultrasound where the dilating capacity of a conduit artery is assessed. The brachial, radial and femoral arteries are the most common vessels examined. A sphygmanometer cuff occludes blood flow in the chosen artery for a few minutes. When the pressure of the cuff is released, a reactive hyperaemia is created, with increased flow through the vessel. This increased flow raises the shear stress against the vessel wall, acting as a stimulus for the endothelial cells to release vasoactive substances. In this way, a flow-mediated dilatation occurs. The vessel diameter is measured *before* and *after* the increase in shear stress. Impaired brachial artery flow-mediated dilatation detected by this method, has been shown to be an independent predictor of future cardiac events (1,2).

Guidelines have been developed for evaluating flow-mediated dilatation of the brachial artery (3) to enable comparison of studies assessing endothelial function. Even so, the variability of the method has been inconsistently reported in the literature, and several earlier studies have suggested high variability of the method. The aim of this study was to investigate the variability of the ultrasound-based assessment of flow-mediated dilatation, and to determine the most efficient way of reducing this variability.

Material and methods

Subjects

Twenty-two healthy adults (seven females, 15 males) with a mean age of 27 years (range 23 to 54) were

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(Received 30 October 2007; accepted 1 March 2008)

ISSN 1401-7431 print/ISSN 1651-2006 online
DOI: 10.1080/14017430802032731

recruited among university students and staff. Exclusion criteria were smoking, diabetes, known cardiovascular disease and pregnancy. All participants provided a written informed consent. The study was conducted in accordance with the Helsinki Declaration and was approved by the Regional Committee for Medical Research Ethics.

Study design

The clinical protocol has been described elsewhere (3). In brief, the subjects rested in supine position for 15 minutes before a baseline status of the left brachial artery was recorded with high resolution ultrasound. After finding a satisfactory transducer position, we marked the position on the skin with a felt tip pen which was ultrasound-gel resistant. The arm remained in the same position throughout the study. To ensure placing the probe in the same position the following day, we measured the distance from the skin mark to the distal olecranon. We occluded the artery through the inflation of a pneumatic cuff to a pressure of 250 mmHg for 5 minutes and depicted the artery again 60 s after pressure release as recommended by the International Brachial Artery Reactivity Task force (3).

In each subject, the procedure was repeated on two consecutive days, at the same hour, to control for circadian variation (4). Avoidance of food intake, coffee, vitamin C/orange juice and exercise within the last 4 hours prior to examination was required. Two observers analysed all images twice. Figure 1 shows the study design.

Image acquisition

The brachial artery was depicted in two dimensional mode with a Vivid 7 scanner (GE Vingmed Ultrasound AS, Horten, Norway). We used a handheld linear array transducer (M12L) with a frequency of 14 MHz, axial resolution of 0.1 mm and depth settings of three cm. Gain was set by visual assessment and five focus points were adjusted to cover the artery, optimising the resolution in the region of the vessel walls. We stored the images digitally as cine loops of five heart beats.

Data analysis

The images were analysed using the software analysis program EchoPAC PC (GE Vingmed Ultrasound AS, Horten, Norway) on a personal computer with a caliper resolution of 0.1 mm.

The vessel diameter was measured at baseline and 60 s after cuff release to evaluate flow-mediated dilatation. Prior to analysis, each cine loop was extracted from the examination file and saved given a random number as file name, so that the observers were unaware of subject identity, day of acquisition or whether it was a recording at baseline or in the dilated state. We measured the vessel from the anterior to the posterior media-adventitia interface (the m-lines) at the R-wave (end-diastole) of the QRS-complex of the electrocardiogram to avoid systolic variation due to compliance (5). The cine loops were scrolled to find the R-wave with the clearest view of the vessel walls. Three different measurements of the diameter were obtained along the vessel. The average of these three measurements was used in the subsequent analysis, corresponding to the lowest level of measurement in Figure 1.

Measurements and statistical analysis

Dilatation is presented in two ways: as *dilatation in millimetres* (mm), i.e. the baseline diameter subtracted from the dilated diameter; and as *percentage dilatation*, i.e. the dilatation in millimetres divided by the baseline diameter and multiplied by 100%. The brachial artery *diameters* are given in millimetres.

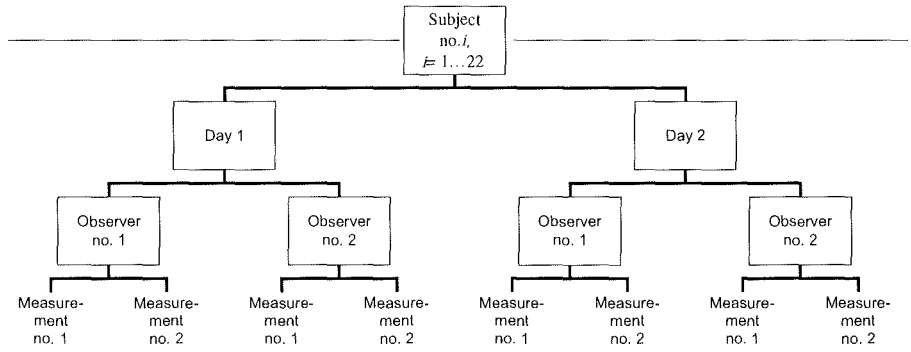

Figure 1. Design of the study. With 22 subjects, a total of $22 \times 2 \times 2 \times 2 = 176$ measurements of the outcome variables were performed. No., number.

The sources of variability were considered as follows. The experimental conditions (observer, day and subject) were considered to be independent random factors, contributing to the total variance of the outcome variables *percentage dilatation, dilatation in millimetres, baseline diameter and dilated diameter.* The resulting variance components model can be summarised as

$$\mathrm{var}(y) = \sigma^2_{subj} + \sigma^2_{day} + \sigma^2_{obs} + \sigma^2_{res}$$

where y is the outcome variable of interest, *subj* denotes subject, *day* denotes day and *obs* denotes observer. The last component, *res*, denotes the residual, or intraobserver (i.e. within observer) variance, that cannot be accounted for otherwise. The variance components were estimated hierarchically, as observer within day within patient, by the method of restricted maximum likelihood (REML), using the *nlme* package of the statistical software R, version 2.5.0 (6). The coefficient of variation for each component was calculated as the standard deviation divided by the mean of all observations of the corresponding outcome variable, multiplied by 100.

Results

For percentage dilatation as outcome variable, the proportional contribution from the variance components *intraobserver, interobserver, interday* and *interpatient* were 0.41, 0.18, 0.25 and 0.15 respectively (Table I). Note that these sum to 1.00 (or 100%). The proportional contributions from the variance components regarding *dilatation in millimetres* were similar: 0.43, 0.20, 0.29 and 0.08, respectively. For the *baseline diameter*, the corresponding figures were 0.03, 0.01, 0.05 and 0.92, and for the *dilated diameter* the figures were close to the latter; 0.03, 0.01, 0.05 and 0.91.

The coefficients of variation regarding *intraobserver, interobserver, interday* and *interpatient* percentage dilatation were 29.1%, 19.4%, 22.9% and 17.9%, respectively (Table II). With respect to the *baseline diameter*, the coefficients of variation were 2.9%, 1.5%, 3.7% and 16.6%. Similar figures were obtained for the *dilated diameter.*

Overall mean *percentage dilatation* was 16.5% with a standard deviation of 6.8 percent points. The mean *dilatation in millimetres* was 0.6 mm with a standard deviation of 0.21 mm, based on a *baseline diameter* of 3.6 mm and a *dilated diameter* of 4.2 mm, both with standard deviations of 0.6 mm. The mean difference in dilatation in percentages *between observers* was 0.22 percent points with a standard deviation of the differences of 7.75 percent points.

We found no obvious bias, nor trend, in Bland-Altman plots of our data (figures not shown) (7). Graphically, the differences between the measurements were independent of their respective mean values.

Discussion

The largest source of variability of percentage flow-mediated dilatation was *intra*observer variability, accounting for a proportion of 0.41 of the total variance (total variance defined as 1.00), (Table I).

The coefficients of variation of the percentage dilatation in this study are in the middle range of earlier reported results (Table III). We measured the diameter from the media-adventitia interfaces. In the studies presenting lower *interday* and *intra*observer coefficients of variation, the diameter was measured from the intima-lumen interfaces. Lower variability when measuring from the intima-lumen interfaces has earlier been shown by Woodman et al. (9) (Table III, *intra*observer). When considering groups using manual placement of electronic calipers for analysis (Table III), variability decreases as the number of diameter measurements per frame increases. We averaged three diameters per frame, fewer than the groups presenting lower variability than us.

The studies presenting lower coefficients of variation are characterised by the use of probes with higher frequency (>10 MHz) (*interday* and *intraobserver*) and a younger study population (*interday*) (Table III), both yielding better image quality. Lower variability by high frequency image acquisition has been shown by Herrington et al. (9) (Table III, *interday*). In this study we both used high frequency image acquisition (14 MHz) and recruited a fairly young study population (mean age 27, range 23–54).

Table I. Variance components tabulated as proportions of total variance.

	Percentage dilatation	Dilatation in millimetres	Baseline diameter	Dilated diameter
Intraobserver*	0.41 (23)	0.43 (0.024)	0.03 (0.011)	0.03 (0.013)
Interobserver	0.18 (10)	0.20 (0.011)	0.01 (0.0029)	0.01 (0.0063)
Interday	0.25 (14)	0.29 (0.016)	0.05 (0.018)	0.05 (0.022)
Interpatient	0.15 (8.6)	0.08 (0.005)	0.92 (0.37)	0.91 (0.42)
Total variance	1.00 (55)	1.00 (0.056)	1.00 (0.38)	1.00 (0.44)

Variance in absolute values in brackets. * Intraobserver variance component equals residual variance component.

Table II. Coefficients of variation.

	Percentage dilatation	Dilatation in millimetres	Baseline diameter	Dilated diameter
Intraobserver*	29.1 (25.1, 33.8)	26.9 (23.2, 31.2)	2.9 (2.5, 3.4)	2.7 (2.3, 3.1)
Interobserver	19.4 (12.1, 31.2)	18.5 (11.7, 29.2)	1.5 (0.7, 2.9)	1.9 (1.2, 2.9)
Interday	22.9 (13.3, 39.5)	22.1 (13.0, 37.5)	3.7 (2.6, 5.3)	3.5 (2.4, 5.2)
Interpatient	17.9 (7.6, 42.0)	11.8 (2.6, 53.0)	16.6 (12.8, 22.7)	15.3 (11.2, 20.9)

95% confidence intervals in brackets. * Intraobserver variance component equals residual variance component.

Automatic edge-detection software might render lower variability, due to consistency and lack of human interpretation. Woodman et al. (8) reported low intraobserver and interobserver variability by automatic analysis, whereas Herrington et al. (9), however, reported high values (Table III). The discrepancy in these cases could be due to different software solutions or differences in image quality. For interday variability, the groups presenting *lower* variability used *manual* placement of electronic calipers (Table III).

The most efficient way to reduce total variability is to reduce *intra*observer variability, being the largest contributor to total variability. *Intra*observer variability can be reduced by repeating the process of analysis and calculating the mean value of observations. Theoretically, the variance of a mean value approaches zero when the number of averaged observations tends towards infinity. However, for practical reasons, three repetitions of image analyses will suffice, as the *intra*observer thereby will become less than *inter*observer variance (in our case, the *intra*observer variance will be reduced from 23 to 7.7, compared to the *inter*observer variance of 10). The total variance will then be reduced by 30% which will make an important impact on study results.

The larger contribution of *intra*observer variability relative to *inter*observer variability may seem surprising, as most conditions are held constant in the *intra*observer setting. Note, however, that we have employed a hierarchical statistical model in which all variance components are accounted for in the same operation. As the *intra*observer variability constitutes the lowest level of the study hierarchy (Figure 1), it embraces all unknown variance components not accounted for elsewhere in the model (i.e. other than day, patient and observer). Hence, the *intra*observer variance might be nominated the *residual* variance. Common practice has been to consider each variance component separately when assessing variability. When applying this approach on our data, *intra*observer variability *does* indeed become less than *inter*observer variability (data not shown). However, such a model assumes that all variability arises from the variance component assessed, which is unreasonable; and makes it impossible to assess the relative contribution from the different variance components. Therefore we find our model more suitable. The confidence intervals for *intra-* and *inter*observer variance overlap (Table II), suggesting that they are roughly of the same magnitude. If we had used more than two observers, precision of the *inter*observer estimate might have improved.

Our results show that the measurement accuracy highly depends on whether *dilatation* (i.e. percentage dilatation or dilatation in millimetres) or *diameter* (baseline diameter or dilated diameter) is analysed (Table II). The method is quite accurate regarding *diameter*, with coefficients of variation ranging from 1.5–3.7% (Table I). (The interpatient component is disregarded, as this component does not add information about the *method's* variability). Such figures of the coefficient of variation are rather low. With regard to *dilatation* the method is less satisfactory. This is shown by the higher coefficients of variation ranging from 19 to 29%. (The interpatient component is disregarded for reasons mentioned above.) Relative to the resolution of the measurement and analysis tools, the scale of *dilatation* is much smaller than the *diameter* (six times the resolution versus 42 times, respectively). It is therefore hardly surprising that dilatation holds more variation than diameter. DeRoos et al. (8) speculate whether some of the earlier reported lower coefficients of variation might refer to baseline or maximal diameters rather than percentage dilatation. Certainly, this would result in a higher apparent accuracy. As dilatation, not diameters, represents the physiology of interest, the variability of the method should also refer to dilatation.

In our study, the interday (intrapatient) coefficient of variation of the percentage dilatation was 22%, implying that the assessment of endothelial function by high resolution ultrasound is a tool of limited value in the clinical setting. Reducing the interday (intrapatient) variance would require averaging repeated measurements over a number of days, a rather time-consuming approach. However, the method performs better as a research tool applied to patient groups. In this setting, the total variability easily can be reduced by repeating the image analysis.

Table III. Studies reporting the coefficient of variation of percentage dilatation, including our own results.

Author, year (reference)	CV	Age of Population mean ±SD or mean (range)	Number of examinations (time between examinations)	Probe freq. (MHz)	Caliper (C)/edge detection software (E)	Layer in vessel (I, M)	Number of diameter measurements	
							No. heart cycles	No. meas./frame
Interday								
Uehata et al. 1997 (11)	1.4	- - -	2 (2 w)	7.5	C*	I	3	15–20
Liang et al. 1998 (12)	10.3	44 (23–69)	2 (2.5 w)	10	- - -	- - -	3	3
Woodman et al. 2001 (8)	14.7	55±10	2 (7 d)	12	C	I	2	2
Tyldum et al.	22.9	27 (23–54)	2 (1 d)	14	C	M	1	3
Juonala et al. 2007 (13)	26.0	- - -	2 (3 mth)	13	C	- - -	1	3
Herrington et al. 2001 (9)	26.3	44.7±17.5	2 (1 w)	13	E	M	mult.	mult.
Malik et al. 2004 (14)	41.0	34±8	2 (max 10 d)	7.5	E	M	6	mult.
Herrington et al. 2001 (9)	45.3	79.3±4.8	2 (1 w)	7.5	E	M	mult.	mult.
De Roos et al. 2003 (10)	50.3	24 (18–43)	2–6 (max 16d)	7.5	C	M	- - -	- - -
Interobserver								
Hijmering et al. 2001 (15)	13.9	26±6		7.5	E	- - -	4–16	mult.
Tyldum et al.	19.4	27 (23–54)		14	C	M	1	3
Herrington et al. 2001 (9)	45	47.1±20.5		7.5	E	M	mult.	mult.
Intraobserver								
Woodman et al. 2001 (8)	6.7	- - -		12	E	I	mult.	mult.
Woodman et al. 2001 (8)	24.8	- - -		12	C	I	2	2
Tyldum et al.	29.1	27 (23–54)		14	C	M	1	3
Woodman et al. 2001 (8)	32.5	- - -		12	C	M	2	2
De Roos et al. 2003 (10)	34.0	24 (18–43)		7.5	C	M	- - -	- - -
Herrington et al. 2001 (9)	44.7	47.1±20.5		7.5	E	M	mult.	mult.

Dashed line (- - -) indicates that information was not reported in manuscript.* Uehata et al. traced the anterior and posterior intimal surfaces manually for a distance of at least 10 mm and an automated algorithm searched for the shortest distance between the two lines. CV, coefficient of variation; d, day/days; freq., frequency; I, intima-lumen interface; M, media-adventitia interface; meas., measurements; MHz, megaHertz; mth, months; mult., multiple, No., number; SD, standard deviation; w, week/weeks.

Limitations

Our results are based on a healthy population. The variability may differ when examining patient populations.

Conclusion

The major source of variability when assessing flow-mediated dilatation was found to be intraobserver variability. While interobserver variability was roughly of the same magnitude, the simplest way to reduce total variability is for the observer to average results from repeated image analyses. We suggest that three repetitions are sufficient. This will reduce the total variance by 30%.

Acknowledgements

The study was financially supported by the Co-operative Body of Central Norway Regional Health Authority and Norwegian University of Science and Technology.

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Paper II

Paper II

Maternal left ventricular and endothelial functions in preeclampsia

Running headline: Cardiac and endothelial functions in PE

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Abstract

Objective. To compare maternal left ventricular and endothelial functions in preeclampsia and normal pregnancy during pregnancy and after delivery.

Design. Observational study with follow-up.

Setting. University hospital and midwife-led antenatal care center.

Samples. Twenty untreated women with preeclampsia and twenty women with normal pregnancy, matched for gestational age and parity.

Methods. The women were examined during pregnancy and three months after delivery. Left ventricular function was assessed by echocardiography, including tissue-Doppler imaging. Endothelial function was assessed by measuring flow-mediated dilation of the brachial artery.

Main outcome measures. Left ventricular diastolic and systolic functions and endothelial-dependent flow-mediated dilation.

Results. The diastolic function was reduced in preeclampsia, with lower early diastolic mitral annular tissue velocity, e', and higher ratio of early diastolic mitral inflow velocity and early diastolic mitral annular velocity, E/e'. Early diastolic mitral inflow deceleration time and isovolumetric relaxation time were similar between the groups, suggesting pseudonormalization and increased filling pressures in preeclampsia. Peak systolic tissue velocity was lower in the preeclampsia group during pregnancy. Both diastolic and systolic left ventricular functions normalized post partum. Flow-mediated dilation was similar between the groups during pregnancy, but lower in the preeclampsia group three months after delivery.

Conclusions. The maternal left ventricular function was impaired during preeclampsia, but had normalized three months after delivery. Flow-mediated dilation was not significantly lower in the preeclampsia group as compared to the normal pregnancy group during pregnancy, but was significantly lower three months after delivery.

Keywords

Hypertension in pregnancy, preeclampsia, maternal left ventricular function, endothelial function, echocardiography.

Introduction

Women with a history of preeclampsia are at increased risk of cardiovascular disease later in life (1-4). The risk is greater after early-onset preeclampsia (3) and after preeclampsia with complications such as intrauterine growth restriction and stillbirth (4).

The underlying link between preeclampsia and cardiovascular disease is not known, but endothelial dysfunction is considered a central feature of both conditions (5-7). Preeclampsia and cardiovascular disease share the same risk factors (8), and may share a common cause, probably involving endothelial dysfunction.

It is not clear whether maternal left ventricular function is affected at the time of pregnancy in preeclampsia. Most previous studies have used conventional echocardiography in their investigations, and have focused on systolic function. The reports from these indicate maintained left ventricular performance in preeclampsia (9-13). One study using tissue-Doppler imaging suggested reduced diastolic function, but tissue-Doppler derived indices of systolic function were not reported in this study (14).

The aim of our study was to investigate whether the diastolic and the systolic left ventricular functions, measured by tissue-Doppler imaging, were impaired during pregnancy in women with untreated preeclampsia, and, whether endothelial function, measured by flow-mediated dilation, was reduced concomitantly. We also wanted to investigate whether the left ventricular and the endothelial functions were reduced three months after delivery.

Material and Methods

The study was performed in accordance with the Helsinki Declaration and approved by the Regional Committee for Medical and Health Research Ethics Mid-Norway. Written informed consent was obtained from all study subjects at study entry.

Twenty-three women with preeclampsia were enrolled at the time of admission to the Department of Obstetrics, St. Olav's University Hospital, Trondheim, Norway. The preeclamptic women were matched with 23 normotensive pregnant women, in terms of gestational age (± one week), and parity (nulliparous versus parous), from a cohort provided by midwives at the municipal antenatal care centers in the city of Trondheim, Norway. The matching was performed due to the dynamic cardiovascular changes in pregnancy and due to

the observed differences in cardiovascular function during first and subsequent pregnancies (15). Women with diabetes, hypertension, known cardiovascular disease, and multiple gestation were excluded. The examinations were carried out at enrolment and repeated three months post partum.

Preeclampsia was defined according to international standards (16), as the development of new-onset hypertension (systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mm Hg) after 20 weeks' gestation, accompanied by proteinuria ≥1+ on a semi-quantitative dipstick. Hypertension and proteinuria should be apparent on two different occasions at least four to six hours apart.

Blood pressure measurements were obtained from the right arm in the sitting position after 10 minutes' rest with a conventional mercury sphygmanometer. The diastolic pressure was recorded at the disappearance of sounds (the Korotkoff phase V). The blood pressure was measured three times, and the mean of the latter two was used in further analysis.

Echocardiography was performed according to the recommendations of the American Society of Echocardiography (17) with a Vivid 7 ultrasound scanner with a 2.5 MHz phased-array matrix transducer (M3S) (GE Vingmed Ultrasound AS, Horten, Norway). The study subjects were resting in the left lateral decubitus position during the examination. Left ventricular end-diastolic volume, end-systolic volume, and ejection fraction were calculated by the modified Simpson's rule. Mitral annular excursion was obtained from M-mode recordings. Stroke volume was calculated both from the Doppler-based velocity time integral of the left ventricular outlet tract, and from the two-dimensional recordings (end-diastolic and end-systolic volumes). Mitral annular velocities in systole (S'), and early (e') and late (A') diastole were measured by tissue-Doppler in the septal, lateral, anterior and posterior margins of the mitral annulus, and the average value from the four positions was used in the further analyses.

Endothelial function was measured by brachial artery flow-mediated dilation, as described by Corretti et al. (18). The right brachial artery was longitudinally scanned in the two-dimensional mode with the same scanner as used for the echocardiographic recordings, but this time with a linear-array transducer (M12L) with a frequency of 14 MHz. A pneumatic blood pressure cuff around the upper arm was inflated to 250 mmHg for five minutes,

inducing increased flow upon release, which stimulates the endothelium to release vasodilating substances through the influence of increased shear stress on mechanosensitive receptors in the endothelial cell membrane (19).

The images were stored at baseline, and 0.5, 1, 1.5, 2, 3, 4, and 5 minutes after cuff release, as cine loops of five heartbeats. Prior to the analyses of the brachial artery images, each cine loop was extracted from its examination file and coded, i.e. it was saved with a random number as file name, but the code was kept for re-identification after the analyses, to ensure that the observer was unaware of study subject and timing of the image. Three measurements of the diameter were obtained along the vessel, and the average of these three measurements was calculated. All images were analyzed on three separate occasions to reduce intra-observer variability, as previously described (20). The average value was used in further analysis. Both cardiac and brachial artery images were stored digitally and analyzed off-line using EchoPacPC (GE Vingmed Ultrasound AS, Horten, Norway).

The statistical analysis was performed using SPSS version 16.0 (SPSS, Inc., an IBM Company, Chicago, Illinois). Standard deviations were used as a measure of variance. The Kolmogorov-Smirnov test was used to assess the normality of the data distributions. The differences between the groups were examined by the use of the paired samples t-test, as the women were matched in pairs according to their gestational age. Supplementary analyses by the means of the independent t-test were also performed.

Results

Three women were retrospectively excluded in accordance with our study protocol, since we learnt, by checking the medical charts, that antihypertensive treatment had already been initiated at the time of our examination during pregnancy. One woman did not show up at the follow-up examination after delivery, due to long travel distance to the hospital. In our final analyses, 20 women in each group during pregnancy, and 19 women in each group three months after delivery were included.

Maternal age and height were similar in the two groups (Table 1). As expected, the systolic and the diastolic arterial pressures were higher during preeclampsia. The systolic pressure was still higher in the preeclampsia group three months after delivery. Body weight and body mass index were higher in the preeclampsia group during pregnancy, but no differences were

found between the groups three months after delivery. Mean gestational age was 35 weeks in both groups, ranging from 27 to 40 weeks. Thirteen of the pairs were nulliparous, and seven were parous. Furthermore, seven women in the preeclampsia group had early-onset preeclampsia (diagnosed <34 weeks' gestation), and 13 had late-onset preeclampsia (34+ weeks' gestation).

The early diastolic mitral annular tissue velocity (e') was lower in the preeclampsia group during pregnancy, whereas the ratio of the early diastolic mitral inflow velocity and the early diastolic mitral annular tissue velocity (E/e') was higher (Table 2, Figure 1). Both values normalized post partum. No difference in late diastolic mitral annular velocity (A') was found between the groups. Late diastolic mitral inflow velocity (A) was higher in the preeclampsia group during pregnancy, but had normalized three months after delivery. There was a tendency of higher early diastolic mitral inflow velocity (E) in the preeclampsia group during pregnancy, but the difference did not reach statistical significance. Three months after delivery, the values were similar between the groups. The ratio of early and late mitral blood flow velocity (E/A), E-deceleration time and isovolumetric relaxation time were similar between the groups, both during pregnancy and post partum.

The peak systolic mitral annular velocity (S') and the heart rate were lower in the preeclampsia group as compared to the normal pregnancy group during pregnancy, but the values were similar between the groups three months after delivery (Table 2, Figure 1). The Doppler-derived stroke volume was similar between the groups during pregnancy, but higher in the preeclampsia group post partum, while the two-dimensional-derived stroke volume was similar between the groups both during pregnancy and after delivery ($p=0.31$). The velocity time-integral of blood flow in the left ventricular outlet tract (LVOT VTI), the cardiac output, and the mitral annular excursion were similar between the groups both during pregnancy and after delivery.

The end-diastolic and the end-systolic volumes were higher in the preeclampsia group during pregnancy, but normalized post partum (Table 3). The left ventricular outlet tract diameter was similar between the groups during pregnancy, but was significantly larger in the preeclampsia group post partum. There was no difference in the interventricular septal wall thickness, the left ventricular posterior wall thickness, the left ventricular inner diameter in

end-diastole, the left ventricular inner diameter in end-systole, or in the ejection fraction between the groups.

The flow-mediated dilation of the brachial artery was not significantly lower in the preeclampsia group during pregnancy (flow-mediated dilation 1.5 minutes after cuff release: 14.9±6.1% versus 18.5±6.6%, p=0.12, standard deviation of the paired differences 9.6), but was significantly lower three months after delivery (flow-mediated dilation two minutes after cuff release: 15.5±5.2% versus 20.0±5.2%, p=0.046, standard deviation of the paired differences 7.7) (Figure 2). However, supplementary analyses by means of the independent t-test showed significant differences both during pregnancy, and after delivery (p=0.04 and p=0.02, respectively). The preeclampsia group had larger brachial arterial diameter than the normal pregnancy group during pregnancy (mean 3.9 mm versus 3.6 mm, p=0.048), but there were no differences in artery diameter between the groups post partum (mean 3.5 mm in both groups, p=0.55).

A bicuspid aortic valve was discovered in one woman, and a mitral insufficiency in another, both in the preeclampsia group. These women were referred to a cardiologist for further evaluation.

Discussion

The diastolic and the systolic left ventricular functions were impaired in the preeclampsia group during pregnancy in our study, with a post partum normalization three months after delivery. The impairment in the diastolic function was evident from the slower relaxation of the left ventricle in early diastole, shown by the lower early diastolic mitral annular tissue velocity (e') in the preeclampsia group. The ratio of the early diastolic mitral inflow velocity and the early diastolic mitral annular tissue velocity (E/e') was higher in the women with preeclampsia than in the women with normal pregnancy, but this was only partly due to the increased early diastolic mitral inflow velocity (E) in the preeclampsia group, which was only borderline significant. As the isovolumetric relaxation time and the E-deceleration time were similar between the groups, pseudonormalization was indicated. Our study thereby supports the elevated filling pressure in preeclampsia, previously suggested by Rafik Hamad et al. (14).

A relative impairment in the systolic function was found in the preeclampsia group during pregnancy, whereas no differences were found between groups after delivery. In the normal

pregnancy group a pregnancy-related increase in peak systolic mitral annular velocity (S') was found, in line with previous reports (21, 22). No such pregnancy-related increase in mitral annular peak systolic velocity was observed in the preeclampsia group. The pregnancy-related increase in systolic function in the normal pregnancy group was not confirmed by the other systolic function indices measured in our study, the ejection fraction and the mitral annular excursion. However, the peak systolic mitral annular velocity has been shown to be closer related to contractility than these indices (23).

The stroke volume and the cardiac output were similar in both the preeclampsia and the normal pregnancy groups during pregnancy. Previously, both higher (12) and lower (13) cardiac output have been reported in preeclampsia, but in these previous studies the gestational age has differed among the groups. Higher cardiac output in the preclinical phase of preeclampsia has been reported in two large longitudinal cohort studies (24,25). In one of these, a cross-over to lower cardiac output was found in week 36 in the preeclamptic group, suggested to coincide with the clinical onset of the disease (25). In our study, cardiac output was measured at the time of diagnosis, which may reflect the time of cross-over reported in this earlier study.

In our study, the stroke volume derived from the Doppler-recordings was found to be higher three months after delivery in the preeclampsia group. However, since the velocity time-integral of blood flow in the left ventricular outlet tract (LVOT VTI) was similar between the groups, this higher Doppler-derived stroke volume was due to the larger left ventricular outlet tract diameter after delivery. As the left ventricular outlet tract diameter was similar between the groups during pregnancy, we suspect that the larger diameter after delivery represents a chance finding. This is supported by the stroke volume based on two-dimensional recordings, showing no difference between the groups after delivery.

We found a tendency towards left ventricular hypertrophy (increased left ventricular wall thickness) during pregnancy in the preeclampsia group, although this was not statistically significant. Increased left ventricular wall thickness has been reported up to one year post partum after preeclampsia (12,26), suggesting a persistent hypertrophy in this group. However, our study did not confirm this. In a within-group analysis in the preeclampsia group, we found that the wall thickness was significantly decreased post partum as compared to the pregnancy-values, which rather indicates reversible hypertrophy in preeclampsia.

Although a tendency towards lower levels of flow-mediated dilation in preeclampsia was found in our study, the difference between groups was not statistically significant, in contrast to what has previously been reported (27,28). Three months after delivery, however, the flow-mediated dilation was found to be significantly lower in the preeclampsia group, in line with previous reports (29,30). In contrast to previous studies, we coded (i.e. un-identified) our images prior to analysis of flow-mediated dilation, a procedure which we consider to be a strength of our study as it ensured unbiased results. However, the coding may have yielded greater variability in our data. Furthermore, in contrast to the comparable studies referred to, we chose to explore our data by means of the paired t-test, due to the matched design of our study. The matched design was chosen as it was assumed that the hemodynamic changes within the groups throughout gestation would be greater than the differences found between the matched pairs of similar gestational age. This turned out not to be the case regarding flow-mediated dilation, where the mean standard deviation of the matched pairs was found to be larger than the standard deviation of the group means, resulting in less statistical power of the paired t-test compared to the independent t-test. In supplementary analyses by means of the independent t-test, a statistically significant lower flow-mediated dilation in the preeclampsia group during pregnancy was found also in our material, The results regarding the left ventricular function were similar both when the paired t-test and the independent t-test were applied, suggesting that the pregnancy-related changes in left ventricular function were more gestational age-dependent than the changes in the flow-mediated dilation.

In conclusion, the diastolic and the systolic left ventricular functions were impaired during pregnancy in preeclampsia, but had normalized three months after delivery. The flow-mediated dilation was not significantly lower in the preeclampsia group during pregnancy, but was found to be significantly lower in the preeclampsia group three months post partum.

Acknowledgements

We thank the midwives at the Department of Obstetrics and Gynecology, St. Olav's Hospital, especially midwife Lillian Andersskog Ekle, for helping us to recruit women with preeclampsia; and the community midwives at the maternal health centers of the City of Trondheim, especially midwife Carina Svensson, for helping us to recruit women with normal pregnancies. The study was funded by the Norwegian University of Science and Technology and the Central Norway Regional Health Authority.

Disclosure of interests

None

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Legends of Figures and Tables

Figure 1. Tissue-Doppler based indices of left ventricular function during pregnancy and post partum. (a), early diastolic mitral annular velocity; (b), ratio of early diastolic mitral inflow velocity and early diastolic mitral annular tissue velocity; and (c), peak systolic mitral annular velocity. Preeclampsia (■); normal pregnancy (▼). Means and standard deviations (whiskers) are given. *, $p<0.05$, **, $p<0.005$.

Figure 2. Flow-mediated dilation in the preeclampsia group (■) and the normal pregnancy group (▼). (a), during pregnancy; (b), post partum. FMD, flow-mediated dilation. *, $p<0.05$.

Table 1

Data given as means (standard deviations). BMI, body mass index; SBP, systolic arterial blood pressure; DBP, diastolic arterial blood pressure.

Table 2

Values given as means (standard deviations). IVRT, isovolumetric relaxation time; E, early diastolic mitral inflow velocity; E-DT, early diastolic mitral inflow deceleration time; A, late diastolic mitral inflow velocity; E/A, ratio of early diastolic and late diastolic mitral inflow velocities; e', early diastolic mitral annular tissue velocity; A', late diastolic mitral annular velocity; E/e', ratio of early diastolic mitral inflow velocity and early diastolic mitral annular tissue velocity; S', peak systolic mitral annular velocity; SV, stroke volume; CO, cardiac output; MAE, mitral annular excursion; LVOT VTI, velocity-time integral of flow in the left ventricular outlet tract; HR, heart rate; bpm, beats per minute..

Table 3

Values given as means (standard deviations). SWT, interventricular septal wall thickness; PWT, left ventricular posterior wall thickness; LVIDd, left ventricular inner diameter in end-diastole; LVIDs, left ventricular inner diameter in end-systole; LVOTd, left ventricular outlet tract diameter; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction.

Table 1. Clinical characteristics in the study groups during pregnancy and post partum.

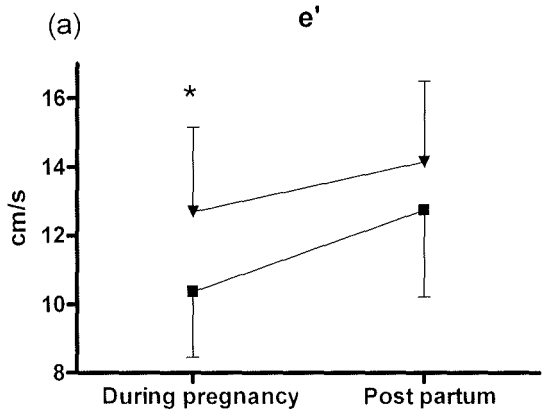
	Pregnancy				Post partum			
Characteristic	Preeclampsia (n=20)	Normal pregnancy (n=20)	95%-CI of differences	p	Preeclampsia (n=19)	Normal pregnancy (n=19)	95%-CI of differences	p
Maternal age (years)	29 (5)	27 (4)	-0.7-4.2	0.16				
Height (m)	1.69 (0.06)	1.68 (0.06)	-0.02-0.05	0.54				
Weight (kg)	93 (12)	80 (14)	2-23	0.02	77 (10)	72 (10)	-7.3-17	0.38
BMI (kg/m^2)	33 (4)	29 (4)	0.4-7.5	0.03	28 (4)	26 (3)	-2.9-7.0	0.38
SBP (mmHg)	161 (9)	116 (8)	38-51	<0.001	122 (12)	109 (7)	4.6-22	0.005
DBP (mmHg)	93 (5)	71 (8)	18-27	<0.001	75 (8)	71 (6)	-1.4-10.7	0.12

Table 2. Systolic and diastolic function parameters in the study groups during pregnancy and post partum.

	Pregnancy				Post partum			
	Preeclampsia (n=20)	Normal pregnancy (n=20)	95%-CI of differences	p	Preeclampsia (n=19)	Normal pregnancy (n=19)	95%-CI of differences	p
Diastolic function parameters								
IVRT (ms)	71 (12)	68 (14)	-5-11	0.41	79 (10)	81 (9)	-7-4	0.48
E (cm/s)	85 (18)	74 (18)	-0.01-0.2	0.08	80 (15)	75 (18)	-0.07-0.2	0.40
E-DT (ms)	173 (27)	186 (32)	-35-9	0.23	165 (30)	171 (27)	-30-18	0.62
A (cm/s)	62 (12)	51 (9)	0.04-0.2	0.005	46 (9)	46 (7)	-0.06-0.06	0.92
E/A	1.41 (0.36)	1.50 (0.37)	-0.3-0.09	0.32	1.79 (0.45)	1.67 (0.54)	-0.3-0.5	0.52
e' (cm/s)	10.4 (1.9)	12.7 (2.5)	-4-(-1)	0.005	12.8 (2.5)	14.2 (2.3)	-4-1	0.20
A' (cm/s)	9.4 (1.1)	9.3 (1.5)	-1-1	0.81	8.6 (0.9)	8.4 (1.8)	-1-1	0.79
E/e'	8.6 (1.5)	5.7 (1.0)	2-4	<0.001	6.4 (1.6)	5.5 (1.3)	-0.2-2	0.11
Systolic function parameters								
S' (cm/s)	8.5 (0.8)	9.4 (1.4)	-2-(-1)	0.03	8.7 (1.9)	8.7 (1.5)	-1-1	1.00
CO (L/min)	5.8 (1.2)	5.4 (1.4)	-0.4-1	0.37	5.5 (1.9)	4.4 (0.9)	-0.3-2	0.12
SV (mL)	76 (11)	68 (23)	-6-21	0.26	82 (18)	68 (13)	0.8-28	0.04
MAE (mm)	15.1 (1.9)	15.3 (2.2)	-1-0.7	0.63	15.5 (1.8)	15.3 (1.8)	-0.8-1.3	0.66
LVOT VTI	23.3 (3.5)	21.5 (5.7)	-1-5	0.22	22.4 (3.8)	21.8 (3.5)	-3-4	0.70
HR (bpm)	74 (9)	80 (11)	-11-(-1)	0.03	64 (11)	65 (11)	-9-7	0.83

Table 3. Echocardiographic parameters in the study groups during pregnancy and post partum.

	Pregnancy				Post partum			
	Preeclampsia (n=20)	Normal pregnancy (n=20)	95%-CI of differences	p	Preeclampsia (n=19)	Normal pregnancy (n=19)	95%-CI of differences	p
SWT (mm)	8.8 (2.0)	7.4 (1.5)	-0.2-3	0.09	6.9 (1.8)	6.6 (1.3)	-1-2	0.73
PWT (mm)	10.0 (2.5)	8.6 (2.2)	-0.8-4	0.19	8.7 (2.3)	8.0 (1.8)	-1-3	0.44
LVIDd (mm)	49 (3)	48 (3)	-2-4	0.62	47 (6)	47 (4)	-4-5	0.97
LVIDs (mm)	31 (4)	32 (4)	-4-3	0.69	32 (6)	31 (3)	-3-5	0.50
LVOTd (mm)	20.5 (1.7)	20.1 (1.5)	-0.8-2	0.44	21.5 (1.6)	19.8 (1.5)	0.4-3	0.02
EDV (mL)	95 (13)	81 (19)	0.4-26	0.04	91 (18)	80 (19)	-4-27	0.13
ESV (mL)	41 (10)	32 (7)	1-16	0.02	40 (9)	33 (9)	-3-16	0.16
EF (%)	57 (7)	60 (6)	-8-2	0.25	56 (7)	58 (5)	-7-3	0.40


(a)
e'
cm/s
16
14
12
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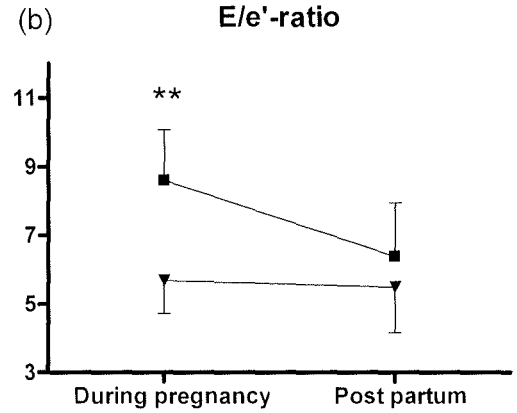
8
*
During pregnancy
Post partum


(b)
E/e'-ratio
11
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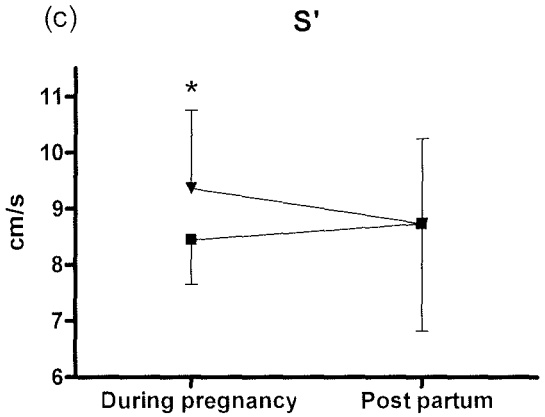
**
During pregnancy
Post partum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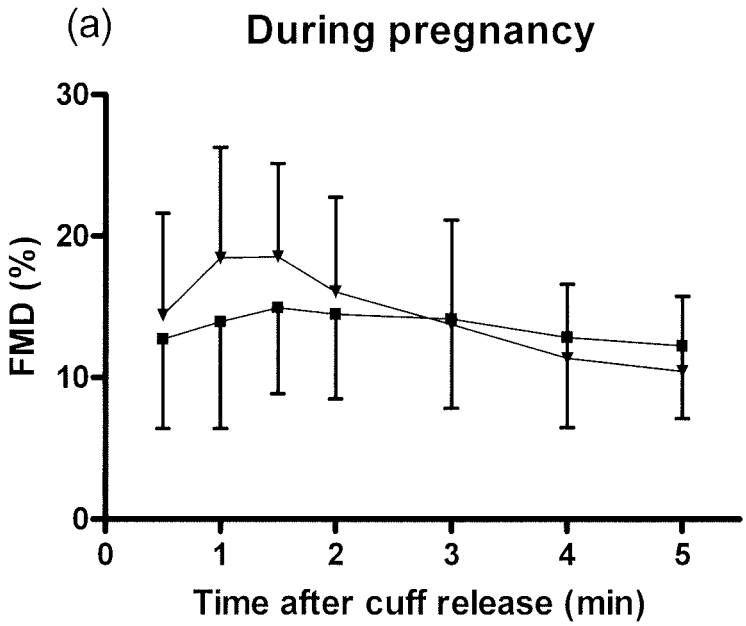
During pregnancy
Post partum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During pregnancy
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FMD (%)
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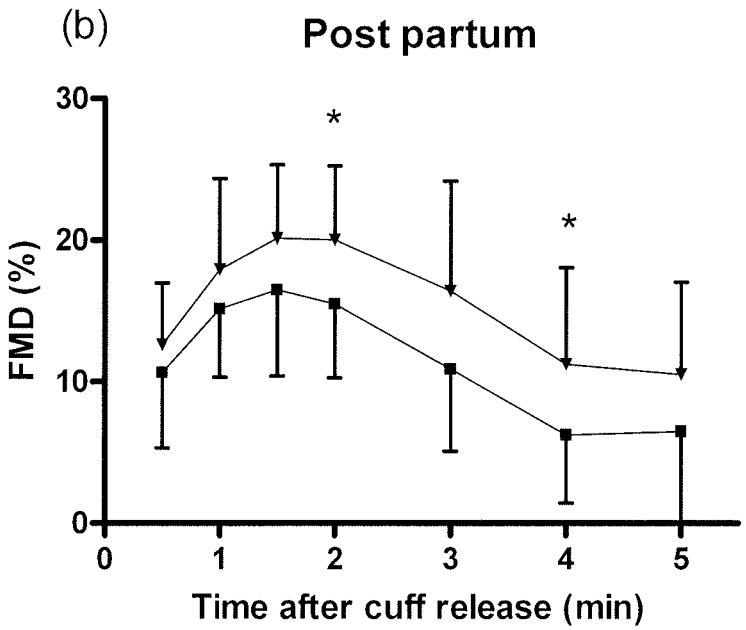
Time after cuff release (min)


(b)
Post partum
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Time after cuff release (min)

Paper III

Acta Obstetricia et Gynecologica. 2010; 89: 315–320

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MAIN RESEARCH ARTICLE

Pre-pregnancy physical activity and preeclampsia risk: a prospective population-based cohort study

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Abstract

Objective. To test the hypothesis that women physically active prior to pregnancy are at reduced risk of preeclampsia. *Design.* Population-based prospective cohort study. *Setting.* Linkage between the HUNT-1 Study (health study) and the Medical Birth Registry of Norway. *Population.* Women with singleton live births after participation in the HUNT-1 Study were included, if the newborn's gestational age was more than 22 weeks or birthweight above 500 g. Women pregnant during participation in the health study were excluded. *Methods.* The physical activity level was measured by a questionnaire. Information on the women's coming birth was provided by the Medical Birth Registry of Norway. Odds ratios of preeclampsia were calculated and adjusted for maternal age, parity, smoking and body mass index. *Main outcome measure.* Preeclampsia. *Results.* Of 3,656 women included, 167 (4.6%) developed preeclampsia. Overall, we found no link between pre-pregnancy physical activity and preeclampsia. Only among the women physically active for 120 min/week or more, a tendency for reduced risk was found (adjusted odds ratio 0.6:95% CI 0.3–1.2). *Conclusion.* Women physically active prior to pregnancy were not at reduced risk of developing preeclampsia.

Key words: *Preeclampsia, physical activity, prospective, cohort study*

Introduction

Physical activity reduces obesity (1) and insulin resistance (2), which are constitutional risk factors for preeclampsia. Furthermore, physical activity improves endothelial dysfunction (3) and reduces oxidative stress (4), both central features of preeclampsia. Cardiovascular mortality, which has been shown to be increased among women with a history of preeclampsia (5), is also reduced by physical activity (6). Against this background we asked: does pre-pregnancy physical activity prevent preeclampsia?

As the incidence of preeclampsia is low, about 3–5% of all births (7), and as the occurrence of pregnancy cannot be controlled in a research setting, randomized controlled trials addressing the effect of pre-pregnancy exercise on preeclampsia risk are difficult to perform. The prospective cohort study design represents an alternative that can overcome these challenges. A large epidemiological study was performed in Norway from 1984 to 1986, registering the level of physical activity of the inhabitants of the county of Nord-Trøndelag (the HUNT-1 Study). Linkage of these data to the Medical Birth Registry of Norway offered us an opportunity to look into the effect of pre-pregnancy physical activity on preeclampsia, through a population-based prospective cohort study. In our study we wanted to test the hypothesis that pre-pregnancy physical activity reduces the risk of preeclampsia.

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(Received 8 January 2009; accepted 23 September 2009)

ISSN 0001-6349 print/ISSN 1600-0412 online
DOI: 10.3109/00016340903370106

Material and methods

Our study is based on a linkage between a population-based health study in Norway serving as baseline study (the HUNT-1 Study) (8) and the Medical Birth Registry of Norway (9). The study was approved by the Regional Committee for Medical Research Ethics of Central Norway and the Norwegian Data Inspectorate.

The HUNT-1 Study is a population-based health study conducted in Nord-Trøndelag county, Norway (1984–1986). All inhabitants aged 20 years or more were invited to participate (n = 85,100); 43,602 of those invited were women. The study consisted of two questionnaires and a physical examination. The first questionnaire was mailed to the participants together with the study invitation. The second questionnaire, requesting information on physical activity, was handed out during the physical examination and requested to be returned by mail. The physical examination included standardized measurements of the participant's height, weight, blood pressure and heart rate, as well as a chest x-ray examination.

Through the physical activity questionnaire the participants were asked how many times per week on average they were physically active. Those active once a week or more were further asked about the average intensity and duration per session. The physical activity questionnaire is shown in Table 1. It was validated in men by Kurtze et al. in 2008 (10) reporting a strong, significant test-retest agreement (weighted kappa for frequency r = 0.80, intensity r = 0.82 and duration r = 0.69) and a moderate, significant correlation between the responses and the maximal oxygen uptake (r = 0.48). Wisløff et al. also performed a validation study of the questionnaire, including women. They found no differences by sex in the reported exercise intensity relative to the maximal oxygen uptake (11).

Table 1. Questions on physical activity in the HUNT-1 Study (with permission from the Faculty of Medicine, NTNU).

Physical activity
By physical activity we mean for example walking, skiing, swimming or exercising/participating in sport activities
How often are you physically active?
(On an average basis)
• Never
• Less than once a week
• Once a week
• 2–3 times per week
• Almost every day
If you are physically active as often as once a week or more
How hard do you push yourself?
(On an average basis)
• I take it easy, do not begin to sweat nor get short of breath
• I begin to sweat and get short of breath
• I push myself to near-exhaustion
For how long do you keep going each time?
(On an average basis)
• Less than 15 min
• 16–30 min
• 30 min–1 hour
• More than 1 hour

Among women in the age group relevant to our study (20–49 years), the participation rate in the baseline study (both responders and non-responders to the physical activity questionnaire) was 83% of the total population (16,966 of 19,586) (Figure 1). The population of Nord-Trøndelag is stable, with sex and age distribution similar to the national average. The county has, however, no large cities and the average income is somewhat lower than the mean national levels.

All deliveries of more than 16 weeks' gestation are registered in the Medical Birth Registry of Norway. The registration is mandatory and is based on a standardized form completed by midwives at the delivery units. The registry holds information on the mother's health before and during pregnancy, as well as perinatal data on the fetus. Preeclampsia is routinely entered on the form as a specified diagnosis. The diagnostic criteria for preeclampsia in Norway follow international classification systems and are defined as a sustained increase in blood pressure (systolic blood pressure of at least 140 mmHg and/or a diastolic blood pressure of at least 90 mm Hg) in a woman normotensive before 20 weeks' gestation accompanied by proteinuria of at least 1+ or more on a semi-quantitative dipstick. Hypertension and proteinuria should be apparent on two different occasions at least 4–6 hours apart (12).

The Medical Birth Registry of Norway provided information on the deliveries among the women in the baseline study (HUNT-1), i.e. the first birth after participation in the baseline study. Our study included women with singleton live births with a gestational age of more than 22 weeks or birthweight above 500 g at least nine months after the baseline study. Those pregnant at the time of participation in the baseline study were excluded. A total of 3,656 women were included.

We used logistic regression to estimate crude and adjusted odds ratios with 95% confidence intervals (95% CIs) to investigate the effect of pre-pregnancy physical activity on preeclampsia. The adjusted odds ratios were controlled for maternal age, parity, smoking and body mass index. We used SPSS for Windows, Rel. 15.0.1. 2006 (SPSS Inc., Chicago, IL) in our statistical analysis.

Women physically active never or less than once a week were used as reference group. For intensity we applied the denomination 'low' to the group

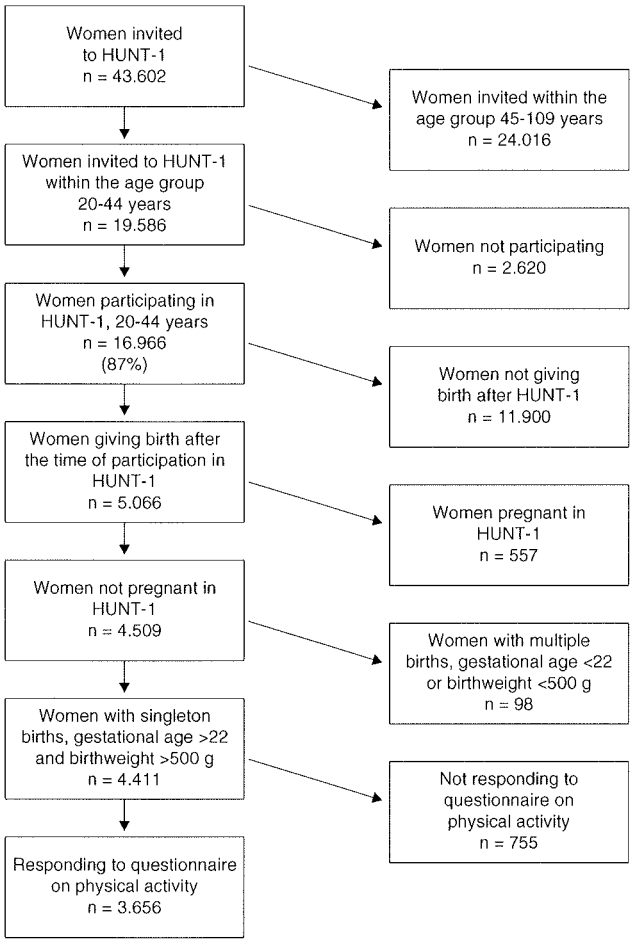

Figure 1. Flow chart of inclusion of study participants.

answering 'I take it easy, do not begin to sweat nor get short of breath', 'moderate' to the group answering 'I begin to sweat and get short of breath' and 'high' to the intensity level 'I push myself to near-exhaustion' (Table 1). We calculated the total duration of exercise per week and used this variable rather than duration per session. The total duration of physical exercise per week was calculated by multiplying the average duration per session with the average number of sessions per week.

Results

There were 167 women (4.6%: 95% CI 3.9–5.3%) who developed preeclampsia. The maternal characteristics from the baseline study in relation to preeclampsia are shown in Table 2.

Overall, we found no link between preeclampsia and physical activity (Table 3). However, the adjusted odds ratios of women active 120 min/week or more was 0.6 (95% CI 0.3–1.2). The adjusted odds ratio for any physical activity (from less than once a week to almost every day) versus none was 1.9 (95% CI 0.8–4.3).

Among the women developing preeclampsia 48.5% were nulliparous versus 35.5% of the women who did not (Table 4). Higher maternal age was associated with a higher risk of preeclampsia, whereas smoking was associated with lower risk compared to non-smoking (adjusted odds ratios of 0.6 for both current and former smokers with 95% CIs 0.3–0.9 and

Table 2. Maternal characteristics in the baseline study (HUNT-1).

Variables	Preeclampsia (n = 167)	No preeclampsia (n = 3,489)	95% CI of the differences
Age at inclusion in baseline study (years)	26.6 (4.0)	26.5 (4.1)	(–0.7 to 0.5)
Age at index birth (years)	31.0 (4.3)	30.6 (4.6)	(–1.1 to 0.3)
Body mass index (kg/m^2)	24.3 (4.3)	22.5 (3.2)	(–2.3 to –1.3)
Heart rate (beats/min)	76.7 (12.9)	74.8 (11.6)	(–3.7 to –0.0)
Systolic blood pressure (mmHg)	123.9 (12.5)	116.6 (11.1)	(–9.1 to –5.7)
Diastolic blood pressure (mmHg)	80.2 (10.4)	74.2 (9.0)	(–7.4 to –4.6)

Values are presented as mean (standard deviation).

Table 3. Association between pre-pregnancy physical activity (frequency, intensity and duration) and risk of preeclampsia.

Variables	Preeclampsia (n = 167) n (%)	No preeclampsia (n = 3,489) n (%)	Odds ratio (95% CI) Crude estimate	Adjusted estimate*
Exercise frequency				
Never/less than once a week	60 (35.9)	1,290 (37.0)	1.0 (reference)	1.0 (reference)
Once a week	51 (30.5)	1,002 (28.7)	1.1	1.1 (0.7–1.6)
2–3 times a week	43 (25.7)	891 (25.5)	1.1	0.9 (0.6–1.4)
Almost every day	13 (7.8)	302 (8.7)	1.0	0.8 (0.4–1.4)
Missing	0 (0)	4 (0.1)		
Exercise intensity				
Not exercising	60 (35.9)	1,290 (37.0)	1.0 (reference)	1.0 (reference)
Low	32 (19.2)	850 (24.4)	0.8	0.8 (0.5–1.2)
Moderate	68 (40.7)	1,193 (34.2)	1.3	1.1 (0.7–1.5)
High	5 (3.0)	109 (3.1)	1.0	0.9 (0.4–2.4)
Missing	2 (1.2)	47 (1.3)		
Exercise duration				
Not exercising	60 (35.9)	1,290 (37.0)	1.0 (reference)	1.0 (reference)
< 60 min per week	62 (37.1)	1,195 (34.3)	1.2	1.1 (0.7–1.5)
60–119 min per week	33 (19.8)	634 (18.2)	1.2	1.0 (0.6–1.5)
120+ min per week	11 (6.6)	323 (9.3)	0.8	0.6 (0.3–1.2)
Missing	1 (0.6)	47 (1.3)		

*Odds ratios adjusted for maternal age, parity, smoking and body mass index from participation in the health study to the index birth.

Table 4. Characteristics of pregnancy and birth in 167 women with preeclampsia and 3,489 without preeclampsia.

Variables	Preeclampsia n (%)	No preeclampsia n (%)
Maternal age at index birth		
20–24.9	8 (4.8)	358 (10.3)
25.0–29.9	71 (42.5)	1,354 (38.8)
30.0–34.9	55 (32.9)	1,146 (32.8)
35.0+	33 (19.8)	631 (18.1)
Parity		
Nullipara	81 (48.5)	1,298 (35.5)
Parous	86 (51.5)	2,358 (64.5)
Gestational age (weeks)		
Term (37+)	127 (76.0)	3,069 (88.0)
Preterm (34–36)	10 (6.0)	107 (3.1)
Very preterm (< 34)	12 (7.2)	57 (1.6)
Missing	18 (10.8)	256 (7.3)
Birthweight (grams)		
2500 +	134 (80.2)	3,374 (96.7)
1,500–2,499	22 (13.2)	82 (2.4)
< 1,500	10 (6.0)	31 (0.9)
Missing	1 (0.6)	2 (0.1)

0.4–0.9, respectively). We found no interaction between physical activity, age and parity with respect to risk for preeclampsia. As expected, more women in the preeclampsia group gave birth preterm, and the birthweight was also lower.

The median time from participation in the baseline study to the women's index birth was 3.2 years, with a minimum time of nine months to a maximum time of 20 years (the first birth occurring in 1984 and the final one in 2004). The odds ratios were unaffected when adjusted for the time from the baseline study to the index pregnancy. Furthermore, our results were also unchanged when re-analysing the data to include only the women who gave birth within five years from the baseline study.

The response rate among those eligible from the baseline study was 83% (Figure 1). The non-responders to the questionnaire were slightly older (26.9 vs. 26.5 years, 95% CI of the difference –0.75 to –0.10), but they had a similar body mass index (22.6 vs.

22.6 kg/m^2) and had similar systolic and diastolic blood pressure (117 vs. 117 mmHg and 75 vs. 74 mmHg, respectively) when compared to the responders.

Discussion

In contrast to what we expected, women physically active prior to pregnancy were not at reduced risk of developing preeclampsia. Overall, we found no link between physical activity and preeclampsia risk. Nevertheless, in the group of women physically active more than 120 min/week a tendency for reduced risk was detected. This group was the smallest group, numbering only 334 women.

Our findings support the results of two other cohort studies, conducted by Rudra et al. (2008) (13) and Saftlas et al. (2004) (14), where no clear link between preeclampsia risk and pre-pregnancy leisure time physical activity was found (physical activity both before and during pregnancy: adjusted odds ratio 0.76 (95% CI 0.34–1.73) and 0.71 (95% CI 0.32–1.56), respectively; and physical activity before but not during pregnancy: adjusted odds ratio of 0.73 (95% CI 0.30–1.77) and 1.12 (95% CI 0.48–2.61), respectively) (13,14). The odds ratios were not adjusted for smoking, and the women were already pregnant at the time of interview; hence, an early influence of preeclampsia on the responses cannot be disregarded. In a retrospective case-control study conducted by Sorensen et al. in 2003, a protective effect of physical activity the year before pregnancy was suggested but not found to be significant (15). Increasing intensity seemed to reduce the risk further. Since this study was retrospective, recall bias may have influenced the results (16).

As our study was prospective, bias is not likely. Misclassification in the reports from midwives and doctors to the Medical Birth Registry of Norway might, however, have occurred. The population-based nature of our study, the participation rate and the sample size strengthen the external validity of our findings. But, as only inhabitants of 20 years of age or more were invited to participate in the baseline study (HUNT-1), the results are only valid for women older than that.

The proportion of the study population developing preeclampsia (4.6%) was higher than the mean annual incidence from 1984 to 2004, both in the county of Nord-Trøndelag (3.9%) and in Norway as a whole (3.5%). The incidence among the women who did not respond to the physical activity questionnaire was 4.2%. We observed a higher maternal age in our study than that observed at national and county levels: 10.0% of the women in our study were less than 25 years of age at their index birth, compared to the national level of 24.9%. The reason for this is presumably the age limit of 20 years at inclusion, the exclusion criterion pregnancy at participation in the baseline study, and the fact that some young people move from the county for educational reasons. As the risk of preeclampsia increases with advanced maternal age (17), this could explain the increased incidence of preeclampsia.

Adjusting for smoking reduced our odds ratios by approximately 10%. This reduction was anticipated, as smoking has been shown to be associated with reduced risk for preeclampsia (18,19) and since we found a decreasing proportion of smokers with physical activity in our material. Adjusting for body mass index, however, only influenced the odds ratios regarding intensity of physical activity, where a small increase was observed. Since high pre-pregnancy body mass index has been found to be a strong predictor of preeclampsia risk (19), this increase was expected. It may be argued whether body mass index should be adjusted for or not, as theoretically, physical activity may act through this factor. Nevertheless, in our study, adjusting for body mass index had only a slight influence on the results.

We chose to combine the group 'never' physically active with the group physically active 'less than once a week' in our main analysis, due to the small number in the first group (n = 266). In a sub-analysis we looked at 'no' versus 'any' physical activity, i.e. comparing those 'never' physically active with the rest. Surprisingly, we found a tendency of lower risk among those never physically active (adjusted odds ratio of the never active 0.5, 95% CI 0.2–1.2). We speculate whether this finding might be unrelated to physical activity and rather due to some other factor common to the inactive, as our other findings did not support this tendency.

In conclusion, our study did not indicate any protective effect of pre-pregnancy physical activity on preeclampsia risk.

Acknowledgments

The authors would like to thank the Medical Birth Registry of Norway for providing information on births and the HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology NTNU), Nord-TrØndelag County Council and the Norwegian Institute of Public Health for allowing to use data from the HUNT-1 Study.

Disclosure of interest: The authors have no potential conflicts of interest to report.

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150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

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158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
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160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.

161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

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178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
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183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
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186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97

191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

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201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
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203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
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216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
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227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

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235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY

243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

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248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

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269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE

271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT
272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
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285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
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292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
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296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN

297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

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299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
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305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS

324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
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