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**CARDIAC FUNCTION IN WOMEN WITH
PREECLAMPSIA**

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MBBS PGDipEcho FANZCA

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ABSTRACT

Preeclampsia is a major cardiovascular system disease with significant short and long term sequelae. It causes considerable morbidity and mortality in women and their babies. There is a relative lack of knowledge with respect to cardiac function in women with preeclampsia and results regarding cardiac output changes are conflicting in previous studies. Therapeutic interventions used in the management of women with preeclampsia have iatrogenic complications due to this lack of cardiovascular system knowledge.

Invasive monitoring devices such as the pulmonary artery catheter which have been used to provide information about cardiac function in historical studies, have significant risks and are currently rarely used as research tools. Their use in clinical practice is limited to the intensive care setting in extremely unwell women thus restricting their clinical applicability.

Transthoracic echocardiography, however, is routinely used in cardiovascular system research in other areas of medicine. It is frequently considered the reference standard for cardiovascular system monitoring. It is a non-invasive, precise device and is validated in pregnancy. It is an ideal device for measuring the cardiac function in women with preeclampsia however is currently rarely used in this setting.

Regarding the pathophysiology of the disease, the baboon is a non-human primate in which both mechanical and pharmacological methods of inducing preeclampsia have been developed. Transthoracic echocardiography has not previously been used as a measurement tool in this setting, however its ability to measure changes serially and after interventions offers similar advantages to those in humans.

The first aim of this thesis was to determine left ventricular systolic and diastolic function and structure in women with untreated preeclampsia, in order to determine the native disease state. The second aim was to determine the applicability of transthoracic echocardiography in the baboon for serial cardiovascular system monitoring.

Using transthoracic echocardiography, four investigations were performed; cardiac function in healthy pregnant women (Human Study 1), cardiac function in women with untreated preeclampsia (Human Study 2), cardiac function in healthy pregnant baboons (Animal Study 1), and baboon cardiac function throughout a relaxin infusion (Animal Study 2).

This thesis determined left ventricular systolic and diastolic function and structure in women with untreated preeclampsia.

The most significant finding was that of increased systolic function in women with untreated preeclampsia caused by increased cardiac output and inotropy. This occurred in the presence of abnormal diastolic function, increased left ventricular mass, increased pericardial effusions, and increased afterload. This implies that there are pathophysiological processes causing both an increase in inotropy and also vasoconstriction. This new finding allows consideration of different mechanisms for the establishment and maintenance of the pathological processes in preeclampsia, and the exploration of different aetiological mechanisms.

The other findings from this work were that transthoracic echocardiography was applicable and acceptable to healthy pregnant women and to women with preeclampsia, and was able to quantify cardiac function in almost all women. This establishes an evidence base for the use of transthoracic echocardiography in pregnant women and allows more widespread introduction of clinician based transthoracic echocardiography into routine clinical practice to measure cardiac function in pregnant women.

This technology was also able to be used to measure cardiac function in baboons. It was found to be applicable and reproducible for the assessment of systolic and diastolic function and structure in baboons. The reference ranges for baboon cardiac function, and serial changes after relaxin, were determined.

DECLARATION

This is to certify that

- i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,
- ii. due acknowledgement has been made in the text to all other material used, and
- iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

The project reported in this thesis was performed through the Department of Pharmacology, University of Melbourne. The clinical studies were performed at the Department of Anaesthesia, Mercy Hospital for Women, Heidelberg, Australia and the Department of Medicine, University of Western Sydney, University of Sydney and Royal Prince Alfred Hospital, and the National Health and Medical Research Council Australian Baboon Colony, during the dates December 2007 – September 2010 while enrolled in the degree of Doctor of Philosophy, University of Melbourne. All human studies, and the transthoracic echocardiography studies in baboons, were conceived and designed by myself. The transthoracic echocardiographic studies reported in this thesis were all performed by me. This included 208 transthoracic echocardiographic examinations in 130 women, and 24 transthoracic examinations in sixteen baboons.

Alicia Therese Dennis (ATD)

September 2010

PREFACE

Work carried out in collaboration with others:

The pregnant and non-pregnant baboon comparative study (Animal Study 1) was performed in collaboration with Professor Annemarie Hennessy and Mr Scott Heffernan at Royal Prince Alfred Hospital Sydney and the Australian National Health and Medical Research Council (NHMRC) Baboon Colony.

The relaxin baboon intervention study (Animal Study 2) was performed in collaboration with Professor Kirk Conrad, University of Florida United States of America (USA), and Professor Annemarie Hennessy and Mr Scott Heffernan at the Royal Prince Alfred Hospital Sydney and the Australian NHMRC Baboon Colony.

Dr Sue Finch assisted with the statistical analysis for the first human study (Human Study 1) and statistical design of the second study. Caroline Carr, clinical research midwife assisted with recruitment of women for the second study (Human Study 2), and Professor John Ludbrook assisted with the statistical analysis of the second study (Human Study 2). Dr Ioana Arhanghelschi (IA) performed the interobserver reliability measurements for Human Study 1. Dr Julian Castro (JC) performed the interobserver reliability measurements for Human Study 2 and the two baboon studies (Animal Study 1 and Animal Study 2).

ACKNOWLEDGMENTS

This work was supported by grants from the Australian Society of Anaesthetists PhD Research Support Grant - LMA PacMed/ASA Fellowship, 2008, and Edwards Lifesciences Educational grant. SonoSite Australia provided the transthoracic echocardiography machines for both the human clinical research and the animal research. Additional resources were provided by the Department of Pharmacology, University of Melbourne, The Heart Research Institute Preeclampsia Laboratories, Sydney, Australia and the Mercy Hospital for Women, Heidelberg, Australia.

I would like to acknowledge the assistance and advice provided to me by my academic supervisors Associate Professor Colin Royse of the Anaesthesia and Pain Management Research Unit, Department of Pharmacology, University of Melbourne, and Professor Michael Permezel of the Department of Obstetrics and Gynaecology, University of Melbourne. I would like to thank them for giving me the opportunity to undertake this work.

For their guidance and support I would like to thank Professor James Angus AO, Dr Scott Simmons, Dr Ioana Arhanghelschi, Dr Julian Castro and Professor John Ludbrook.

Particular thanks goes to Caroline Carr who worked with me for nine months during the intense phase of the second human study and shared with me many of the joys and revelations of the science whilst herself being pregnant. Her pictures and echocardiographic images are included in this thesis; Professor Annemarie Hennessy who introduced me to the amazing world of the baboons and some great science, and Scott Heffernan whose instinct for anaesthesia and the care of animals was so appreciated and refreshing.

Special thanks goes to my family for their love and support; my husband Chris for his care, his understanding of what it was all about, his scientific insights and his help with the creation of the diagrams in this thesis; my two wonderful daughters Freya and Monique who now know more about preeclampsia than most of my colleagues, and for

giving me a daily reason to do this work; my mum and dad for educating me well, being wonderful role models, and instilling in me a belief that anything is possible; and to Sara and Justin, Baard and Barb, Daphne, Cam and Heidi, and Andy and Lisa for their understanding and care during this time.

Finally I would like to acknowledge and thank all the women who participated in these studies; both healthy women and extremely unwell women, for their enthusiasm, their questions, their interest and their hope for a better future for other women and their families who experience this disease.

“It’s this really weird disease. It is the sick that you get sick with when you are pregnant.”

Freya and Monique Solnordal
aged 8 and 5 in 2009 when asked about preeclampsia.

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NOMENCLATURE

Abbreviation	Definition
A4C	Apical 4 chamber
A5C	Apical 5 chamber
ACE-I	Angiotensin converting enzyme inhibitor
ANOVA	Analysis of variance
APO	Acute pulmonary oedema
ART	Assisted reproductive technology
ASA	American Society of Anesthesiologists
ASE	American Society of Echocardiography
ATP	Adenosine triphosphate
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
BSA	Body surface area
CO	Cardiac output
CWI	Cardiac work index
DBP	Diastolic blood pressure
DICOM	Digital imaging communications in medicine
ECG	Electrocardiograph

FAC	Fractional area change
FS	Fractional shortening
HELLP	Haemolysis elevated liver enzymes low platelets
HP	Healthy pregnant
HUS	Haemolytic uraemic syndrome
ICD	International classification of disease
IUGR	Intrauterine growth restriction
IVCT	Isovolumetric contraction time
IVRT	Isovolumetric relaxation time
IVST	Interventricular septum thickness
LA	Left atrium
LAD	Left atrial diameter
LAP	Left atrial pressure
LDH	Lactate dehydrogenase
LV	Left ventricle
LVEDA	Left ventricular end diastolic area
LVEDD	Left ventricular end diastolic diameter
LVEDP	Left ventricular end diastolic pressure
LVEDV	Left ventricular end diastolic volume
LVESA	Left ventricular end systolic area

LVESD	Left ventricular end systolic diameter
LVID	Left ventricular internal diameter
LVOT	Left ventricular outflow tract
MAP	Mean arterial pressure
MgSO ₄	Magnesium sulphate
M-mode	Time motion scanning mode (echocardiography)
MV	Mitral valve
MV A	Mitral valve A pulse wave Doppler (echocardiography)
MV DT	Deceleration time of the mitral valve E pulse wave Doppler waveform (echocardiography)
MV E	Mitral valve E pulse wave Doppler (echocardiography)
NHMRC	National Health and Medical Research Council
NP	Non-pregnant
PA	Pulmonary artery
PACU	Post anaesthesia care unit
PLAX	Parasternal long axis
PPV	Positive predictive value
PSAX	Parasternal short axis
PWT	Posterior wall thickness
RV	Right ventricle

sa'	Septal a' tissue Doppler wave (echocardiography)
SBP	Systolic blood pressure
SD	Standard deviation
se'	Septal e' tissue Doppler wave (echocardiography)
sENG	Soluble endoglin
sFlt1	Soluble FMS-like tyrosine kinase
SGA	Small for gestational age
ss'	Septal s' tissue Doppler wave (echocardiography)
SV	Stroke volume
SVR	Systemic vascular resistance
SWI	Stroke work index
TD	Thermodilution
TDI	Tissue Doppler indices (echocardiography)
TTE	Transthoracic echocardiography
TTP	Thrombotic thrombocytopenic purpura
UnRxP	Untreated preeclampsia
Vcf	Velocity of fibre shortening (echocardiography)
VEGF	Vascular endothelial growth factor
VTI	Velocity time integral

LIST OF PUBLICATIONS AND PRESENTATIONS

In the course of this thesis the following scientific papers, invited speaker presentations, and scientific abstracts of which the candidate is the sole author, lead author or presenter, have been generated.

Publications – journal publications and abstracts

1. Dennis AT, Castro J, Simmons SW, Carr C, Permezel M, Royse CF. Left ventricular systolic and diastolic function and structure in women with untreated preeclampsia. Accepted abstract and oral presentation. International Society of Hypertension in Pregnancy (ISSHP) Meeting, Melbourne, Australia, October 2010. Zuspan Award and Young Investigator Travel Award. in press.
2. Dennis AT, Castro J, Heffernan S, Hennessy A. Cardiac Function using Transthoracic Echocardiography in Healthy Non-Pregnant and Pregnant Baboons (*Papio hamadryas*). Accepted abstract and Poster P82. International Society of Hypertension in Pregnancy (ISSHP) Meeting, Melbourne, Australia, October 2010. in press.
3. Dennis AT Transthoracic echocardiography in obstetric anaesthesia and obstetric critical illness (Invited review). Int J Obstet Anesth - under review.
4. Dennis AT, Castro J, Simmons SW, Carr C, Permezel M, Royse CF. Left ventricular systolic and diastolic function and structure in women with untreated preeclampsia. Int J Obstet Anesth 2010; May 19(S) S11. Best Presentation of Meeting Award, Obstetric Anaesthetists Association (OAA) of United Kingdom (UK).
5. Dennis AT, Heffernan S, Hennessy A. Cardiac Function using Transthoracic Echocardiography in Healthy Non-Pregnant and Pregnant Baboons (*Papio hamadryas*). Accepted abstract and Poster 10. Society of Obstetric Anesthesiologists and Perinatologists (SOAP) Meeting, Texas, United States of America (USA), May, 2010.

6. Dennis AT, Castro J, Simmons S, Carr C, Permezel M. The Emerging Application of Transthoracic Echocardiography in Obstetric Anaesthesia and Obstetric Critical Illness. Accepted abstract and Poster 21. SOAP Meeting, Texas, USA, May 2010.
7. Dennis AT The Rapid Obstetric Screening Echocardiographic Examination. Accepted abstract, Australian and New Zealand College of Anaesthetists (ANZCA), Annual Scientific Meeting (ASM), Christchurch, New Zealand, May, 2010.
<http://www.anzca.edu.au/events/asm/asm2010/abstracts/saturday/the-rapid-obstetric-screening-echocardiography.html>
8. Dennis AT, Arhanghelschi I, Simmons SW, Royse CF. Prospective observational study of serial cardiac output by transthoracic echocardiography in healthy pregnant women undergoing elective caesarean delivery. *Int J Obstet Anesth* 2010;19:142-8.
9. Dennis AT, Walker S, Crowhurst J, Drinkwater P. Intravenous labetalol is available in Australia. *Anaesth Intensive Care* 2010 38(2): 397.
10. Dennis AT Transthoracic echocardiography and obstetric critical illness. Accepted abstract and oral presentation ANZCA ASM, Cairns, Australia 2009.
<http://www.anzca.edu.au/events/asm/asm2009/abstracts/transthoracic-echocardiography-in-obstetric.html>
11. Duley L, Henderson-Smart DJ. Magnesium sulphate versus diazepam for eclampsia. *Cochrane Database of Systematic Reviews* 2003, Issue 4. Art. No.: CD000127. DOI: 10.1002/14651858.CD000127. Comment Dennis AT 12 May 2008.
12. Obstetric Anaesthesia: Scientific Evidence Working Party 2008, ANZCA Management of Pre-eclampsia and Eclampsia.
<http://www.anzca.edu.au/fellows/sig/obstetric-anaesthesia-sig/obstetric-anaesthesia-scientific-evidence>
13. Dennis AT Preeclampsia: Issues for Anaesthetists Accepted abstract and oral presentation. ANZCA ASM, Sydney, Australia, 2008.

<http://www.anzca.edu.au/events/asm/asm2008/abstracts/preeclampsia-issues-for-anaesthetists.html>

National Teaching Workshops

Dennis A Transthoracic Echocardiography in the pregnant patient. Workshops US1 and US2, Australian Society of Anaesthetists (ASA) National Scientific Congress, Melbourne, Australia, October 2010.

Dennis A Transthoracic Echocardiography in Obstetric Anaesthesia. Workshop W39 ANZCA, ASM, Cairns, Australia, May, 2009.

http://www.anzca2009asm.com/docs/ANZCA2009_Workshop110209.pdf

Invited speaker at National/International Meetings

1. Dennis AT The Rapid Obstetric Screening Echocardiographic Examination. ANZCA ASM, Christchurch, New Zealand, May, 2010.
2. Dennis AT Preeclampsia – How, When, Where. Mercy Hospital for Women Multidisciplinary Obstetric Critical Care Meeting, Melbourne, Australia, November, 2009.
3. Dennis AT Cardiac Function in healthy term pregnant women. University of Western Sydney School of Medicine Research Colloquia, Sydney, Australia, August, 2009.
4. Dennis AT Cardiac Function in healthy term pregnant women. Heart Research Institute Sydney, Australia, August, 2009.
5. Dennis AT Transthoracic echocardiography in obstetric anaesthesia. ANZCA, Annual Scientific Meeting, Cairns, May, 2009.
6. Dennis AT Preeclampsia – Issues for Anaesthetists. ANZCA Regional Committee Canberra, Australia, September, 2008.

7. Dennis AT Preeclampsia – Issues for Anaesthetists. ANZCA, ASM, Sydney, Australia, May, 2008.

Presentations at National Meetings

1. Dennis AT Eclampsia. Collaborative Education Seminar, Melbourne, Australia, February, 2010.
2. Dennis AT Defining a new normal – Challenging our understanding of the cardiovascular system in pregnancy. ASA/New Zealand Society of Anaesthetists Combined ASM (ASA/NZSA CASM), Wellington, New Zealand, October, 2008.
3. Dennis AT Cardiovascular system function in healthy pregnant women Society of Obstetric Medicine Australia and New Zealand meeting Adelaide, October, 2008.

Research awards related to this work

1. Zuspan Award. International Society Study Hypertension in Pregnancy International Congress Melbourne, Australia, September, 2010.
2. Young Investigator Travel Award. International Society Study Hypertension in Pregnancy International Congress Melbourne, Australia, September, 2010.
3. Best scientific paper/Best Presentation of Meeting Award at Obstetric Anaesthetists Association (OAA) of United Kingdom meeting, Newcastle, United Kingdom. “Left ventricular systolic and diastolic function and structure in women with untreated preeclampsia” May, 2010.
4. Preeclampsia Research Laboratories (PEARLS) Scholar Sydney Australia 2009 – 2010 – August 2009.
5. Gilbert Troup Prize – ASA/NZSA CASM Wellington, New Zealand. “Serial transthoracic echocardiography of cardiac function in healthy term pregnant women on the day of their caesarean birth”, October 2008

Chapter 1. Introduction

1.1. Overview, hypothesis and aims

Cardiovascular or haemodynamic state is a complex interplay of left and right ventricular systolic and diastolic function, and integration with the vascular system. There is limited knowledge with respect to parameters of cardiac function in pregnancy and even less in the presence of pregnancy complications such as preeclampsia. In the latter condition, there is currently no consensus on the systolic and diastolic parameters of cardiac function and the literature is conflicting regarding whether there is increased, decreased or any change in cardiac output (CO).

Current clinical diagnostic and monitoring tools for assessing the cardiovascular system are limited in pregnant women to blood pressure, electrocardiograph (ECG), pulse oximetry and rarely invasive pulmonary artery pressure or cardiac output monitoring. The rather gross end point of cardiac output often clouds the more subtle perturbations of mild or moderate diastolic dysfunction or other parameters of systolic dysfunction. Overall, these are blunt tools in differentiating the underlying cardiac function, and may be misleading. Transthoracic echocardiography (TTE) is becoming increasingly widespread in the anaesthesia and critical care community, and has the potential to provide far greater diagnostic insight into the cardiovascular abnormality associated with preeclampsia, thereby offering improved patient care. The technology is especially applicable in pregnant women where the use of more invasive monitoring and assessments of the cardiovascular system are now rarely used due to their known risks. The characteristics of the pregnant women make the use of transthoracic echocardiography both easy and acceptable. This is however, an area in which transthoracic echocardiography is not routinely used.

Anaesthetists are frequently involved in the peripartum management of critically ill women with preeclampsia. Management of these critically ill women is hindered by a deficit in the knowledge base regarding cardiovascular performance in healthy pregnant

women and in women with preeclampsia and the absence of an effective cardiovascular assessment tool.

The implications of this lack of cardiovascular system knowledge, and also the absence of the use of transthoracic echocardiography in this setting, are that many of the therapies are empirical and have side effects related to the cardiovascular system. These include acute pulmonary oedema, cardiac failure or ongoing severe hypertension and intracerebral hemorrhage or infarction, which result in significant morbidity and mortality.

The aims of this thesis are to

- establish a robust, reliable and reproducible method of measurement using transthoracic echocardiography in the peripartum setting in pregnancy,
- assess the acceptability and applicability of transthoracic echocardiography in pregnant women in this setting,
- establish a reference range for cardiovascular system variables in healthy term pregnant women,
- investigate the differences in systolic and diastolic function in women with untreated preeclampsia who are gestationally matched with healthy pregnant women to determine native disease state, and
- apply transthoracic echocardiography technology in the setting of the baboon to investigate the same systolic and diastolic variables to enable translatable animal to human data.

Chapter 2. Literature review

2.1. Introduction

Many methods for searching and critical appraisal of the literature were performed. These included online searching through Pubmed database, Cochrane Central Library searching database and hand searches of key references. Review of work and acquisition of references from key groups in the various fields and review of recently published theses with extraction of key references was undertaken. Most references were from sources in English, however if a paper was found in another language translation was sought. Where level of evidence for intervention studies have been included the NHMRC classification has been used (NHMRC 2008).

Keywords used for searches were as follows: Pregnancy, Pre-eclampsia / preeclampsia, eclampsia, Transthoracic echocardiography, Echocardiography, Transesophageal / transoesophageal echocardiography, Obstetric anaesthesia/anesthesia, Eclampsia, Haemolysis Elevated Liver Enzymes, Low Platelets (HELLP) syndrome, Cardiac output/cardiac function/heart function/systolic/diastolic function/cardiac failure/heart failure, Animal models.

This literature review will first explore the morbidity and mortality caused by the disease, its definition, and key elements of preeclampsia, the complications of the disease and the complications of therapy. Transthoracic echocardiography as a measuring and monitoring device and research tool in healthy pregnancy and in preeclampsia is then discussed. A summary is then given of the cardiovascular system in healthy women followed by a summary of the known information about the cardiovascular system in women with preeclampsia. Finally the baboon animal preparation is discussed in relation to its use as a research modality in understanding the human disease.

2.2. Preeclampsia

2.2.1. Morbidity and mortality

Preeclampsia is a multisystem disease unique to human pregnancy characterized by hypertension and organ system derangement. The disease is responsible for considerable morbidity and mortality complicating 5 - 8% of pregnancies and remains in the top three causes of maternal morbidity and mortality globally. These morbidity and mortality implications are most marked in poor, underprivileged, remote and rural communities and indigenous populations (WHO 2004; WHO 2007a; WHO 2007b). Deaths are due to intracranial haemorrhage and cerebral infarction, acute pulmonary oedema, respiratory failure, hepatic failure or rupture, and bleeding such as may occur with disseminated intravascular coagulation or placental abruption. It is the leading cause of fetal growth restriction, intrauterine fetal demise and planned preterm birth (Khan, Wojdyla *et al.* 2006; Lewis 2007). In Australia, preeclampsia accounts for approximately 20% of maternal deaths (Sullivan, Hall *et al.* 2007). It is estimated that approximately 50,000 women die each year of preeclampsia worldwide and approximately 300,000 babies die predominantly due to premature birth in women with preeclampsia.

Despite the World Health Organization (WHO) promoting the concept of the Millennium Development Goals, Goal 5, which was to decrease maternal mortality by 75% by 2015, has barely changed. Worldwide maternal mortality has not significantly decreased in the past ten years.

As well as the immediate short term mortality and morbidity that occurs from the disease during pregnancy, there are also increased long term risks associated with preeclampsia. These include ischaemic heart disease, ischaemic stroke and chronic hypertension (Lewis 2007; Sibai 2008a; WHO 2007a; WHO 2007b). Population based studies performed in Iceland and Scotland give a relative risk of dying of ischaemic heart disease of 1.7 and a relative risk of dying due to a cerebrovascular accident of 3.6 if a woman had the diagnosis of preeclampsia (Kestenbaum, Seliger *et al.* 2003; Newstead, von Dadelszen *et al.* 2007).

Unfortunately regarding preeclampsia little is known about cardiovascular system changes, pathophysiology or aetiology thus making new treatment interventions and improvement in disease outcomes difficult. This is also coupled with a low profile status of the disease. Whilst preeclampsia is commonplace in discussions relating to pregnancy and pregnancy complications, at a broader population level it is frequently absent from government policy documents and general publications about the importance of the management of hypertension. Despite small sections regarding preeclampsia appearing in recent editions of key general circulatory journals (Maron, Towbin *et al.* 2006; Mosca, Banka *et al.* 2007) the profile of this disease is very low. In the most recent National Heart Foundation (Australia) document published in 2006 and reporting on the burden of cardiovascular disease in Australia, preeclampsia is not mentioned (Vos 2006). Similarly in the New England Journal of Medicine's 2009 Shattuck lecture "The Hypertensive Paradox" (Chobanian 2009) preeclampsia is not discussed despite hypertensive cardiac failure and hypertensive encephalopathy being responsible for a significant amount of maternal death and mortality.

2.2.2. Defining the disease

Preeclampsia has many different definitions (ACOG 2002; Brown, Hague *et al.* 2000; Chesley 1985; Geller, Ahmed *et al.* 2004; Khan, Wojdyla *et al.* 2006; Lewis 2007; RCOG 2006). Despite some differences in the detail there are features common to all of these. The most important elements are elevation of blood pressure plus the additional involvement of one or more organ systems. In addition there is a common definitional requirement for the condition to resolve at some time in the postpartum period, i.e. it is specifically a complication of pregnancy. Hence there is the rather peculiar quality of the condition that management occurs when the diagnostic criteria have not been fulfilled. This specifically distinguishes preeclampsia from chronic pre-existing hypertension and gestational hypertension (Figure 1). From a management perspective clinicians should always be alert to the possibility of alternative diagnoses. The importance of defining levels of blood pressure and other objective measures of proteinuria, biochemical abnormalities and seizures lies with guiding therapy and as an aid in decision-making regarding the timing of the birth. Ending the pregnancy and removing the placenta is currently the only definitive way of curing the condition. Despite the absence of a

biomarker and at times diagnostic uncertainty of the disease what is clear is that hypertension at or beyond a systolic blood pressure 140 mmHg or a diastolic blood pressure at or higher than 90 mmHg is abnormal in pregnancy, and is outside the normal reference range for pregnancy. Blood pressure > 160/100 mmHg is critically high and even in the absence of other organ system involvement requires immediate attention.

The disease itself is known by many different names. Terms that have been used in the past such as pregnancy induced hypertension (PIH) and pre-eclamptic toxæmia (PET) must now be considered to be outmoded. The multitude of terms for the disease, its many and varied risk factors (Duckitt and Harrington 2005), as well as imprecision regarding its definition, means that historical studies or retrospective review of cohort or case control studies require cautious interpretation.

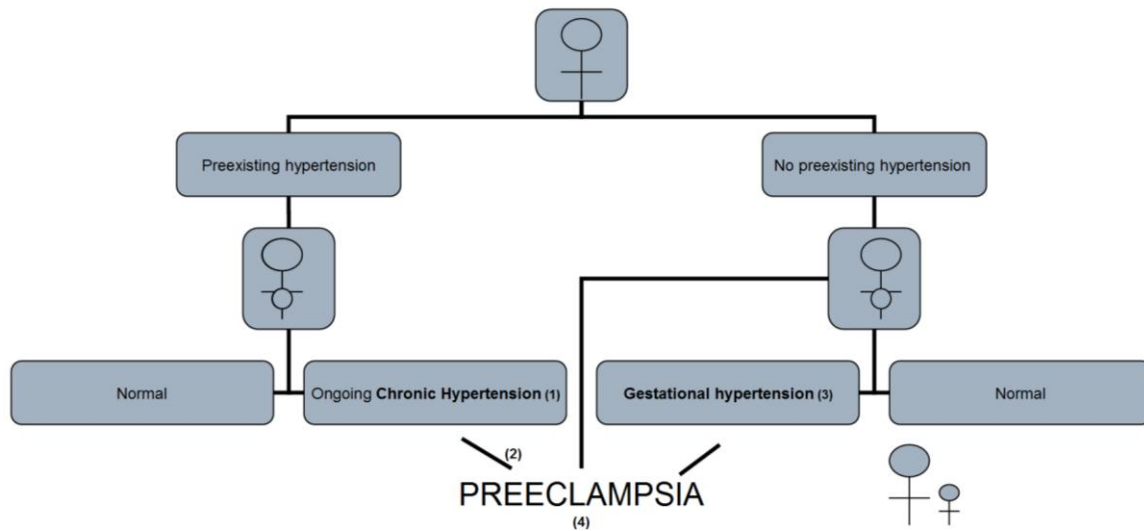


Figure 1 Classification of hypertension in pregnancy

1. chronic hypertension 2. gestational hypertension 3. preeclampsia superimposed on chronic hypertension 4. preeclampsia

2.2.3. Australasian definition

In Australian and New Zealand, the International Classification of Disease (ICD) published and regularly updated by the World Health Organization is adapted for local conditions and is referred to as ICD10 – Australian Modification (AM) (National Centre for the Classification in Health (NCCCH) 2008). This classification is used for clinical coding purposes and may be relevant from a financial and international research perspective. However, for practical clinical purposes in Australia, an appropriate definition is that of the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP) which has recently been updated (Brown, Hague *et al.* 2000; Lowe, Brown *et al.* 2009). This defines preeclampsia as hypertension arising after 20 weeks gestation with subsequent resolution of the disease by three months postpartum and with the following specific features:

- systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) (Korotkoff V) ≥ 90 mmHg, (repeated measures at rest)
plus one or more of:
- proteinuria > 0.3 g/24 hours,
- renal insufficiency,
- liver disease,
- neurological problems,
- haematological disturbances,
- fetal growth restriction.

The spot urinary protein:creatinine ratio is frequently measured and is based on the principle that the daily excretion rates of protein and creatinine are similar. Normal values are defined as ≤ 0.03 g/mmol (Cote, Brown *et al.* 2004; Leanos-Miranda, Marquez-Acosta *et al.* 2007; Price, Newall *et al.* 2005).

Clinically significant proteinuria should be suspected when urinary dipstick proteinuria is $\geq 2+$. There is insufficient information to make a recommendation about the accuracy of the urinary albumin:creatinine ratio (Rowe 2008). Serum urate levels are often elevated in

preeclampsia. Reference values are up to 0.42 mmol/l. Hyperuricaemia has an association with perinatal complications and although elevated levels have not predicted adverse maternal outcomes it is frequently measured in clinical practice (Cnossen, de Ruyter-Hanhjarvi *et al.* 2006; Rowe 2008). Terminology is often confused regarding the diagnostic criteria with some authors reporting 0.3 g protein/litre of urine rather than per 24 hours i.e. concentration rather than an excretion rate.

Severe preeclampsia is a term applied to a condition with marked elevation of blood pressure (Systolic Blood Pressure \geq 160 mmHg, Diastolic Blood Pressure \geq 110 mmHg) and/or extreme derangements of organ function. These may include central nervous system problems of seizures (eclampsia), impaired conscious state and visual disturbances, renal dysfunction (urinary protein \geq 5 g protein/24 hours) and haematological complications. Severe disease may occur at any gestation and any parity and is estimated to occur in approximately 25% of women with preeclampsia (Sibai 2004).

Haemolysis Elevated Liver enzymes Low Platelets (HELLP) syndrome is considered a variant of severe preeclampsia. The exact levels of biochemical and haematological values and criteria that are used to make the diagnosis of HELLP are debated in the literature. The most widely used classifications are those of Sibai and Martin (Martin, Blake *et al.* 1991; Sibai, Taslimi *et al.* 1986). Common to both are evidence of haemolysis as indicated by a raised lactate dehydrogenase (LDH), elevated liver transaminases, plus a platelet count of $< 100 \times 10^9/l$.

Preterm preeclampsia is often severe and associated with abnormalities of placentation and intrauterine growth restriction.

The definition is summarized in Table 1, based on various sources (ACOG 2002; Brown, Hague *et al.* 2000; RCOG 2006; Sibai, Taslimi *et al.* 1986).

Table 1 Preeclampsia – Definition

	MILD PREECLAMPSIA	SEVERE PREECLAMPSIA
Hypertension	✓	✓
OR		OR
Hypertensive crisis	nil	SBP ≥160 mmHg and/or
	nil	DBP ≥110 mmHg
	AND one or more of	AND one or more of
CENTRAL NERVOUS SYSTEM		
Seizures (eclampsia)	nil	✓
Headache	nil	✓
Visual disturbances	nil	✓
Papilloedema	nil	✓
Clonus	nil	✓
GASTROINTESTINAL SYSTEM		
Liver tenderness	nil	✓
Nausea and vomiting	nil	✓
Epigastric pain	nil	✓
Elevated Liver enzymes(AST/ALT)	≥40 iu/l	≥70 iu/l
HAEMATOLOGICAL SYSTEM		
Low Platelets (thrombocytopenia)	≤150 x 10 ⁶ /l	<100 x 10 ⁶ /l
Haemolysis (visualised on blood film, Lactate dehydrogenase or elevated total bilirubin or falling haematocrit or bleeding diathesis)	nil	✓
Disseminated Intravascular Coagulation	nil	✓
CARDIORESPIRATORY SYSTEM		
Pulmonary oedema	nil	✓
RENAL		
Proteinuria	> 0.3 g/24 hours, 1+ protein on dipstick (0.3 g/l)	> 5 g/24 hours, 3+ on dipstick
Protein/creatinine ratio	0.03 g/mmol (equiv to 0.3 g/24 hours)	> 0.5 g/mmol
Urine output	normal	< 500 ml/24 hour
UTEROFETAL		
Fetal compromise/placental compromise		nonreassuring cardiotocograph /intrauterine growth retardation with absent umbilical artery Doppler velocimetry - absent or reversed end diastolic flow

Definition of preeclampsia with divisions into mild and severe. Clinical symptoms, signs and haematological, biochemical and urinary abnormalities are grouped into colour coded organ systems. Note that severe disease can occur in the presence of non-critical hypertension (i.e. Systolic Blood Pressure (SBP) < 160 mmHg / Diastolic Blood Pressure (DBP) < 110 mmHg) if other organ systems are grossly deranged, and that proteinuria is not mandatory for defining preeclampsia. SBP = systolic blood pressure; DBP = diastolic blood pressure; AST = aspartate amino transferase; ALT = alanine amino transferase

2.2.4. Diagnostic challenge

Hypertension in pregnancy can be caused by a variety of different pathologies and it is important to consider other aetiologies. These include renal disease, acute fatty liver and cholestasis of pregnancy, haemolytic uraemic syndrome (HUS) / thrombotic thrombocytopenic purpura (TTP), phaeochromocytoma, illicit drug use (e.g. cocaine, amphetamines), and cardiovascular diseases such as coarctation of the aorta, subclavian stenosis, aortic dissection and vasculitis. Extremely rarely, preeclampsia may develop prior to the arbitrary cut-off of 20 weeks gestation in the setting of hydatidiform mole, multiple pregnancies, fetal or placental abnormalities, antiphospholipid syndrome or severe renal disease (Broekhuizen, Elejalde *et al.* 1983; Brown, Hague *et al.* 2000).

Definitions for research purposes and stratification have often been more rigid imposing strict blood pressure criteria and the mandatory presence of proteinuria.

2.2.5. Preterm versus term preeclampsia

Controversy exists regarding whether preeclampsia is more than one disease and in particular whether preterm preeclampsia (defined as that occurring prior to 34 weeks gestation) is different to term disease. Evidence for this assertion is derived from studies that have examined placental tissue and birth weight at various gestations in women with preeclampsia (Moldenhauer, Stanek *et al.* 2003). Associations have been found between low birth weight infants, placental ischaemia and preterm preeclampsia, as well as relatively normal placentas and normal or large birth weight infants in women with term preeclampsia (Vatten and Skjaerven 2004). Some of these studies have also attempted to define maternal haemodynamics early in pregnancy and to use this information in order to predict the likelihood of preeclampsia occurring. Further work is needed in this area before conclusions can be made and ideally serial studies of maternal haemodynamics throughout pregnancy are needed (Sibai 2008b; Valensise, Vasapollo *et al.* 2008). Mild disease can occur in the preterm women and fulminant severe disease may occur in term or post term women.

2.2.6. Therapeutic interventions

Pharmacological interventions in preeclampsia are aimed at reducing the complications relating to hypertension, and treating and preventing seizures. Preeclampsia occurring at term is generally managed by stabilizing the woman and early delivery of the fetus whereas preeclampsia occurring remote from term is often a more difficult situation to manage. This is due to the competing requirements of the woman and the fetus with respect to the complications of prematurity. In this situation, fetal lung maturation with steroids and methods of controlling the disease as a temporizing measure in the woman may be employed in order to allow an increased survival and reduced morbidity in the fetus. This however always needs to be closely monitored as preeclampsia will not resolve whilst the woman remains pregnant and any temporary improvement with therapy will inevitably be followed by later progression in disease severity. It is also important to be aware that conservative management may also be a threat, not only to the woman, but also to the fetus. Severe fetal hypoxia, sometimes as a consequence of placental abruption, may occur at any time during conservative management with possible fatal consequences for the fetus.

The Confidential Enquiries into Maternal and Child Health (CEMACH) 2007 report highlights the importance of the reduction of critically high blood pressure in women with preeclampsia (Lewis 2007). Of particular relevance to obstetric anaesthetists, there is a recommendation that the anaesthetist needs to be given as much time as possible to stabilize the woman (Figure 2). This is because deaths have occurred in the setting of inadequate blood pressure optimization prior to general anaesthesia for caesarean birth.

Learning points: Preeclampsia and Eclampsia

1. Fulminating preeclampsia occurs at term and post term, as well as preterm
 2. Systolic blood pressure over 160 mmHg must be treated
 3. Syntometrine should not be given for the active management of the third stage if the woman is hypertensive, or her blood pressure has not been checked
 4. The anaesthetist should be given as much time as possible to try and prevent the pressor effects of intubation in the woman with preeclampsia, even when there are pressing fetal reasons for urgent caesarean section under general anaesthesia
-

Figure 2 Learning points for Preeclampsia and Eclampsia

Adapted from Confidential Enquiry into Maternal and Child Health (Lewis 2007)

Mild to moderate hypertension in preeclampsia is treated with various interventions with the aim of preventing progression to severe hypertension, systolic blood pressure > 160 mmHg and/or diastolic blood pressure > 110 mmHg and thereby improving outcome for both the woman and the baby. Inevitably, increasing hypertension will lead to a need for increased surveillance, perhaps in hospital, and early delivery on maternal grounds, due to risks to the woman associated with severe hypertension which include intracranial haemorrhage and infarction. Whilst a direct fetal benefit of treating mild to moderate hypertension has not been shown, this is likely where gestational age is increased by treatment.

Risks of untreated severe hypertension include placental abruption, accelerated hypertension leading to hospitalization and target or end organ damage such as intracerebral haemorrhage or infarction. The fetal risks include preterm birth and intrauterine growth restriction. Severe hypertension is a medical emergency and requires rapid treatment usually with an intravenous antihypertensive agent.

In other areas of medicine it has been possible to apply a scientific or mechanistic approach to the management of hypertension (Blumenfeld and Laragh 2001; Walker and Geniton 1989). However in pregnancy the choice of antihypertensive medication is

limited and frequently based on historical usage and the associated evidence of safety for the fetus.

Non-severe hypertension

Non-severe hypertension is defined as systolic blood pressure 140-159 mmHg / diastolic blood pressure 90-109 mmHg. In a recent systematic review there were no clear differences when antihypertensive intervention was compared with placebo or with no intervention or when two antihypertensives were compared. Due to the risk of haemorrhagic stroke in the presence of systolic hypertension however, most guidelines recommend lowering of non-severe blood pressure to levels of systolic blood pressure 140-150 mmHg / diastolic blood pressure 90-100 mmHg (Martin, Thigpen *et al.* 2005). Thresholds vary depending on the existence of co-morbidities.

Agents with established evidence of fetal safety include methyldopa, labetalol and nifedipine. Atenolol is not recommended due to evidence of fetal growth restriction. Whilst other β -adrenoceptor blockers (metoprolol, pindolol, propranolol) have not yet been shown to have the same association, they are generally not used as first line antihypertensive medications in pregnancy. Angiotensin converting enzyme inhibitors (ACE-I) and angiotensin type II receptor blockers are contraindicated due to their effects on the fetal kidney (Abalos, Duley *et al.* 2007; Podymow and August 2008; Rowe 2008) (Level IV).

Severe hypertension

Severe hypertension is defined as a systolic blood pressure ≥ 160 mmHg and/or a diastolic blood pressure ≥ 110 mmHg. Based on maternal mortality reports this degree of hypertension if left untreated is associated with an increased risk of intracerebral haemorrhage (Lewis 2007). Reducing blood pressure in severe hypertension decreases the risk of death (Podymow and August 2008).

Drugs that can be safely used include labetalol, nifedipine and hydralazine. There are different formulations of each of these and they change over time including availability for different routes of administration. The choice should be made on clinician familiarity

and experience with a particular agent. Particular care should be taken to avoid precipitous falls in blood pressure which may induce maternal or fetal complications as a result of falling below critical perfusion thresholds. Blood pressure should be lowered to levels of systolic blood pressure 140-150 mmHg / diastolic blood pressure 90-100 mmHg at a rate of 10-20 mmHg every 10-20 minutes. Consideration should also be given to the extent of placental transfer of the administered drug and the direct effect of the agent and any metabolites.

There is extensive experience with the safety and efficacy of intravenous hydralazine. This is usually administered by intermittent bolus of 5 mg intravenously (IV) or intramuscularly (IM) and repeated as necessary; it has an onset of action of 10-15 minutes. A continuous infusion of 0.5-10.0 mg/hr is typically used in more refractory cases. The use of hydralazine is often accompanied by maternal tachycardia. There is an absence of robust trials comparing hydralazine with intravenous labetalol or oral nifedipine. These latter agents may be preferable due to reduced maternal and fetal complications (Magee, Cham *et al.* 2003) (Level I). Labetalol should be avoided in women with severe asthma. Drugs that should be avoided for the reduction of blood pressure are diazoxide, ketanserin, nimodipine and magnesium sulphate (MgSO₄) (Duley, Henderson-Smart *et al.* 2006). Continuous fetal heart rate monitoring should be employed until the blood pressure is stable (Rowe 2008).

Sodium nitroprusside is rarely used in pregnancy and cannot be recommended for routine use due to known adverse effects of hypotension, paradoxical bradycardia in women with severe preeclampsia and the unknown risk of fetal cyanide toxicity. It should be viewed as a last resort to be used in situations of life threatening hypertension immediately prior to delivery and in circumstances where clinicians are very familiar with its use (Podymow and August 2008).

Intravenous labetalol has recently become available in Australia and this allows another option of the acute reduction of severe hypertension in preeclampsia. Intravenous labetalol is a competitive non-selective β - adrenoceptor antagonist and a competitive α_1 -

adrenoceptor antagonist. It also has membrane stabilising properties at higher doses. It is $\sim 10 \times$ more potent for β than α receptors, $\sim 10 \times$ less potent than propranolol for the β receptor and $\sim 10 \times$ less potent than phentolamine for the α receptor. Its actions address the elevated systemic vascular resistance problem at a local level and block the reflex tachycardia associated with reduction of vasoconstriction. It does not result in fetal compromise or result in decreased uteroplacental blood flow. Labetalol may cause hypotension, bradycardia and hypoglycaemia in the neonate but no adverse long term sequelae have been reported.

The first reports of labetalol were in the 1970 (Aggerbeck, Guellaen *et al.* 1978; Prichard and Richards 1979) however until recently intravenous labetalol has not been available in Australia. Thus the safest choice of intravenous agents was limited primarily to hydralazine. Previously full dose diazoxide was associated with hyperglycaemia, arresting of labour and hypotension requiring treatment. However mini dose diazoxide has recently been shown to be safer than the previously used full dose (Hennessy, Thornton *et al.* 2007). Other agents such as sodium nitroprusside, glyceryl trinitrate, clonidine and prazosin have been used however their side effect profile inhibits their routine use (ANZCA(ScientificEvidenceWorkingParty) 2008; Duley, Henderson-Smart *et al.* 2006). Refractory hypertension has often been difficult to treat safely as the combination of two safe agents has not been available.

In the United Kingdom and United States of America, intravenous labetalol has been available for many years and is becoming the first line drug for the treatment of severe hypertension in pregnancy. This is due to its reduced side effect profile with less maternal hypotension and tachycardia when compared with hydralazine and other agents (Podymow and August 2008). Neither labetalol nor hydralazine however has been shown to be the obvious drug of first choice as reported in a recent Cochrane systematic review (Duley, Henderson-Smart *et al.* 2006).

In the correct setting and with the appropriate monitoring of the hypertensive woman and fetus, labetalol is given as an intravenous bolus dose of 20 mg with repeated doses of 20

mg every 10 minutes to a maximum of 160 mg. It may be run as an infusion of 20 mg/hr increasing every 20 minutes by 20 mg/hr to a maximum of 160 mg/hr. End points would be a reduction in blood pressure to the desired level i.e. systolic blood pressure 140-150 mmHg / diastolic blood pressure 90-100 mmHg. It is contraindicated in women with asthma or cardiac failure. The maternal blood pressure should be regularly monitored and so too should the fetal heart rate. Conversion to oral labetalol is possible after stabilization (Ashe, Moodley *et al.* 1987; Harper and Murnaghan 1991; Mabie, Gonzalez *et al.* 1987).

The availability of intravenous labetalol in Australia is a major step forward in the treatment of hypertensive emergencies in women with preeclampsia, thereby giving the clinician the choice of two safe rapidly acting agents, which may be used separately or in combination to treat women with severe hypertension in pregnancy.

Magnesium sulphate

Magnesium sulphate was first reported to be of benefit in women with preeclampsia in the early 1920's (Lazard 1925; Lazard 1933) and subsequently has been shown to reduce the chance of the first seizure in women with preeclampsia and also to reduce morbidity and mortality when used as treatment of seizures in preeclampsia (Duley, Gulmezoglu *et al.* 2010; Duley, Gulmezoglu *et al.* 2003; Duley and Henderson-Smart 2003a; Duley and Henderson-Smart 2003b). It is estimated that the incidence of seizures in women who have severe preeclampsia and are untreated is approximately 3-4%, whilst those receiving prophylaxis with magnesium sulphate is approximately 1%. Therefore prophylaxis is not 100% effective and some women receiving prophylaxis will still have seizures. Despite its widespread use in high infrastructure countries, especially after the publication of the Magnesium sulphate for the Prevention of Eclampsia (MAGPIE) trial in 2002 (Altman, Carroli *et al.* 2002), the mechanism of action is unknown and it is associated with some significant side effects, cost and infrastructure implications. In low infrastructure countries, there is often a lack of support services for safe administration of magnesium sulphate and thus may be unsafe to administer despite its morbidity and mortality advantages. This has led some groups to search for an alternative agent to magnesium sulphate and Belfort and colleagues present an argument for the use of labetalol in this

setting (Belfort, Tooke-Miller *et al.* 2002). Their group is currently undertaking a multicentre randomized control trial comparing magnesium sulphate with labetalol for the prevention of eclampsia (LAMPET trial) (Belfort 2008).

Previously antihypertensive agents have been administered to reduce the blood pressure and have not been specifically investigated to measure their effect in the prevention and treatment of seizures i.e. antihypertensives have been used to reduce the blood pressure, magnesium sulphate has been used to treat and prevent seizures. There is wide variation in treatment of severe preeclampsia throughout the world with some groups having a low threshold for administering magnesium sulphate whereas other groups preferentially use antihypertensive medication and rarely administer magnesium sulphate. As with most interventions, the greatest benefits are seen with the safe observation of women and fetal surveillance antenatally, early detection of the disease and multiprofessional involvement. The provision of infrastructure such as clean water, sanitation and vaccination is absolutely paramount. Belfort and colleagues argue that there is a need for a safe alternative to magnesium sulphate especially in low infrastructure countries (Belfort, Clark *et al.* 2006).

Treatment of seizures

Of the interventions that have been examined, magnesium sulphate is the pharmacological agent that is the treatment of choice for treatment of eclampsia (Duley and Henderson-Smart 2003a; Duley and Henderson-Smart 2003b). Magnesium sulphate reduces mortality when compared with diazepam (Duley and Henderson-Smart 2003a), (Level I). Magnesium sulphate is superior to diazepam, phenytoin and lytic cocktail (chlorpromazine, promethazine, pethidine) in reducing significantly the risk of seizure recurrence (Duley, Gulmezoglu *et al.* 2010; Duley and Henderson-Smart 2003a; Duley and Henderson-Smart 2003b) (Level I). Morbidity related to pneumonia, mechanical ventilation and admission to an intensive care unit are significantly reduced with the use of magnesium sulphate compared with phenytoin (Duley and Henderson-Smart 2003a; Duley and Henderson-Smart 2003b) (Level I). Both IV and IM routes of administration have been used effectively. The regimen used in the Collaborative Eclampsia Trial which is the largest randomized controlled trial (RCT) in this area was magnesium sulphate 4 g

or 5 g IV over 5 minutes, followed by an infusion of 1 g/hr for 24 hr. If recurrent seizures occurred magnesium sulphate 2 g IV was given (CollaborativeEclampsiaTrialGroup 1995).

Prevention of seizures

Magnesium sulphate is recommended as prophylaxis for eclampsia in women with severe preeclampsia (Duley, Gulmezoglu *et al.* 2003). Compared with placebo or no treatment, the use of magnesium sulphate more than halved the risk of eclampsia and the number needed to treat (NNT) to prevent one seizure in this group of women was 50. Magnesium sulphate was also advantageous in reducing the first seizure when compared with other agents.

There is controversy regarding the use of magnesium sulphate in mild (non-severe) disease. The NNT was approximately 100 and side effects were more common in the magnesium sulphate group, although none were life threatening (Duley, Gulmezoglu *et al.* 2003). There was also an increase in the caesarean births in the magnesium sulphate group.

When magnesium sulphate was selectively administered only to women with severe preeclampsia instead of to all women with gestational hypertension, there were more women with eclampsia who then required general anaesthesia and experienced adverse neonatal outcomes compared with their historical control (Alexander, McIntire *et al.* 2006) (Level III-2).

Mechanism of action

Magnesium is a metallo-coenzyme and is involved in many enzymatic reactions including those involved in the formation and utilization of adenosine triphosphate (ATP). Magnesium may also alter ion channels, N-methyl-D-aspartate (NMDA) receptors and calcium metabolism. Its effect on cerebral vasculature is unclear and there may be differences in its effect in pregnant women with mild versus severe preeclampsia. Simultaneous but proportionately greater systemic blood pressure reduction with reliable cerebral vasodilation may be the reasons why magnesium sulphate decreases risk of

seizures. Also it may reduce cerebral perfusion pressure (CPP) and decrease the risk of cerebral barotrauma. At a cellular level magnesium sulphate is known to reduce intracellular calcium concentration (Touyz, Laurant *et al.* 1998). Little is known about the cardiac effects of magnesium sulphate. It has been used for many years in the treatment of torsades de pointes associated with long QT syndrome. Magnesium is also a weak calcium channel antagonist that regulates calcium influx through the NMDA receptor in neuronal tissue and may inhibit ischaemic neuronal damage caused by anion flux (Lyall and Belfort 2007).

Clinical practice issues related to magnesium sulphate

There are several clinical practice points that have been identified:

1. Magnesium sulphate does not reverse or prevent the progression of the disease, nor does it significantly lower blood pressure and it is not recommended as an antihypertensive agent (Podymow and August 2008; Rowe 2008).
2. Patient safety and clinical effectiveness are enhanced when hospitals, health centres, and emergency transport vehicles have guidelines for the safe use of magnesium sulphate (Lewis 2007).
3. Monitoring of magnesium sulphate should utilise clinical parameters of urinary output, respiratory rate, oxygen saturation and patellar reflexes. Serum magnesium levels should be measured if toxicity is suspected and is most apparent with levels of > 3.5 mmol/l. Features of toxicity include suppression or loss of patellar reflexes, respiratory depression, and drowsiness and ultimately loss of consciousness. Complications may be significant and may require mechanical ventilation (McDonnell, Muchatuta *et al.* 2010). Toxicity is particularly likely in the presence of significant renal insufficiency. The drug treatment for magnesium sulphate toxicity is 10% calcium gluconate (1 g) 10 ml over 10 minutes.
4. The haemodynamic effects of magnesium sulphate given by slow intravenous bolus are a reduction in peripheral vascular resistance by approximately 20 – 35% thus causing a slight reduction in blood pressure and an increase in heart rate. The approximate drop in blood pressure is 10 mmHg with a compensatory increase in cardiac output.

2.2.7. Complications of therapies

Some of the current therapies for treatment of the disease such as magnesium sulphate, fluid therapy and antihypertensive treatment can have unpredictable haemodynamic effects such as acute pulmonary oedema (APO) which can further cause complications (Christiansen and Collins 2006). Preterm birth as a treatment for the disease is also associated with significant complications for the neonate. The medical uncertainty and unpredictability of therapies at times is contributed to by a fundamental lack of data regarding the cardiovascular system function in these women and lack of standardized non-invasive monitoring to record key data. The issues of acute pulmonary oedema and hypertensive cardiac failure are particularly pertinent to the obstetric anaesthetist.

Acute pulmonary oedema is a leading cause of death in women with preeclampsia (Duley, Williams *et al.* 1999; Ganzevoort, Rep *et al.* 2005) and is a frequent cause for admission to an intensive care unit (Sriram and Robertson 2008). Pulmonary oedema may occur in up to 2.9% of women with preeclampsia with only 30% of cases occurring prior to birth (Norwitz, Hsu *et al.* 2002). In addition to the usual goals of management of stabilization of the woman and expediting resolution of the acute pulmonary oedema, consideration needs to be given to delivery of the fetus if acute pulmonary oedema occurs in the antenatal period.

The mechanism for acute pulmonary oedema is poorly understood in preeclampsia, however it may be similar to heart failure that occurs in other medical disease unrelated to pregnancy and associated with severe increases in blood pressure (Lip, Felmeden *et al.* 2000). Acute pulmonary oedema may therefore represent a form of hypertensive cardiac failure (Kindermann, Reil *et al.* 2008). There is also the alteration in fluid balance and pulmonary artery and venous pressures (Starling's forces) in the pulmonary vasculature frequently associated with hypoproteinuria. Various authors have observed changes in the cardiovascular system in women with acute pulmonary oedema. The cardiac function shows significant heterogeneity from women in profound global cardiac failure with low cardiac output to some women experiencing diastolic cardiac changes (Benedetti, Cotton

et al. 1980; Benedetti, Kates *et al.* 1985; Desai, Moodley *et al.* 1996; Sibai, Mabie *et al.* 1987).

The usual investigations and monitoring techniques should be employed. Treatment follows similar practices to those employed in the non-obstetric population. Oxygen saturation monitoring and oxygen supplementation either via non-invasive ventilation devices or intubation and ventilation are used depending on the severity of the respiratory compromise. Ventilation strategies incorporating the known cardiorespiratory and metabolic changes of pregnancy need to be considered when ventilating a pregnant or recently pregnant woman. Intravenous frusemide (bolus 20 - 40 mg over two minutes) is used to promote diuresis, with repeated doses of 40 - 60 mg after approximately 30 minutes if there is inadequate diuretic response (maximum dose 120 mg/hour). Intravenous morphine 2-5 mg, fluid restriction and strict fluid balance and positioning such that the head is elevated and antenatal uterine displacement is maintained, should also be used (Barton and Sibai 1992; Sciscione, Ivester *et al.* 2003).

Intravenous fluid therapy in preeclampsia may be associated with acute pulmonary oedema. There is however no evidence available to specifically guide therapy so as to reduce the incidence and severity of this complication. However the appropriate use of intravenous fluids in terms of both fluid type and quantity may influence morbidity and mortality. In observational studies the use of either crystalloid or colloid solutions has been associated with transient improvements in maternal cardiovascular system parameters. However in one large trial (Ganzevoort, Rep *et al.* 2005) and a systematic review (Duley, Williams *et al.* 1999), volume expansion demonstrated no advantages over no plasma volume expansion in women with preeclampsia.

The use of intravenous fluids to increase plasma volume or treat oliguria in a woman with normal renal function and stable serum creatinine levels cannot be recommended (Duley, Williams *et al.* 1999). Oliguria in the postpartum period is multifactorial and in the presence of normal renal and respiratory function requires no treatment. The use of frusemide or low dose dopamine for the management of oliguria in a woman with normal renal function is not recommended (Steyn and Steyn 2007) (Level I).

2.3. Transthoracic echocardiography

Transthoracic echocardiography is used throughout medicine as a research and diagnostic tool. Transthoracic echocardiography utilizes the properties of sound waves as a form of kinetic energy to determine the structure and function of the heart and great vessels. The frequency of the sound waves emitted and received by the unit are in the ultrasound range. The ultrasound waves are reflected from the human body in a similar way to an audible echo reflecting from a large surface such as a canyon wall. Thus the term echo reflecting from the cardiac structures and generating a picture is incorporated into the description of the device (*echo cardio graphy*).

The device measures and records distances and times as the fundamental units and this enables calculations such as area, velocity, and flow. Other fundamental properties of sound waves are employed such as the Doppler shift enabling velocity to be calculated (Coman 2005). Pressure within the heart is not directly measured however the relationship between pressure and velocity is described by the modified Bernoulli equation, in which the pressure gradient is approximated to four times the square of the velocity (Yoganathan, Cape *et al.* 1988). Invasive monitoring devices are rarely used as research tools or in clinical practice due to their risks (Harvey, Young *et al.* 2006). Doppler echocardiography, as an alternative, has been compared with invasive devices such as cardiac catheterization for estimating pulmonary artery pressure, left atrial pressure and left ventricular pressure, and has been found to be an accurate measure of this physiology. It has also been compared to other techniques for determining cardiac output and stroke volume including thermodilution, electromagnetic and roller pump methods and has been found to be accurate (Anderson 2007).

Regarding pregnancy, the cardiovascular system can be monitored in a variety of ways. These range from non-invasive techniques (clinical assessment using simple observation, blood pressure assessment, heart rate monitoring, electrocardiography, respiratory rate, temperature, pulse oximetry, urine output) and minimally invasive techniques (intra-arterial blood pressure measurement, cardiac output assessment) to highly invasive

techniques (central venous pressure measurements using a central venous catheter or pulmonary artery catheter, and transoesophageal echocardiography). Except in the situation of critically ill women, the use of invasive devices is uncommon in current clinical practice due to the high risks associated with their use (bleeding, perforation, infection, death). Other authors have summarized the advantages and disadvantages of these invasive, minimally and non-invasive monitors in the setting of pregnancy, obstetric anaesthesia and the non-pregnant population (Cholley and Payen 2005; Dyer, Piercy *et al.* 2008; Jhanji, Dawson *et al.* 2008; Kager, Dekker *et al.* 2009; Langesaeter 2008; Langesaeter, Rosseland *et al.* 2008; Laupland and Bands 2002).

Most of these monitors are described in comparison to transthoracic echocardiography, as transthoracic echocardiography is frequently regarded as the reference standard for cardiovascular system diagnosis, monitoring and research purposes. It is a valid, precise and reproducible measurement device in research studies providing information not only about cardiac output, which the perioperative literature is currently focusing on, but also on other measurements of systolic function, and diastolic, structural and functional information. It has been validated for use in pregnancy (Easterling, Carlson *et al.* 1990; Easterling, Watts *et al.* 1987; Robson, Boys *et al.* 1988; Robson, Dunlop *et al.* 1987c; Robson, Murray *et al.* 1988). Its main disadvantage is that of operator dependence and the significant amount of additional training that is required to perform the precise technique. In the clinical setting it is not a continuous monitoring technique but rather provides diagnostic snapshots at discrete time intervals. Technical and diagnostic errors may be overcome by precise training in the technique, accurate patient positioning, and uniform measurements with optimal images (Klimczak 2008).

Transthoracic echocardiography has many advantages in the pregnant woman. It is a non-invasive and highly precise device. Its ability to not only derive volumetric and flow data from the device but also its ability to actually demonstrate the functioning heart with advanced graphical images makes it a powerful tool. Advances in device portability, ease of use, connectivity and data storage, in combination with reduced size, weight and increased durability and battery life, enable these devices to be used in a range of settings. These include the birthing suite, emergency department, intraoperatively, in the post-

anaesthetic care unit (PACU), in intensive care situations, patient transport vehicles, and in remote and rural settings. These monitors provide qualitative and quantitative data comprising left and right ventricular systolic and diastolic function, ventricular filling and contractility information. Transthoracic echocardiography gives the clinician who asks the clinical question the ability to answer that question at the point of patient care.

The pregnant woman has unique characteristics that facilitate transthoracic echocardiography examination. Anterior and left displacement of the heart combined with an elevated diaphragm and partial left lateral tilt means that the pregnant woman is the ideal subject for parasternal and apical transthoracic echocardiography views. In the intraoperative setting with no sedation, spontaneous ventilation, left lateral tilt and a thoracic sensory block with regional anaesthesia, the transthoracic echocardiography examination is sensation free with minimum lung interference. Most pregnant women are accepting of and knowledgeable about ultrasound. Ultrasound has an excellent safety profile and is a non-invasive device with fetal ultrasound routinely used in pregnancy and birth. As many acute obstetric critical illnesses occur on the background of normal physiology, pregnant women can act as their own healthy reference if a routine transthoracic echocardiography is performed during pregnancy or preoperatively. Additionally transthoracic echocardiography is advantageous in the situations of advanced maternal age or preexisting cardiac disease with cardiac disease now one of the leading causes of maternal mortality (Lewis 2007). Transthoracic echocardiography can be used in obese women as image quality especially in the parasternal view is minimally affected by this body habitus. The ability to use these devices in diverse settings including rural and remote areas also creates the opportunity for the linking of the immediate point of care clinical observations with teaching, training and education in these settings.

There are case reports highlighting the usefulness of transthoracic echocardiography in the obstetric setting (Ferguson, Paech *et al.* 2006; Filipovic, Seeberger *et al.* 2000; Okutomi, Saito *et al.* 2005), key groups investigating the use of transthoracic echocardiography in pregnancy and providing validation of transthoracic echocardiography in pregnancy and preeclampsia (Easterling, Watts *et al.* 1987; Robson,

Dunlop *et al.* 1987c) and descriptions of a technique for assessing the cardiovascular system in critically ill obstetric patients (Belfort, Rokey *et al.* 1994). Despite these advantages and reports of usage, this technology is not widely used in obstetric anaesthesia or obstetric critical care. With obstetric critical illness often occurring in an unpredictable, life threatening fashion, the application of a focused or screening transthoracic echocardiography examination in these settings would be advantageous. Transthoracic echocardiography is beginning to be used in other critical care areas (Salem, Vallee *et al.* 2008) with examples being the focused assessment with sonography for trauma (FAST) for detection of free intra-abdominal fluid in emergency medicine, the focus assessed transthoracic echocardiography (FATE) for cardiopulmonary monitoring in intensive care (Jensen, Sloth *et al.* 2004) and the haemodynamic echocardiographic assessment in real time scan (HART scan) (Royse 2008; Royse, Seah *et al.* 2006; Veltman 2007). These techniques can be taught to and performed by clinicians and paramedical staff. These examinations do not replace the formal quantitative transthoracic echocardiography examinations, which can still be performed in an elective setting at a later time.

Both quantitative and qualitative data can be obtained from transthoracic echocardiography and Table 2 shows the range of information that is obtainable using modern transthoracic echocardiography devices (Cheitlin, Armstrong *et al.* 2003; Nagueh, Appleton *et al.* 2009). There are recommendations about both the appropriateness of the use of echocardiography in clinical circumstances and its use in research (Cheitlin, Alpert *et al.* 1997; Cheitlin, Armstrong *et al.* 2003; Douglas, Khandheria *et al.* 2007; Nagueh, Appleton *et al.* 2009; Salem, Vallee *et al.* 2008). There are published guidelines about how to perform calculations and measurements using echocardiography in order to create uniformity and maintain quality of studies, and also the important issues of device safety, bioeffects and cleaning and infection control (Anderson 2007; Baumgartner, Hung *et al.* 2009; Cerqueira, Weissman *et al.* 2002; Cheitlin, Armstrong *et al.* 2003; Corriere, Hoyle *et al.* ; Douglas, Khandheria *et al.* 2007; Gottdiener, Bednarz *et al.* 2004; Lang, Bierig *et al.* 2005; Price, Via *et al.* 2008; Quinones, Otto *et al.* 2002; Schiller, Shah *et al.* 1989).

In addition to these capabilities, the new modality of tissue Doppler recording has been introduced. This is a reliable and reproducible measurement of the movement of the ventricular wall during systole and diastole. It records the systolic velocity (s') and the diastolic velocities (e' and a') (Bess, Khan *et al.* 2006; Ho and Solomon 2006; Salem, Vallee *et al.* 2008; Sturgess, Marwick *et al.* 2007; Yu, Sanderson *et al.* 2007).

A large amount of information has been published regarding the normal systolic function in particular cardiac output, in healthy pregnant women using various invasive and non-invasive devices. There are a wide variety of methodologies used for the different studies and the overall results show a wide range of cardiac outputs with large variability within each of the studies for cardiac output. Despite their high risks, studies of healthy women using invasive devices have been performed (Groenendijk, Trimbos *et al.* 1984). The invasive nature of the devices, however, may lead to the potential confounding issue that these devices may artificially raise cardiac output by increasing anxiety, causing pain or causing arrhythmias and lead to inaccurate results and altered basal resting states (Clark, Cotton *et al.* 1989; Young and Johanson 2001).

Reference values for the adult non-pregnant population of transthoracic echocardiography parameters are frequently limited to small studies. Regarding reference values for healthy pregnant women the data are limited and regarding reference values for women with preeclampsia the data are very sparse. These issues will be addressed in the next two sections.

Table 2 Doppler Echocardiography capabilities in the adult patient

Transthoracic echocardiography					
	M-mode	Two Dimensional imaging	Spectral (Pulse wave and continuous) Doppler	Tissue Doppler	Colour Doppler
<i>Structural</i>					
Chamber size	++++	++++	-	-	-
Wall thickness	++++	+++	-	-	-
Relation of chambers	+	++++	-	-	-
Early closure of MV	++++	+	-	-	-
Systolic anterior motion of MV	++++	+++	-	-	-
LV mass	++++	++++	-	-	-
LV masses (tumour, clot, vegetation)	+	+++	-	-	-
Masses in atria and right ventricle	+	++	-	-	-
Anatomic valvular pathology	++	++++	-	-	-
Septal defects	+	++++	++	-	++++
Pericardial effusion	++	++++	-	-	-
<i>Functional</i>					
Global LV systolic function	++	++++	++	++	-
Regional wall motion	+	+++	-	-	-
Severity of valve stenosis	+	++	++++	-	+++
Severity of valve regurgitation	+	+	+++	-	+++
Site of right to left, left to right shunt	-	+++	+++	-	++++
		together			together
RV and PA systolic pressure	-	-	++++	-	-
LV filling pressure	-	-	++	+++	-
Stroke volume and cardiac output	+++	++	+++	-	-
	together		together		
LV diastolic function	+	+	+++	+++	-
Identify ischaemia and viable myocardium with exercise or pharmacological stress	-	+++	-	+	-
Diseases of the aorta	-	++	-	-	++
Prosthetic valve evaluation	-	++	++++	-	+++

++++ indicates most useful; + least useful; - not useful; LV = left ventricular; MV = mitral valve; RV = right ventricular; PA = pulmonary artery

Adapted from ((Cheitlin, Alpert *et al.* 1997).

2.4. The cardiovascular system in healthy pregnant women

2.4.1. Cardiac function in healthy pregnancy

Cardiovascular system changes that occur during pregnancy can be broadly divided into the four categories: the effects of circulating hormones; mechanical pressure of the enlarging uteroplacental fetal unit; increasing metabolic demands of the uteroplacental fetal unit, and the presence of the uteroplacental circulation. Cardiac murmurs, mitral and tricuspid regurgitation and small pericardial effusions have been reported in pregnancy and are asymptomatic.

Studies have been designed to attempt to answer the two fundamental questions of what are the normal cardiovascular system changes that occur in pregnancy and what is their longitudinal relationship to the developing fetus and gestation.

Non-invasive assessments of cardiac output during pregnancy using transthoracic (Doppler) echocardiography have been studied. Transthoracic echocardiography is a preferred technique due to its relative ease of use, high quality and range of data. and its safety profile and accuracy (Augoustides, Hosalkar *et al.* 2005; Ferguson, Paech *et al.* 2006; Filipovic, Seeberger *et al.* 2000; Larsen, Torp *et al.* 2007; Okutomi, Saito *et al.* 2005).

Doppler echocardiography has been validated against other methods of determining cardiac output such as Fick, dye dilution, thermodilution and electromagnetic flow probe techniques and has been found to be reproducible and accurate (Coats 1990). Validity studies of cardiac output have been performed in pregnancy comparing thermodilution and dye dilution with Doppler echocardiography, and Doppler echocardiography has been found to be an acceptable measurement of cardiac output in pregnancy (Easterling, Carlson *et al.* 1990; Easterling, Watts *et al.* 1987; Robson, Dunlop *et al.* 1987c)

Regarding systolic function, the range of normal cardiac output in the literature in healthy term pregnant women is approximately 5 l/min to 8 l/min with the peak of cardiac output being achieved at a range of different gestations in different studies (28 weeks to 38

weeks). Some studies report a reduction in cardiac output from second trimester (Atkins, Watt *et al.* 1981; Chestnut 2004). There is general agreement that cardiac output increases during early pregnancy, however the precise mechanism of increase in cardiac output is controversial. There is a tendency for an increased cardiac output in second trimester compared with first and third trimester, mainly due to an increase in heart rate which increases shortly after conception. This is thought to most likely be mediated by the corpus luteum and is related to increasing circulating levels of oestrogens or vasodilatory peptides and factors such as calcitonin-gene-related peptide and nitric oxide. There are also observed changes in blood pressure, blood volume and systemic vascular resistance. Some authors have reported a reduction in both systolic and diastolic function near term. Systolic function, as measured by the septal Doppler indices of the septal s' velocity was significantly reduced to 6.7 cm/s compared with early pregnancy values. It would appear that normal pregnancy at term is associated with a mild impairment of systolic and diastolic function (Mone, Sanders *et al.* 1996; Zentner 2006; Zentner, du Plessis *et al.* 2009). Some of the reasons for the disparity in reports of cardiac outputs between studies are discussed below and when attempting to design an ideal study these aspects need to be considered.

Despite an increase in cardiac output during pregnancy, there is not an increase in blood pressure. This is due to a reduction in systemic vascular resistance during pregnancy. This reduction is attributable to blood flow through the low resistance region of the uterine intervillous space acting in a similar way to a shunt. There is also consideration that receptor down regulation of the α and β adrenoceptors occur in pregnancy and that prostacyclin mediates the increase in regional blood flow. There is considerable debate about the relative effects of the sympathetic nervous system and the parasympathetic nervous system in pregnancy (Hughes, Levinson *et al.* 2002), however most studies investigating the sympathetic nervous system report increases in heart rate.

2.4.2. Methodological considerations

The study design

Studies are frequently divided into single occasion during pregnancy (cross-sectional studies) or serial or longitudinal studies on the same women. Some study designs include non-pregnant controls. The benefit of longitudinal or serial studies on the same women throughout pregnancy is that the women act as their own control and temporal changes are more easily detectable (Mabie, DiSessa *et al.* 1994). Serial studies are however more difficult to perform and complete data sets are often not obtained for each patient.

A review (van Oppen, Stigter *et al.* 1996) summarized the data of cardiac output in pregnancy up until 1994. Cardiac output data from the reviewed cross-sectional and serial studies show a wide degree of variability. A wide variety of techniques are used including invasive and non-invasive methods, varying gestations and different maternal positions employed during the studies which makes pooling of data and generalization about cardiac function very difficult.

The statistical elements of many of the studies are not clearly defined. Sample sizes are rarely calculated and often the sample sizes are relatively small (e.g. less than 30 women). A recent study (Desai, Moodley *et al.* 2004) examined 35 healthy (defined as normotensive with a singleton pregnancy) pregnant women using transthoracic echocardiography. Only fourteen women of this group were studied at term (37-40 weeks gestation) and only ten were serially followed during second/third trimester. The average cardiac output in the fourteen women at term was 6.9 l/min with a standard deviation of 1.8 l/min.

Table 3 and Table 4 summarise systolic, diastolic and structural data obtained using transthoracic echocardiography in healthy pregnant women in the lateral position during the third trimester.

Table 3 Cardiac output in healthy pregnant women using Doppler echocardiography

Author	Cardiac output (l/min)	Number of study participants
Easterling (1987)	6.6 ± 1.8*	18
Robson (1987)	7.6 ± 0.13	15
Robson (1987a)	6.99 ± 0.18	15
Robson (1989)	7.2 ± 0.09	30
Robson (1989b)	7.22 ± 0.11	13
Vered (1991)	6.5 ± 1.5 *	15
Nisell (1992)	6.1 ± 0.3	16
Sorensen (1992)	8.1 ± 1.8*	23
Thomsen (1993)	7.53 (median)	40
Duvekot (1993)	5.78 (median)	10
Mabie (1994)	8.7 ± 1.4	18
Simmons (2002)	4.2 ± 0.9^	44
Desai (2004)	6.9 ± 1.8*	33
Zentner (2006)	5.7 (4.7 - 6.3) ⁺	32
Bamfo (2007a)	7.3 ± 1.2*	17

Mean ± standard error of the mean (SEM)

*standard deviation instead of SEM

⁺ median (interquartile range)

[#] median (5th and 95th percentiles)

[^]cardiac index

Table adapted from (Bamfo, Kametas *et al.* 2007a; van Oppen, Stigter *et al.* 1996; Zentner, du Plessis *et al.* 2009). Studies in the third trimester only. Participants in the lateral position.

Table 4 Diastolic function and cardiac structure in healthy pregnant women using Doppler echocardiography

Author	Measured diastolic variables	Value obtained	Number of study participants
Simmons (2002)	LV mass (g)	136 ± 33	44
	MV E (cm/s)	77 ± 15	
	MV A (cm/s)	55 ± 10	
	MV DT (ms)	193 ± 33	
	MV IVRT (ms)	72 ± 16	
Zentner (2006)	LV mass (g)	139 (119 -168)	32
	MV E (cm/s)	84 (72 – 100)	
	MV A (cm/s)	57 (44 – 72)	
	MV E/A	1.4 (1.1 - 01.8)	
	MV DT (ms)	198 (175 – 240)	
	Septal e' (cm/s)	9.0 (7.8 – 10.6)	
	Septal a' (cm/s)	6.5 (5.7 – 7.4)	
	Lateral e' (cm/s)	11.4 (9.6 – 13.0)	
	Lateral a' (cm/s)	5.4 (4.6 – 7.4)	
Bamfo (2007a)	MV E (cm/s)	72 ± 11	17
	MV A (cm/s)	55 ± 9	
	MV E/A	1.4 ± 0.3	
	Septal e' (cm/s)	12.4 ± 3.2	
	Septal a' (cm/s)	10.0 ± 2.1	
	Lateral e' (cm/s)	16.5 ± 3.5	
	Lateral a' (cm/s)	8.4 ± 2.3	
	Tei index	0.37 ± 0.11	
	MV E/septal e'	6.0 ± 1.6	

Studies in the third trimester only. Participants in the lateral position.

LV = left ventricular; MV = mitral valve; DT = deceleration time; IVRT = isovolumetric relaxation time;

Tei index = myocardial performance index; Mean ± standard deviation; Median (interquartile range)

Table adapted from (Bamfo, Kametas *et al.* 2007a; Simmons, Gillin *et al.* 2002; Zentner 2006).

These studies have served as the basis for assumptions about the cardiovascular system in healthy pregnant women (Atkins, Watt *et al.* 1981; Droste, Sorensen *et al.* 1992; Duvekot, Cheriex *et al.* 1993; Mabie, DiSessa *et al.* 1994; Nisell, Carlstrom *et al.* 1992; Sorensen, Easterling *et al.* 1992; Thomsen, Fogh-Andersen *et al.* 1993; Van Hook, Gill *et al.* 1993; Vered, Poler *et al.* 1991). More recent studies have shown some differing results with a reduction in systolic and diastolic function in the third trimester and at term in healthy women (Bamfo, Kametas *et al.* 2008; Bamfo, Kametas *et al.* 2007a; Bamfo, Kametas *et al.* 2007b; Schannwell, Schoebel *et al.* 2000; Zentner, du Plessis *et al.* 2009). Whilst these studies show the ranges of cardiac output obtained in the third trimester, few studies report values of cardiac output beyond 37 weeks gestation, at the time of birth or immediately postpartum. Few studies report complete data sets for systolic, diastolic and structural changes in healthy pregnant women. There is particularly a paucity of data from pregnant women at term.

The patient characteristics and homogeneity

Cardiac function and especially cardiac output can be influenced by many physiological (body temperature, fasting status, body mass index, resting state, gestation, height, weight, parity), pharmacological (vasoactive medications including recreational substances i.e. nicotine), pathological (preexisting cardiac, endocrine, respiratory disease) and environmental conditions. In pregnancy it is also important to consider the influence of the fetus (gestation, parity) and the influence of the uterus (presence of fibroids, vascular abnormalities) and placenta (placenta praevia, accrete, increta) as these may also influence cardiac output and cardiac function. When examining cardiac function in the peripartum period, there are limited but important studies in this area. There are four studies by Robson and colleagues (Robson, Boys *et al.* 1992; Robson, Hunter *et al.* 1989b; Robson, Hunter *et al.* 1987; Robson, Samsoon *et al.* 1993). Three of these studies examined changes in cardiac output during regional anaesthesia using transthoracic echocardiography and the fourth study examined longitudinally cardiac output until 38 weeks gestation and two, six, twelve and 24 weeks postpartum. In addition work by this group has provided important information about serial changes in cardiac output and changes during anaesthesia (Robson, Boys *et al.* 1989; Robson, Dunlop *et al.* 1987a; Robson, Dunlop *et al.* 1989a; Robson, Dunlop *et al.* 1987b; Robson, Dunlop *et al.* 1989b;

Robson, Dunlop *et al.* 1987c; Robson, Hunter *et al.* 1991; Robson, Murray *et al.* 1988; Robson, Redfern *et al.* 1992; Robson, Richley *et al.* 1992).

The study conditions

The achievement of resting basal state and appropriate positioning in order to reduce the risk of aortocaval compression is very important for accurate assessment of cardiac output. Inability to obtain the basal resting state with minimal time for rest prior to the study commencing has been a significant methodological problem in many previous studies. Lower heart rates reflected in studies where the participants had specified periods of rest prior to commencement of the examination may indicate adequate rest periods and the achieving of true basal resting states (Zentner 2006). Fundamental to examining cardiac output in pregnancy is the need to avoid aortocaval compression and supine hypotension (Kiefer, Ploppa *et al.* 2003; Kinsella 2003). It is important that studies in this area specify the postural condition and minimise this complication of mid to late pregnancy. Studies on the day of birth allow for the assessment of cardiac output to be correlated with fetal weight, and studies examining cardiac output immediately antepartum, intrapartum and postpartum are important to assess changes in the cardiovascular system that occur in the peripartum period. Robson and colleagues have performed studies at this time which remain the key studies in this area.

Cardiovascular system responses to postural changes during pregnancy have demonstrated hypotension due to compression of the aorta and vena cava by the gravid uterus (Bieniarz, Yoshida *et al.* 1969; Kerr, Scott *et al.* 1964; Kinsella 2003). Right or left lateral positions compared to supine positions have demonstrated variability in subjective and objective responses (Clark, Cotton *et al.* 1991; Danilenko-Dixon, Tefft *et al.* 1996). Hypotension in the presence of neuraxial anaesthesia may be particularly marked and prevention and treatment of this may involve postural changes although studies specifically examining this intervention are small (Bamber and Dresner 2003; Cyna, Andrew *et al.* 2006). One study of sixteen women assessed with transthoracic echocardiography during the mid-third trimester demonstrated no significant changes in cardiac output between supine and standing positions (Del Bene, Barletta *et al.* 2001). Other small studies have compared the supine and lateral positions or the left lateral and

erect positions (Dyer, Anthony *et al.* 2004). Though variable, the magnitude and direction of cardiac output can be altered by posture; therefore vigilance is required by both anaesthetists and pregnant women to change posture with the onset of symptoms or changes in blood pressure or heart rate.

No studies have been identified that examine the effects of changing posture serially from left lateral level to left lateral 10° head-up, then left lateral 20° head-down positions on cardiac output in healthy pregnant women.

The method of measurement and measurement technique

The importance of the measurement device accuracy and repeatability has been the subject of many papers. Attention to study design detail and minimization of biases and confounding variables is important. This is a complex area of medicine which is still evolving as evidenced by the development of the Cochrane Collaboration Systematic Reviews of Diagnostic Test Accuracy in addition to Systematic Reviews of Intervention Studies and levels of evidence for diagnostic test accuracy studies (CEBM 2009). There are robust discussions in the medical literature regarding how best to compare measurement devices. The key issue is that of study methodology including statistical methodology, the use of clear and concise language and the accurate and precise reporting of what has actually been measured (Bland and Altman 1986; Ludbrook 2002).

Devices need to be accurate, reliable and give repeatable results. Regarding cardiac output assessment in pregnancy, cross sectional area measurement in combination with Doppler measurement of velocity is reproducible, validated and reliable in pregnancy. Other modalities such as M-mode measurements during dynamic situation of birth, and impedance cardiography have not been validated in pregnancy.

The important aspects of the calculation of cardiac output by transthoracic echocardiography are where and when the two components of the cardiac output are measured. Both measurements must be temporally and spatially related such as occurs with the left ventricular outflow tract (LVOT) during systole from the parasternal long axis view (PLAX) + LVOT velocity time integral (VTI) during systole from the apical

five chamber view. The final measurement is averaged over at least three consecutive heart beats and the method of tracing the velocity time integral should be by tracing the outer aspect of the velocity profile from an image that minimizes the Doppler interrogation angle i.e. $< 20^\circ$.

Methods of measuring diastolic function are relatively new compared with measurements of systolic function. Historically the focus in the literature has been systolic function with an emphasis on the ejection and forward flow functioning of the heart. More recently it has become apparent that diastolic function of the heart is important for complete functioning of the heart, thus methods have been established to measure this aspect of cardiac performance.

The importance and meaning of the intraobserver and interobserver measurements

Crucial to accurately reporting data from measurement studies are the answering of two important questions: do measurements from Observer A match measurements from Observer B, and how consistent or reliable is the primary observer in measuring the characteristic of a group under constant conditions? Addressing these two questions and obtaining numbers for agreement and reliability quantifies the accuracy of the data and enables the studies to be repeated and results confirmed (Myles and Cui 2007).

Ideally two or more observers measure the same characteristic of all the subjects and then an evaluation is made of how close each of their measurements are to each other. This is the interobserver reliability. Limits of agreement, or how well the two observers' measurements agree with each other, are generated.

The second question is an example of intraobserver reliability and is a measure of the consistency and reliability of the primary observer. Few studies in the area of cardiac output assessment in pregnancy address these issues completely. Almost all the studies use a single operator and interobserver reliability is rarely reported (Bland and Altman 1995).

Studies in the peripartum period

Transthoracic echocardiography studies of cardiac function in the immediate peripartum period are extremely limited and have often included only unwell women. Data relating to term pregnant women especially on the day of birth is small. There are limited studies of the normal cardiovascular changes that occur in healthy women during labour and delivery, and studies during caesarean section are also few. The main studies in this area have been performed by Robson and colleagues who examined cardiac output by Doppler and cross sectional area at the aortic valve. Their studies were examining the haemodynamic effects of single shot spinal injection and continuous catheter techniques and epidural anaesthesia for caesarean section. Patients had a baseline cardiac output measurement in the operating room and then measurements of cardiac output after intravenous fluid, and either spinal or epidural neuraxial blockade at ten and fifteen minutes. All patients received an ephedrine infusion. Mean cardiac output prior to interventions in these 32 women was 6.5 l/min for the epidural group and 6.7 l/min for the spinal group. Subsequent studies by this group examined changes in cardiac output during caesarean birth (Robson, Boys *et al.* 1992; Robson, Dunlop *et al.* 1989b; Robson, Hunter *et al.* 1989a; Robson, Samssoon *et al.* 1993) and also the effect of labour on haemodynamics (Robson, Dunlop *et al.* 1987a). There are no studies examining diastolic function in the immediate pre, intra or postpartum periods.

2.4.3. Summary of cardiovascular system changes in healthy pregnancy

There is significant heterogeneity amongst cardiac function studies in healthy pregnant women. From the literature there is wide variation in cardiac output throughout pregnancy. It is not possible to generate the ideal cardiac output vs. gestation graph. There is evidence that systolic function may deteriorate towards term. There is limited information regarding diastolic function in normal pregnancy but evidence supports a reduction in diastolic function at term. Cardiac function beyond 38 weeks gestation and at the time of birth has rarely been reported. Reference ranges for transthoracic echocardiography parameters in pregnancy are rarely reported in any textbook of echocardiography.

2.5. The cardiovascular system in women with preeclampsia

As can be seen from the previous section there is a paucity of general information about systolic and diastolic function in healthy pregnant women and many transthoracic echocardiography parameters are still to be quantified for this population group. It is however imperative to understand both the healthy pregnant women and the women with preeclampsia with respect to their cardiovascular system function as many interventions used in the management of preeclampsia have consequences either directly or indirectly with regard to the cardiovascular system.

Many different interventions are used to manage women with preeclampsia. Currently knowledge relates to historical use of drugs and perceived effects without actually measuring with modern equipment the effect on the cardiovascular system. In particular it is not known whether there are changes in systemic vascular resistance (SVR) or cardiac output from a baseline understanding of the cardiac output and systemic vascular resistance changes in women with untreated preeclampsia. The use of intravenous fluids is a contentious issue with a diversity of opinion regarding the appropriateness of intravenous fluids. There is debate about the appropriateness of one form of antihypertensive agent over another, and the cardiovascular effects of magnesium sulphate. There are cardiovascular system complications of preeclampsia and there are complications of therapeutic interventions to treat preeclampsia. The aim of a clinician in the management of women with preeclampsia is to maximise the beneficial interventions whilst minimizing complications related to the disease and the therapies. Inappropriate intravenous therapies can lead to the iatrogenic complication of acute pulmonary oedema. A reduction in uterine artery blood flow compromising the fetus can occur with antihypertensive medications. Systolic cardiac failure can occur in some women with severe preeclampsia. It is therefore important to define the native disease state in women with untreated preeclampsia in order to better manage therapeutic interventions. The following section outlines what is currently known regarding the mechanism of hypertension in preeclampsia and cardiac function and structure in women with preeclampsia.

2.5.1. Mechanisms of hypertension in preeclampsia

Hypertension defined as systolic blood pressure (SBP) \geq 140 mmHg and/or diastolic blood pressure (DBP) \geq 90 mmHg is the hallmark of preeclampsia. Blood pressure is dependent upon cardiac output and systemic vascular resistance. In the clinical situation and in the research setting regarding preeclampsia, blood pressure and cardiac output are measured, and the systemic vascular resistance is the calculated value. When considerations are given to mechanism of hypertension, increases and decreases in both cardiac output and systemic vascular resistance must be examined and theoretical aspects need to be confirmed by rigid clinical observations.

Cardiac output itself is controlled by heart rate and stroke volume changes. Vascular tone is mediated by three main mechanisms: direct innervation by the sympathetic nervous system; control by circulating mediators, and control by local mediators. In addition the system is linked through appropriate venous return to the heart with matching of venous return to cardiac output elegantly explained by Guyton (Guyton, Lindsey *et al.* 1955). In attempting to discuss the aetiology of preeclampsia it is also important to consider the issue of the primary event versus compensatory mechanisms that occur after disease initiation. This is a common source of confusion regarding this disease as the observations made in the disease may be the result of the primary event or events that cause the disease, or alternatively compensatory events that aim to restore homeostasis. For example, changes in circulating mediators, and local tissue changes including inflammation, may be a secondary reaction to the primary disease process. Finally when making observations about effects on blood pressure of particular interventions it is also important to understand the primary event as well as the compensatory cardiovascular system event. This is particularly important in the case of systemic vasodilating agents which will often cause a reflex tachycardia and increase in stroke volume which do not significantly reduce overall blood pressure.

The initial events and aetiology of preeclampsia have been extensively reviewed by other authors (Feinberg 2006; LaMarca, Alexander *et al.* 2008; LaMarca, Gilbert *et al.* 2008; Lyall and Belfort 2007; Widmer, Villar *et al.* 2007). Table 5 summarizes the proposed

mechanisms of hypertension in preeclampsia. As blood pressure and therefore high blood pressure/hypertension, is determined by cardiac output and systemic vascular resistance, Table 5 is divided into these broad mechanisms. Table 6 translates the laboratory substances thought to be involved in the aetiology of preeclampsia into the Cochrane systematic reviews of randomised control trials that have measured the effect of these substances as interventions for preventing preeclampsia. Table 7 summarizes the regional vascular bed changes that are thought to occur in women with preeclampsia. The definitive treatment of preeclampsia is termination of the pregnancy and removal of the placenta.

The aetiology of preeclampsia is not well understood. It appears that one of the initiating events is abnormal trophoblastic invasion, the cause of which remains unknown, with an absence of the normally occurring spiral vessel remodeling, known as pseudovasculogenesis, in the placenta. This leads to reduced perfusion and ischaemia of the placental and to placental hypoxia. It is thought that the pathophysiology of preeclampsia involves an increase in maternal systemic vasoconstrictive substances released from the ischaemic/perfusion reduced, uteroplacental unit.

Circulatory factors in combination with locally released mediators generate hypertension in the woman. There is also evidence from some animal preparations that indicate that placental ischaemia leads to the production of vasoactive substances that both cause vasoconstriction and inhibition of vasodilation leading to hypertension. Similar preparations in non-pregnant animals do not lead to hypertension.

Widespread endothelial dysfunction affecting vascular beds throughout the body occurs in women with preeclampsia. The clinical manifestations of preeclampsia can vary depending on the degree of vascular involvement of each particular vascular bed.

Two of the key mediators which are currently being extensively investigated are soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sENG) (Levine, Lam *et al.* 2006). These are soluble receptors for vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF β) and these are increased in the circulation of

women with preeclampsia. The binding of VEGF and TGF β in the plasma of women with preeclampsia leads to their inability to bind with the transmembrane receptors Flt1 and endoglin/TGF β receptor. Thus sFlt1 antagonizes the actions of VEGF and placental growth factor (PlGF). VEGF is responsible for decreasing vascular tone and reducing blood pressure. Therefore antagonism of these proteins would be expected to induce many of the pathological changes in preeclampsia.

Table 5 Mechanisms of hypertension in preeclampsia

Determinants of hypertension	Key elements	Proposed substances	References
Cardiac output	Heart rate	Acetylcholine, noradrenaline, adrenaline, thyroid hormones	(Easterling 1992; Easterling and Benedetti 1989; Easterling, Benedetti <i>et al.</i> 1989b; Kjeldsen, Eide <i>et al.</i> 1985; Minagawa, Narita <i>et al.</i> 1999; Nisell and Lunell 1984)
	Cardiac contractility	Adrenaline, noradrenaline	
	Venous tone	Sympathetic nervous system	
	Stroke volume	Intravascular volume	Aldosterone, antidiuretic hormone, progesterone ¹ , oestrogen ² , sodium
Systemic vascular resistance	Direct innervation	Noradrenaline ³	(Aya, Mangin <i>et al.</i> 2003; Visalyaputra, Rodanant <i>et al.</i> 2005)
	Circulating mediators (increase in vasoconstrictive mediators, reduction in vasodilatory mediators)	Catecholamines, angiotensin II ⁴ , vasopressin, relaxin, soluble FMS-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sENG), plasma lipids, IL-10, homocysteine, free radicals, nicotine antagonism (oestrogen), arachidonic acid derivatives, cytokines Nitric oxidase inhibitor N-nitro-L-arginine methyl ester (L-NAME) (animal preparations), tumour necrosis factor α (TNF- α) (animal preparations)	(Berends, Teunkens <i>et al.</i> 2005; Cezar-de-Mello, Vieira <i>et al.</i> 2008; Collins, Rosano <i>et al.</i> 1993; Dowling, Rochelson <i>et al.</i> 2007; Duley, Henderson-Smart <i>et al.</i> 2007; Feinberg 2006; Gerritsen, Tomlinson <i>et al.</i> 2003; Levine, Lam <i>et al.</i> 2006; Meher and Duley 2006a; Meher and Duley 2007; Rumbold and Crowther 2005a; Rumbold and Crowther 2005b; Rumbold, Duley <i>et al.</i> 2008; Sakairi, Ishida <i>et al.</i> 2008; Salafia and Shiverick 1999; Shah 2005; Sherwood 2004; Shiverick and Salafia 1999; Zhou, Ahmad <i>et al.</i> 2008)
	Local mediators	Nitric oxide, prostaglandins, Endothelin, adenosine, Na/K ATPase channel angiotensin II	(Conde-Agudelo, Villar <i>et al.</i> 2004; Duley, Henderson-Smart <i>et al.</i> 2007; Gilbert, Ryan <i>et al.</i> 2008; Hofmeyr, Lawrie <i>et al.</i> 2010; Makrides, Duley <i>et al.</i> 2006; Maynard, Min <i>et al.</i> 2003)

1. Progesterone stimulates aldosterone production.

2. Oestrogen increases the activity of the renin angiotensin system.

3. The sympathetic nervous system (SNS) controls release of noradrenaline from postganglionic nerve terminals which binds to α_1 -adrenoceptors on the arteriolar smooth muscle cell causing vasoconstriction however SNS blockade by regional anaesthesia produces less hypotension in women with preeclampsia than healthy women.

4. In women with preeclampsia, plasma renin activity and aldosterone are suppressed. Women with preeclampsia demonstrate an increased sensitivity to angiotensin II.

Na = sodium; K = potassium; ATPase = adenosine triphosphatase

Table 6 Cochrane Systematic Reviews examining interventions based on proposed aetiological substances and their role in the development of preeclampsia

Author	Substance investigated	Summary ¹	Recommendation
Bamigboye (2003)	Oestrogen	When used for the prevention of miscarriages, diethylstilbestrol was of no benefit. No observed change in the rate of preeclampsia.	This intervention caused harm to women and their offspring and is not recommended.
Meher (2006b)	Progesterone	Insufficient evidence for reliable conclusions about the role of progesterone in prevention of preeclampsia.	Not recommended for this purpose.
Meher (2006a) Rumbold (2008) Rumbold (2005a) Rumbold (2005b)	Antioxidants	Vitamin C and Vitamin E do not reduce risk of preeclampsia or other serious consequences of the disease.	The review does not support routine antioxidant supplementation during pregnancy to reduce the risk of preeclampsia or its consequences.
Hofmeyr (2010)	Calcium	Calcium supplementation halves the risk of preeclampsia and reduces the composite outcome of death or serious morbidity.	Recommendation: calcium 1.5- 2.0 g/day > 12 weeks gestation to reduce the incidence of preeclampsia.
Makrides (2006)	Prostaglandins	Prostaglandins in the form of marine oil decreased the risk of preterm birth before 34 weeks gestation.	Not enough evidence to support routine use of marine oil or other prostaglandin precursor supplements during pregnancy to reduce the risk of preeclampsia.
Meher (2007)	Nitric oxide	Insufficient evidence to draw conclusions regarding the benefits or risks of L-arginine or glyceryl trinitrate.	None made.
Duley (2007)	Prostacyclin/thromboxane	Low dose aspirin has moderate benefits when used for the prevention of preeclampsia and its consequences.	Recommendation: 75 mg aspirin/day > 12 weeks gestation to reduce incidence of preeclampsia.
Duley (2000) Duley (2005)	Sodium	Reduced salt diet or reducing the amount of salt in the diet during pregnancy was not shown to be of benefit or have risks therefore it was concluded that more data is required.	No specific recommendations.

1. adapted from (Hofmeyr, Neilson *et al.* 2008).

Table 7 Regional vascular bed involvement in preeclampsia

Regional vascular bed	Clinical manifestations	Mechanisms	References
Cerebral	Hypertensive encephalopathy, posterior reversible encephalopathy syndrome, headache, visual disturbances, seizures, intracerebral haemorrhage and infarction	Two opposing theories: 1. Cerebral barotrauma and increased cerebral perfusion pressure overcomes normal autoregulatory vasoconstriction ¹ or 2. Cerebral overregulation leading to vasoconstriction as the predominant effect.	(Belfort, Anthony <i>et al.</i> 2003; Belfort, Clark <i>et al.</i> 2006; Belfort, Tooke-Miller <i>et al.</i> 2002; Martin, Thigpen <i>et al.</i> 2005)
Renal	Glomerulosclerosis, reduced renal function, rarely acute renal failure, proteinuria, oliguria, acute renal failure	Renal artery and arteriole oedema, deposition of inflammatory cells	(Karumanchi, Maynard <i>et al.</i> 2005; Kincaid-Smith 1973; Makris, Thornton <i>et al.</i> 2007)
Gastrointestinal/hepatic	Abdominal pain, nausea, vomiting, hepatic haematoma and rupture, alteration in hepatic enzymes associated with haemolysis elevated liver enzymes low platelet (HELLP)	Oedema, hypertension, activation of coagulation cascade, local inflammatory response	(Fischer, Schneider <i>et al.</i> 2000; Gilbert, Estelles <i>et al.</i> 1990; Katz, de Amorim <i>et al.</i> 2008; Martin, Blake <i>et al.</i> 1991)
Uterine/placenta/umbilical	Intrauterine growth restriction, high resistance changes in the uterine artery on Doppler studies 22-24 weeks gestation increases risk of preeclampsia, uterine artery Doppler studies at 35 -37 weeks gestation indicates placental insufficiency and increased risk for the fetus if notched waveform present	Failure of pseudovascularization in the uteroplacental circulation- failure to change from a high resistance circulation to a low resistance circulation. The uterine artery Doppler test has been used to predict development of complications in pregnancy. Approximately half of the women with abnormal uterine artery Doppler waveforms progress during pregnancy to develop preeclampsia, preterm birth or pregnancies complicated by intrauterine growth restriction.	(Barker, Beaves <i>et al.</i> 2009)
Pulmonary	Acute pulmonary oedema	Cardiomyopathy with reduced cardiac output or hypertensive cardiac failure	(Sibai, Mabie <i>et al.</i> 1987)
Coronary	None described		
Cutaneous	None described	Connective tissues diseases and vasculitides are frequently associated with skin changes and rashes as well as fever in acute exacerbations. Preeclampsia is not associated with skin changes or fever.	

1. Favoured by Belfort and colleagues - In an acute setting the homeostatic mechanisms are overwhelmed by sustained vasoconstriction leading to damage of the endothelium and blood brain barrier and diffusion of fluid into the interstitium, the accumulation of substances that lead to vasodilation and ongoing damage. This transient damage leads to a reduction in the seizure threshold and fitting occurs. Blood pressure is often within the reference range for pregnancy shortly after the fit presumably due to outpouring of vasodilatory substances from the brain. The treatment of seizures in the context of pregnancy and presumed preeclampsia/eclampsia is MgSO₄. The most likely mechanism for its effectiveness is that of a neuronal membrane stabilizer; a calcium antagonist which decreases cellular excitability and possibly a systemic action of a slight reduction in mean arterial pressure caused by vasodilation thus reducing cerebral perfusion pressure

2.5.2. Cardiac function in preeclampsia

A wide range of haemodynamic conditions have been described in preeclampsia with no agreement as to the overall cardiac function in the native disease state. Invasive studies have been more common in these women as they have often been critically unwell and their use has been justified. As previously discussed the use of invasive monitoring devices may alter the underlying cardiac manifestations of preeclampsia due to sympathetic nervous system activation, pain and cardiac stimulation by the device.

There are studies that have reported that women with severe preeclampsia have lower cardiac outputs than healthy pregnant women (Bosio, McKenna *et al.* 1999; Cotton, Gonik *et al.* 1984; Groenendijk, Trimpos *et al.* 1984; Mabie, Hackman *et al.* 1993; Mabie, Ratts *et al.* 1989; Visser and Wallenburg 1991; Wallenburg and Visser 1994; Young and Johanson 2001) and also studies that show preservation of left ventricular systolic function at term in women with preeclampsia (Simmons, Gillin *et al.* 2002).

The hypothesis that preeclampsia may be a hyperdynamic state prior to diagnosis is supported by studies by Easterling and Bosio (Bosio, McKenna *et al.* 1999; Easterling and Benedetti 1989; Easterling, Benedetti *et al.* 1990). These studies demonstrate that there may be a greater rise in cardiac output prior to clinical manifestations of preeclampsia than the cardiac output rise that may occur in healthy women.

Groups have also attempted to predict preeclampsia and define haemodynamic states prior to the development of preeclampsia using echocardiography in the first trimester. As these studies are designed to investigate predictors of preeclampsia rather than define the native disease state, these groups do not measure cardiac function at the time of the diagnosis of preeclampsia and so the native disease state is not defined in these studies (Sibai 2008b; Valensise, Vasapollo *et al.* 2008).

Sibai and Mabie, Young and Johanson and Hibbard, Shroff and Lang have presented important reviews on this topic (Hibbard, Shroff *et al.* 2004; Sibai and Mabie 1991; Young and Johanson 2001). In these reviews it can be seen that the ability to obtain

accurate data regarding cardiovascular system function is limited by study design, the problem of patient heterogeneity as well as the significant problem of attempting to enrol women in studies prior to treatment interventions.

There are however, a small number of studies that use Doppler echocardiography to examine the cardiovascular system in women with preeclampsia. These studies have small participant numbers and have been performed in women who have been treated. Regarding cardiac output in women with preeclampsia using Doppler echocardiography in the third trimester, Simmons and colleagues report a mean cardiac index (SD) of 4.1 ± 1.1 l/min/m² and mean heart rate (SD) of 71 ± 14 BPM in fifteen women with treated preeclampsia. In the same study the group reports diastolic function using Doppler echocardiography mean (SD) values for left ventricular (LV) mass 164 ± 37 g, mitral valve (MV) E 92 ± 20 cm/s, mitral valve A 55 ± 13 cm/s, mitral valve deceleration time (DT) 187 ± 36 ms and mitral valve isovolumetric relaxation time (IVRT) 72 ± 6 ms (Simmons, Gillin *et al.* 2002). Easterling and colleagues report mean (SD) cardiac output using Doppler echocardiography in a group of 36 women with both treated and untreated preeclampsia of 7.4 ± 2.0 l/min. Mean (SD) heart rate in this study was 83 ± 14 BPM (Easterling, Watts *et al.* 1987).

Similar methodological issues such as position, attainment of basal conditions, controlling for interventions, method of measurement and intra- and interobserver reliability are also important in the study design when investigating women with preeclampsia. There are also other important factors to consider, which will now be discussed.

2.5.3. Methodological considerations

Measurement devices in women with preeclampsia

The techniques of echocardiography and invasive devices for measuring cardiac function demonstrate excellent correlation in the non-pregnant population. Validation studies have been performed in women with preeclampsia and the devices have been well correlated allowing the use of transthoracic echocardiography as a measuring device to be utilized in this setting as well (Belfort, Rokey *et al.* 1994; Easterling, Watts *et al.* 1987).

Recommendations from the Canadian Hypertension Society and the American Heart Association regarding techniques for blood pressure measurement highlight the need for the use of correctly calibrated devices, for measurements to be made at rest and also in pregnancy for measurements to be made with careful attention to minimizing aortocaval compression (Pickering, Hall *et al.* 2005). Regarding transthoracic echocardiography techniques many older studies utilized only M mode (Kuzniar, Piela *et al.* 1983; Kuzniar, Piela *et al.* 1982) and derived values for cardiac output from these measurements. Whilst these techniques were appropriate at the time they were employed it has now been recognized that Doppler echocardiography is the more accurate method of measuring cardiac output.

Defining the study group

Defining the study group is crucial to study design. The absence of a diagnostic test or reliable biomarker for preeclampsia, and the range of severity of the disease mean that selection into studies may include a heterogeneous group.

One of the most important issues in determining the underlying systolic and diastolic function in women with preeclampsia is obtaining data prior to any treatment intervention. Women are often started on antihypertensive therapy or magnesium sulphate at the time of diagnosis which makes assessment of their cardiac function in the untreated state difficult. The effects of treatment frequently alter the fundamental characteristic of blood pressure and therefore their haemodynamics (Sibai and Mabie 1991; Visser and Wallenburg 1991; Young and Johanson 2001).

The treatment interventions used in preeclampsia include antihypertensive medication, intravenous fluids, insertion of intravenous cannula, the use of labour suppression agents, and the use of magnesium sulphate (MgSO₄). All of these agents have cardiovascular system effects and especially regarding antihypertensive medication, there are both direct and indirect consequences of treatment. For example hydralazine causes systemic vasodilation and a reflex tachycardia and it is feasible that the underlying systolic and diastolic cardiac function in women with preeclampsia may be altered by the administration of these medications.

Easterling and colleagues have performed a large amount of work in the area of non-invasive examination of the cardiovascular system in women with preeclampsia and their work highlights some of the difficulties in performing studies in this group of women. In a validation study performed by Easterling and colleagues women with severe preeclampsia who required a pulmonary artery catheter had their cardiac output measured by two techniques (Doppler transthoracic echocardiography and thermodilution). This was a heterogeneous group of women as they were receiving many vasoactive medications, therefore conclusions regarding haemodynamic changes purely associated with preeclampsia are difficult to make. In the related study the same group measured cardiac output by Doppler transthoracic echocardiography in 36 women with preeclampsia and eighteen normal women. Preeclampsia was defined as blood pressure $\geq 140/90$ mmHg associated with 1+ or greater proteinuria. However the gestations of the women with preeclampsia were not stated and nine of the women with preeclampsia had received magnesium sulphate prior to measurements. The mean cardiac output in this group of 36 women was found to be 7.4 l/min with a standard deviation of 2.0 l/min and the data from the women receiving magnesium sulphate was not analysed separately. This cardiac output did not differ significantly from the group of healthy women (Easterling, Watts *et al.* 1987). This same group has performed a longitudinal study following 179 women from at least 22 weeks gestation to 6 weeks postpartum with aortic diameter measurement and aortic Doppler flows. This study found that of the nine women who developed preeclampsia, all the women had statistically significantly elevated cardiac output throughout their pregnancy compared with women who remained normotensive. The women who developed preeclampsia were statistically significantly heavier at 23 weeks gestation. Data from the 81 women who developed gestational hypertension (45.3%) is not presented (Easterling, Benedetti *et al.* 1990).

Again one of the most important factors in study design is that of correct identification of the study group. Heterogeneity of participants makes data difficult to assimilate. This is illustrated in a recent paper by Bamfo and colleagues examining cardiac function in normotensive women and in women with preeclampsia and intrauterine growth restriction. Unfortunately in this study there is no healthy comparison group, a wide range of

gestations are included, only prepregnancy weight is included and the data is presented as Z scores between the two groups which makes interpretation very difficult (Bamfo, Kametas et al. 2008). This study is one of the few that has examined diastolic cardiac function utilising tissue Doppler indices. Reduced diastolic performance has recently been reported in healthy term pregnant women (Zentner 2006).

The characteristics of the women

Women with preeclampsia often have coexisting medical diseases. These conditions included obesity, renal disease, preexisting hypertension and diabetes (preexisting or gestational). Women with severe preeclampsia often present before 37 weeks gestation and therefore matching these women's cardiovascular characteristics with a subgroup of women without preeclampsia and with one or more of these other characteristics is often difficult and few studies compare similar groups. Ethnicity may contribute to differences in cardiovascular system performance (Hinderliter, Light *et al.* 1992). Gestational age, medication administration, height, weight and parity are also important characteristics of the women that need to be relatively homogenous for comparison studies.

Coexisting obesity in women with preeclampsia is an important associated disease. Regarding the cardiovascular system, the increases in cardiac output observed in women with preeclampsia and who have coexisting obesity may be due to increase weight rather than the underlying disease mechanism of preeclampsia.

The timing of recruitment and the characteristics of the investigators

Recruitment of patients often occurs in emergency situations and is of an unpredictable nature. Ideal studies need to be designed such that monitoring is commenced prior to any interventions so that the pure, native disease state is observed. Ideal studies need to have a system in place to perform immediate cardiovascular investigations upon presentation of the women to hospital prior to institution of therapy. This is one of the major impediments to data acquisition in this group of women as studies examining the pathophysiological changes in preeclampsia need to be done when the woman has had no treatment interventions thus necessitating immediate investigation on arrival in hospital or at the time of diagnosis. This makes study design and the availability of researchers absolutely

essential but has been difficult to achieve given the urgent nature of the disease. Longitudinal studies following women from before pregnancy, during, and after pregnancy are ideal, however these are difficult to perform. A study by DePaco and colleagues examined cardiac output in 4617 consecutive women having singleton pregnancies in an antenatal clinic at 11-13 weeks gestation. They examined the relationship between cardiac output, demographics, social and biological risk factors and the development of preeclampsia with and without small for gestational age (SGA) infants and small gestational age infants without preeclampsia by retrospective examination of questionnaires. The statistical analysis and presentation of the data is very difficult to interpret (De Paco, Kametas *et al.* 2008).

2.5.4. Summary of cardiac changes in women with preeclampsia

There is very little information about the native disease state regarding cardiac function in women with preeclampsia. To date, no adequately powered studies have examined the untreated disease state to determine systolic, diastolic and structural changes, using Doppler echocardiography techniques in a non-invasive way after adequate resting and appropriate matching to controls.

2.6. Animal preparations of preeclampsia

2.6.1. General considerations with animal preparations

There are a wide variety of animal preparations of preeclampsia utilising small animals including mice, rats, rabbits and guinea pigs to larger animals such as sheep, baboons, and monkeys. A range of techniques is used to establish a syndrome of hypertension with additional organ system involvement, usually renal.

Animal preparations can be divided into three main types: mechanically generated disease (Anderson, Lopez *et al.* 2005; Anderson, Lopez *et al.* 2006; Losonczy, Brown *et al.* 1992; Makris, Thornton *et al.* 2007; Sholook, Gilbert *et al.* 2007), biochemically generated disease (Coates, Broderick *et al.* 2006; Ganzevoort, Rep *et al.* 2004; LaMarca, Wallukat *et al.* 2008; Maynard, Min *et al.* 2003; Maynard, Venkatesha *et al.* 2005; McElvy, Greenberg *et al.* 2002), and genetically generated disease (Sakairi, Ishida *et al.* 2008; Takimoto, Ishida *et al.* 1996). All animal preparations have their limitations and emulation of the preeclamptic state in an animal preparation enables intense examination of the cardiovascular state in pregnant healthy and pregnant animals with preeclampsia in a longitudinal fashion, however there is minimal published work in this area (Eremina, Jefferson *et al.* 2008; Gilbert, Ryan *et al.* 2008; Karumanchi and Stillman 2006; Makris, Thornton *et al.* 2007; Maynard, Min *et al.* 2003; Shah 2005). Animal preparations utilising these three different techniques have been described by other authors (Nama, Antonios *et al.* 2007; Podjarny, Losonczy *et al.* 2004).

2.6.2. The Baboon preparation (*Papio hamadryas*)

In Australia, the Australian National Baboon Colony, established in 1982, is considered a national scientific treasure and is supported by the National Health and Medical Research Council of Australia (NHMRC). This colony has been used for many years for research into preeclampsia with well defined reference ranges for biochemical and physiological values as well the achievement of a stable colony with rigid published experimental protocols (Garner, Phippard *et al.* 1985; Maclean, Phippard *et al.* 1987; Maclean, Phippard *et al.* 1990; Phippard, Horvath *et al.* 1986). Both mechanical (using the uteroplacental ischaemia model) and pharmacological (using the nitric oxide inhibitor, nitro-L-arginine)

methods, of generating preeclampsia have been developed in this setting (Birrell, Hennessy *et al.* 1996; Harewood, Gillin *et al.* 1999; Harewood, Gillin *et al.* 2000; Hennessy, Gillin *et al.* 1999; Hennessy, Whitworth *et al.* 1994; Holmes, Paull *et al.* 1996; Orange, Rasko *et al.* 2005; Thomson, McLennan *et al.* 2008).

The baboon, as opposed to many other animals, demonstrates spontaneous development of hypertension associated with pregnancy making these animals suited to investigations of preeclampsia (Hennessy, Gillin *et al.* 1997). In addition, the baboon is closely related to the human and shares many genomic similarities (Carter 2007). Importantly the human and the baboon also share similarities regarding placentation. These include

1. Primary interstitial implantation in a simplex uterus
2. The absence of an allantoic yolk sac
3. The occurrence of an allantoic stalk rather than on allantoic sac
4. Deep implantation and deep trophoblastic invasion of the endometrium
5. Anti-gravity uterine blood flow

Regarding birth of offspring, humans birth relatively mature offspring, usually have singleton pregnancies, have a long gestation and small numbers of neonates (precocial young), which is similar to baboons. The gestation of the baboon is 182 days. This is in contrast to small animal preparations which have significant differences in their placentation compared with humans and therefore may not be the ideal preparation in which to study or generate preeclampsia (Carter 2007).

2.6.3. Transthoracic echocardiography in baboon preparations

Within the area of animal experimentation transthoracic echocardiography has been utilized in baboons predominantly only in its M-mode capability for calculation of end systolic and end diastolic measurements, wall thicknesses and fractional shortening as an indicator of systolic function (Crawford, Walsh *et al.* 1987). Calculation of cardiac output has been employed in small animal preparations (Stypmann 2007) using fractional shortening M-mode methodology as the cardiac long axis is difficult to obtain by echocardiography in small animals (Slama and Maizel 2006). The echocardiography

devices now available have the same theoretical advantages in the non-human primate as they do in humans. They are low risk, non-invasive and have the ability to give qualitative and well as quantitative data. Also they have the benefit of being able to obtain diastolic variables which traditionally could only be obtained with highly invasive monitors such as invasive pressure devices.

Baboons have similar anatomy to humans, are of comparable size to children and transthoracic echocardiography devices used on the human paediatric population can in theory be used on baboons. The differences in thoracic anatomy with the baboon having wider intercostal spaces may be advantageous by increasing the size of the acoustic windows. The apex of the heart lies to the left of the sternum at the level of the sixth intercostal space. The base of the heart is only slightly to the right of the sternum in the second and third interspaces. The long axis of the heart is orientated in a more craniocaudal direction compared with the human (Swindler and Wood 1973). These differences should not alter the image quality or interfere with image acquisition.

Using the same measuring device in humans and non-human primates may enable better translation of animal results to human conditions, thereby improving the relevance of animal studies to the human disease state. Quantification of systolic and diastolic function by transthoracic echocardiography is important to determine cardiac function prior to pregnancy and serially during induced preeclampsia. It can also be used to examine the effect of the disease on cardiac function and to examine changes that occur due to interventions. The use of transthoracic echocardiography utilizing full systolic and diastolic measurement capabilities has not previously been reported in baboons.

2.7. Review summary

2.7.1. Current gaps in the knowledge

1. There is limited understanding of the systolic and diastolic function and cardiac structure in healthy pregnant women at term.
2. There is limited understanding of the effect of postural changes (head-up and head-down) in addition to lateral tilt on cardiac output in term pregnant women.
3. Reference ranges for transthoracic measurements in the pregnant healthy population are lacking.
4. There is very limited understanding of the systolic and diastolic function and cardiac structure in women with untreated preeclampsia using transthoracic echocardiography.
5. Reference ranges for transthoracic echocardiography measurements in the pregnant population with preeclampsia are lacking.
6. There are no published studies utilising transthoracic Doppler echocardiography in the baboon preparation of preeclampsia.

Therefore four investigations will be performed to address these gaps in the literature within the context of the central research question of defining the native disease state in preeclampsia:

Human Study 1 – Cardiac output in healthy pregnant women

Human Study 2 – Cardiac function in women with untreated preeclampsia

Animal Study 1 – Cardiac function in healthy pregnant and non-pregnant baboons

Animal Study 2 – Cardiovascular and renal effects of relaxin in baboons

Chapter 3. Human experimental methods

3.1. Introduction

This section of the thesis outlines the human experimental design, methodology, generation of data end points and specific measurement details.

The normal cardiac cycle is divided into the two phases of systolic ejection and diastolic filling. The terms systole and diastole need defining depending on what they are referring to as they can refer to times, locations or pressures. Ventricular systole and diastole is the contraction and relaxation of the left ventricle. There is also the term systolic and diastolic blood pressure which refers to the vascular system. A visual representation of the phases of the cardiac cycle with the echocardiographic waveforms is shown in Figure 6, page 65.

Systolic ejection occurs at the opening of the aortic valve or R wave on the electrocardiograph (ECG) and comprises isovolumetric contraction, rapid ejection and then reduced ejection flow and ends at the end of the T wave of the ECG. It is the time in which blood is pumped into the circulation. In transthoracic echocardiography (TTE) this is recorded as the left ventricular outflow tract velocity time integral. It is dependent on preload, left ventricular contractility and left ventricular afterload incorporating aortic valve resistance. The s' wave of the tissue Doppler signal records the systolic movement of the myocardium at the same time. Echocardiography allows measurement of time, flow and volume during systole: isovolumetric contraction time (IVCT), tissue Doppler s' wave, left ventricular outflow tract velocity time integral (LVOT VTI), fractional shortening (FS), and fractional area change (FAC).

Diastolic filling commences at the closure of the aortic valve and is composed of four phases: isovolumetric relaxation; rapid ventricular filling; reduced ventricular filling or diastasis, and atrial contraction. Diastolic function of the heart refers to the ability of the heart to relax and fill with the shortest rapid filling time and the lowest intraventricular pressure in order to maintain stroke volume. The overall purpose of the heart is to expel

the correct volume at the correct rate to efficiently deliver oxygen to the tissues and remove by-products of metabolism. It attempts to do this at the lowest possible pressures with the use of the least amount of energy. The electrical signal from depolarization is converted to mechanical energy thus enabling the ejection of the stroke volume and the generation of the cardiac output.

Echocardiography machines measure time, flow and volume during diastole: isovolumetric relaxation time (IVRT), mitral valve (MV) E wave velocity, mitral valve A wave velocity, mitral valve deceleration time (DT), mitral valve A wave duration, tissue Doppler e' wave velocity, and the tissue Doppler a' wave velocity.

3.2. Experimental design

This thesis is composed of two observational investigations in humans and two observational studies in animals. The body of work contained in this thesis explores cardiac function in women with untreated preeclampsia and the application of the technology in baboons to further explore the aetiology and effects of the disease. As such Human Study 1 is the prelude to the main Human Study 2. Animal Study 1 establishes the applicability and baseline value which then leads on to Animal Study 2, an intervention study observing the cardiovascular and renal effects of relaxin in two non-pregnant baboons. The human studies were conducted at the Mercy Hospital for Women, Melbourne, Australia. The Mercy Hospital for Women is a tertiary referral obstetric hospital with approximately 5,800 births per year. The animal (baboon) studies were conducted under the supervision of Professor Annemarie Hennessy, University of Western Sydney, at Royal Prince Alfred Hospital, Sydney, Australia and in collaboration with the University of Florida with Professor Kirk Conrad.

3.3. Ethical considerations

Human Study 1 and Human Study 2 received prospective full institutional ethics committee approval through the Mercy Hospital for Women's Human Research Ethics Committee (HREC). The Human study protocols and Patient Information and Consent Forms (PICF) are listed in Appendix 1. For Human Study 2, echocardiography was performed at the time of diagnosis of preeclampsia with hospital-wide awareness of the study and immediate notification of the investigator (ATD). This did not delay treatment interventions. Written informed consent was obtained from all women. Animal Study 1 and Animal Study 2 were prospectively approved through the Sydney South West Area Health Service (SSWAH) Animal Welfare Committee and are listed in Appendix 1.

3.4. Selection of patients

3.4.1. Healthy women

Human Study 1 consisted of 30 healthy pregnant women at term. Human Study 2 consisted of 40 women with untreated preeclampsia gestationally matched to 40 healthy pregnant women and twenty healthy non-pregnant female controls.

Healthy women were defined as American Society of Anesthesiologists (ASA) Classification I or II, with no significant medical or surgical illness, non-smokers, singleton pregnancy with no uterine abnormalities and normally defined placentation. They were not receiving any vasoactive medication including salbutamol or thyroid replacement hormones.

3.4.2. Women with preeclampsia

Women with preeclampsia were recruited at the time of diagnosis according to accepted definitions of preeclampsia (ACOG 2002; Brown, Hague *et al.* 2000; RCOG 2006; Sibai, Taslimi *et al.* 1986). They had to be healthy (ASA I or II) with no underlying cardiac disease including preexisting hypertension prior to their current pregnancy. The diagnosis was based on Table 1 (page 9). Preeclampsia was classified as mild or severe with severe

disease defined as clinically important derangements of organ function (including a hypertensive crisis) in the presence of hypertension. These may include central nervous system problems of seizures (eclampsia), impaired conscious state and visual disturbances, renal dysfunction (urinary protein ≥ 5 g protein/24 hours) and haematological complications or Haemolysis Elevated Liver enzymes Low Platelets (HELLP) syndrome. The current definition of preeclampsia does not require the presence of proteinuria. It is the presence of hypertension plus an additional organ system involvement that leads to the diagnosis of preeclampsia. The women were untreated which meant that they had received no medication (oral or intravenous) for preeclampsia at any time prior to the study. They did not have intravenous access and were not receiving intravenous fluids.

The presence or absence of the disease of preeclampsia, rather than its severity, was used as the inclusion criteria for the study. This was because the definition of severe preeclampsia is currently relatively subjective and less precise than the definition of the presence of the disease itself.

3.5. Exclusion criteria

3.5.1. Healthy women

Exclusion criteria included current administration of vasoactive drugs including salbutamol and thyroxine, pre-existing or gestational diabetes, smoking, pre-existing or gestational hypertension or preeclampsia, and a known uterine abnormality. Women in labour were excluded.

3.5.2. Women with preeclampsia

Exclusion criteria were any woman with treated disease at any time prior to the study, women in labour, inability to consent to study or pre-existing cardiac disease, including hypertension, prior to pregnancy. Women in labour were excluded.

3.6. Experimental conditions

3.6.1. Women

All women rested in the left lateral flat position measured with an inclinometer, on a comfortable bed in a quiet, temperature-controlled environment for a minimum of ten minutes before the measurements. A single baseline systolic and diastolic blood pressure was obtained non-invasively using a calibrated sphygmomanometer on the left arm recording the diastolic value as Korotkoff V according to the American Heart Association (Pickering, Hall *et al.* 2005) (Figure 3, Figure 4, and Figure 5). An ECG was attached. No medication or anaesthesia was given before any TTE examinations and there was no intravenous access or intravenous fluid given.

3.6.2. Environmental

All measurements were performed in the hospital environment on a comfortable bed in the resting position. Noise, lighting and temperature were all of a comfortable level.



Figure 3 Experimental setup - Healthy pregnant woman



Figure 4 Inclinometer

Figure 5 Resting blood pressure measurement

3.6.3. Investigator

The investigator (ATD) performed all the TTE imaging seated on the left hand side of the patient and with the minimum required pressure to obtain high quality images.

3.6.4. Transthoracic echocardiography machine accuracy

A validation experiment was performed to confirm that two similar TTE devices achieved the same measurements in the same subject thereby confirming the accuracy of the TTE machine used for the entire experimental program (Table 8).

Table 8 Transthoracic echocardiography machine accuracy

Measured variable	Machine 1 – study machine	Machine 2 – control machine
LVOTd (cm)	2.01	2.01
VTI1 (cm)	21.2	22.1
VTI2 (cm)	21.5	22.7
VTI3 (cm)	22.0	21.0
Average VTI (cm)	21.6	21.9
Septal s' (cm/s)	7.29	7.54
Septal e' (cm/s)	11.2	11.6
Septal a' (cm/s)	5.34	5.46
MV E (cm/s)	66.8	67.5
MV A (cm/s)	40.2	40.5
Heart rate from R-R interval (BPM)	56	56
Heart rate measured by palpation (BPM)	56	56

The value recorded is the average of three consecutive beats. Single subject.

LVOTd = left ventricular outflow tract diameter; VTI = velocity time integral; MV = mitral valve; RR = R-R interval on the electrocardiograph.

Difference machine 2 value – machine 1 value for LVOTd = 0 (0%), Average VTI = 0.3 (1.4%)

3.6.5. Postural changes

For studies involving postural changes the following procedure occurred. A TTE examination was performed in the left lateral flat position (P1 baseline position). Whilst remaining on her left side, the woman was placed 20° head-up and the TTE examination was repeated within five min (P2). She was then placed in the 10° head-down position and the TTE examination repeated again within five minutes (P3). Inclinations were quantified precisely using an inclinometer. The fourth position was postoperatively supine in the post anaesthesia care unit within one hour of birth (P4).

3.7. General Measurement techniques

TTE measurements used in this thesis, along with the experimental photographs, are outlined in the next sections and were according to published guidelines by the American Society of Echocardiography (ASE) (Gottdiener, Bednarz *et al.* 2004; Lang, Bierig *et al.* 2005; Nagueh, Appleton *et al.* 2009; Quinones, Otto *et al.* 2002; Schiller, Shah *et al.* 1989). The values recorded for the systolic, diastolic, structural and overall myocardial performance data is the average of the two sets of recordings for the two observers (Human Study 1) or the average of three consecutive beats for one observer (Human Study 2). In both Human Study 1 and Human Study 2, the interobserver reliability data was obtained using the average of three consecutive beats or measurements, for the two observers.

Acquisition of images

All TTE studies were performed by one observer, the principal investigator (ATD) using a MicroMaxx P17 5 – 1 megahertz (MHz) transducer SonoSite®. For studies involving postural changes, four serial TTE studies were performed under standardized conditions in three different positions preoperatively and one position postoperatively. All positions were quantified by an inclinometer and the time to perform the examination was recorded. The four positions were left lateral level (P1), left lateral 20° head-up (P2), and left lateral

10° head-down (P3), performed preoperatively; and supine (P4) performed postoperatively within one hour of delivery in the postanesthetic care unit (PACU).

A parasternal long axis (PLAX), parasternal short axis (PSAX) and apical 4- and 5-chamber (A4C, A5C) TTE examination including two-dimensional imaging and continuous, pulse wave, colour flow and tissue Doppler and M-mode images were performed with the aim of acquiring the best images (Table 9).

Image analysis

Images were converted to *Digital images and communications in medicine* (DICOM) format and analysed off-line using ProSolv® software. Two investigators (ATD, IA/JC) independently reviewed and measured the stored images to calculate cardiac output (LVOT diameter and LVOT VTI). The second investigator IA/JC, reviewed the images blinded to the disease status of the woman and haemodynamic measurements, in a randomly allocated order and at a remote time and location to ATD. All other measurements were made by the principal investigator (ATD) from the stored images.

Reference values, data acquisition and calculations summary

Reference values for Doppler derived diastolic parameters according to age group are given in Table 10 page 80. The mitral valve E/septal e' relationship to left ventricular filling pressure is shown in Table 11 (page 80). Acquisition and measurement methods for haemodynamic and systolic data, and diastolic and structural data are shown in Table 12 (page 88) and Table 13 (page 89) respectively. Reference values, abbreviations and units for haemodynamic and systolic data, and diastolic and structural data are given in Table 14 (page 90) and Table 15 (page 91) respectively. A summary of the haemodynamic calculations is given in Table 16 (page 92).

Table 9 Images recorded and measurements performed for the parasternal and apical acoustic windows in pregnant women

Transthoracic view	Axis or chamber view	Image recorded	Measurements performed
Parasternal	Long axis	Two dimensional video	
		Left ventricular outflow tract zoomed	Left ventricular outflow tract diameter
		M-mode left atrium	Left atrial diameter
	M-mode left ventricle	Left ventricular end diastolic and left ventricular end systolic diameter, posterior wall thickness, internal diameter left ventricle, interventricular septum thickness	
	Short axis	M-mode at mid-papillary region	Left ventricular end diastolic and left ventricular end systolic diameter
		Two dimensional video	Left ventricular end diastolic area and left ventricular end systolic area
Apical	4 chamber	Septal tissue Doppler waveform	Septal s' , e' , a' measurements, septal s' time, isovolumetric relaxation time, isovolumetric contraction time
		Two dimensional video	
	5 chamber	Two dimensional video	
		Mitral valve pulse wave Doppler	Mitral valve E and A wave, Mitral valve deceleration time
		Left ventricular outflow tract pulse wave Doppler	Left ventricular outflow tract velocity time integral, left ventricular outflow tract velocity time integral time, heart rate

3.8. Conceptualizing cardiac function

The work of Carl J Wiggers established the visual understanding of the cardiac cycle in relation to the electrocardiograph (ECG). The time course of the relationships between ventricular volume and pressure changes are described in the Wigger's diagram. This has assisted clinicians, physiologists and others to understand the cardiac cycle (Wiggers 1951).

Modern echocardiography techniques utilize Doppler echocardiography and enable the determination of velocity changes within the cardiac cycle. Velocities have not yet been incorporated into the Wigger's type diagram in journal publications or textbooks. However combining velocity, pressure, volume and time into the one diagram enables an understanding of the cardiac cycle pertinent to non-invasive, clinically relevant, measurement techniques (Figure 6).

In Figure 6, incorporation of the systolic cardiac velocities of

- left ventricular outflow tract velocity and
- septal tissue Doppler systolic movement of the myocardium (s'),

and the diastolic cardiac velocities of

- mitral valve inflow Doppler velocities (E wave and A wave) and
- septal tissue Doppler diastolic movements of the myocardium (e' and a'),

in addition to volume and pressure changes in the ventricle, enable an understanding of the velocities that are recorded by transthoracic echocardiography and their relationship with ventricular pressure, ventricular volumes and the ECG.

The following section discusses the detail of the specific cardiovascular system measurements.

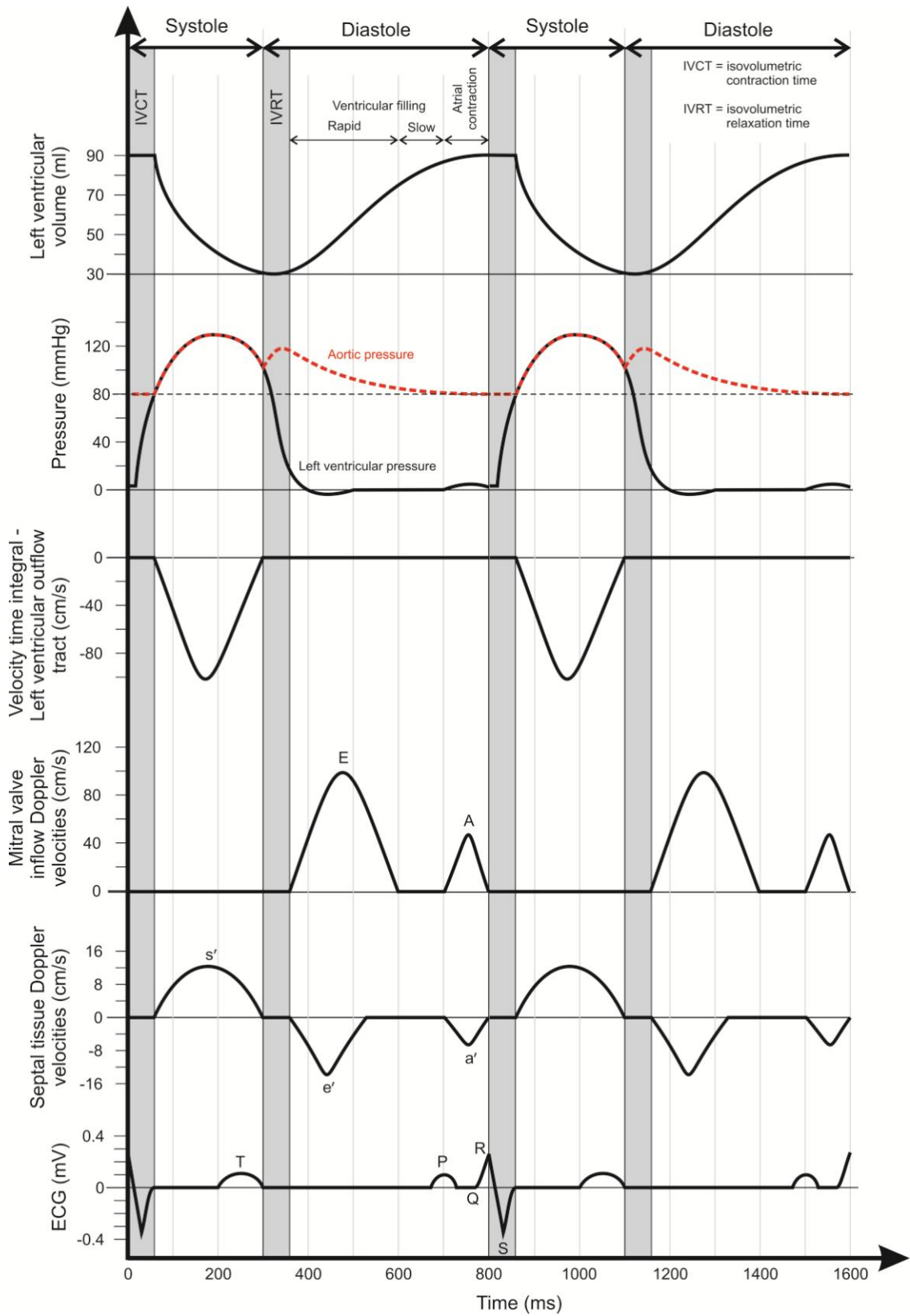


Figure 6 Cardiac function waveforms

3.9. Systolic function

3.9.1. Measurements of systolic function

Left ventricular outflow tract measurement

In the left lateral level position the left ventricular outflow tract (LVOT) image was obtained from the PLAX view. A good quality image was defined as one in which the aortic valve could be seen and the structure looked tubular. A zoomed two-dimensional image in systole during quiet breathing was recorded (Figure 7, and Figure 8).

From the PLAX image, the zoomed LVOT image was frozen during systole. End systole was defined as the frame preceding early diastolic mitral valve opening. End diastole was defined as the onset of the Q wave of the QRS complex. LVOT was measured perpendicular to the aortic root with the anterior caliper positioned at the junction of the anterior coronary cusp and the interventricular septum and the posterior caliper was positioned at the junction of the posterior non-coronary aortic cusp and the anterior mitral valve leaflet. Three measurements were averaged for each observer. The overall LVOT measurement that was used to calculate cardiac output was the average of these two observers' LVOT measurements for position 1.

$$[\text{Observer 1 (measure 1 + measure 2 + measure 3)/2} + \text{Observer 2 (measure 1 + measure 2 + measure 3)/2}] / 2$$
. LVOT was assumed to be a rigid fixed structure therefore LVOT measurement is constant throughout changes in posture (Human Study 1).

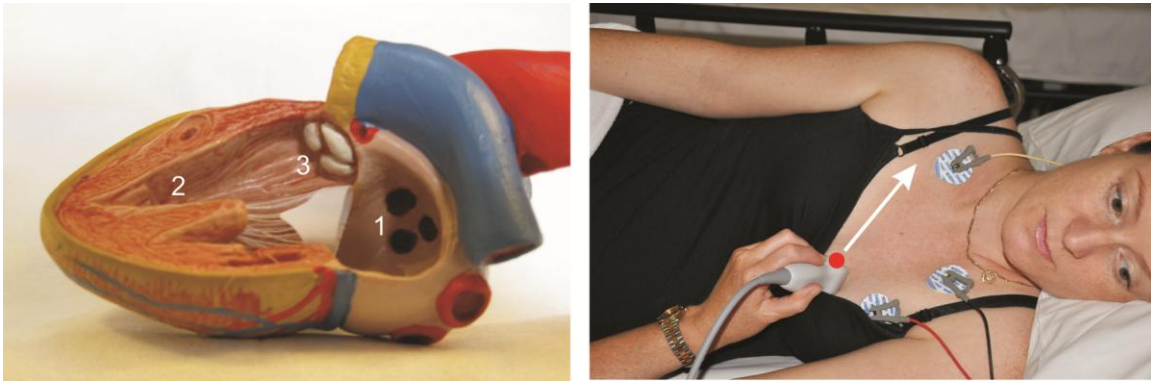


Figure 7 Parasternal long axis view in a healthy pregnant woman - Cardiac model and probe position

The image on the left shows the left atrium (1), mitral valve, left ventricle (2), left ventricular outflow tract (3) and aortic valve in a model of the heart (the parasternal long axis image). The image on the right shows the position of the cardiac probe (transducer) on the chest to acquire the parasternal long axis image. Note the index marker (red dot) on the probe is directed towards the right shoulder tip and the probe is held softly and perpendicular to the chest.

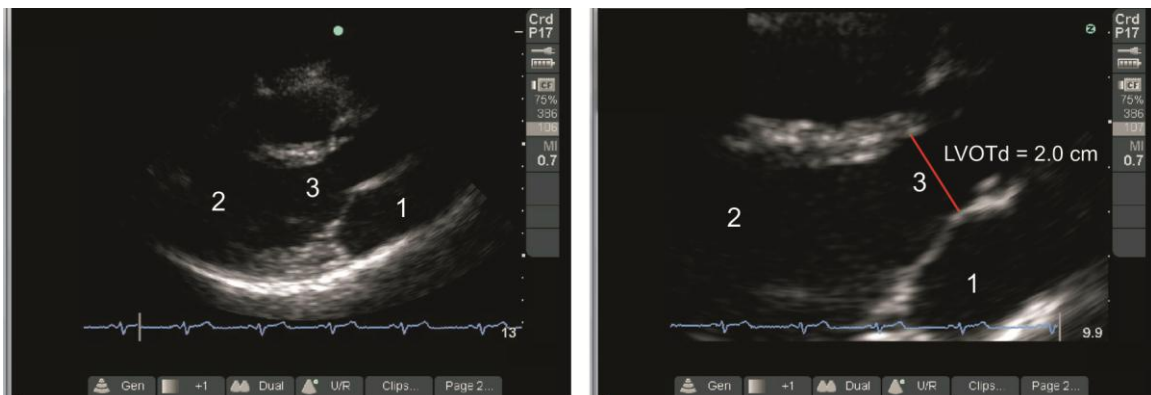


Figure 8 Parasternal long axis image in a healthy pregnant woman – Transthoracic echocardiography images

The view on the left is obtained by freezing the two dimensional video image. The image on the right is the frozen zoomed image of the left ventricular outflow tract (3) during systole with the red line showing the left ventricular outflow tract measurement. The left ventricular outflow tract diameter is 2.0 cm.

1 = left atrium; 2 = left ventricle; 3 = left ventricular outflow tract

Velocity time integral measurement

The LVOT velocity time integral (VTI) was recorded using the apical 5 chamber (A5C) view, with the Doppler integration angle $< 20^\circ$ to flow. An optimal image was assessed by maximal chamber size, a vertical long axis and maximal mitral valve opening size. Pulse wave Doppler was used with a 3 mm sample volume placed within the LVOT approximately 0.5 cm proximal to the aortic valve. At least three consecutive beats were recorded (Figure 9 and Figure 10).

Each observer measured the VTI by tracing the leading edge of the velocity spectrum of three consecutive beats. The VTI used to calculate cardiac output was the average of these three beats from the two observers for each of the four different positions. Heart rate was measured from the Doppler spectral display from the time between two consecutive beats in the VTI tracing. The velocity (VTI time) waveform time can also be measured. It is the duration of the VTI waveform in milliseconds. It is also recorded in oesophageal Doppler where it is known as the corrected flow time.

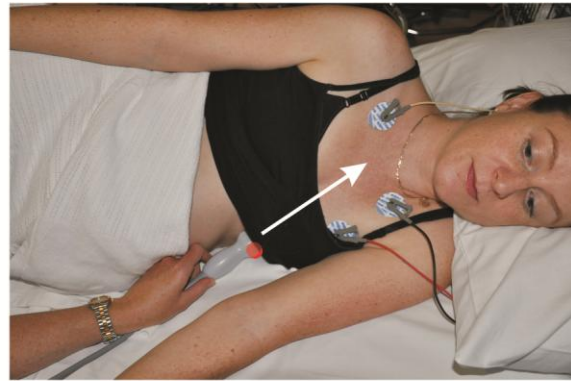
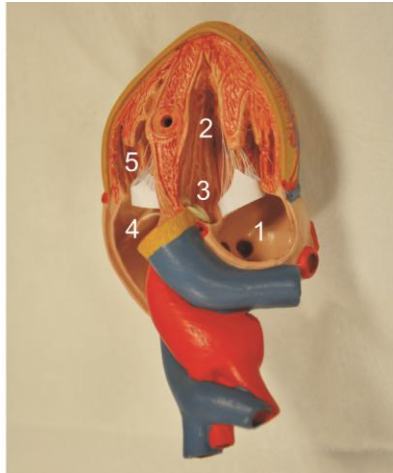


Figure 9 Apical 5 chamber view in a healthy pregnant woman – Cardiac model and probe position

The image on the left shows the left atrium (1), left ventricle (2), the left ventricular outflow tract (3), the right atrium (4) and right ventricle (5) in a model of the heart (the apical 5 chamber image). The image on the right shows the position of the cardiac probe on the chest. Note the index marker on the probe is directed towards the left hand side of the subject adjacent to the bed. The probe is held softly and directed under the left breast towards the sternal notch at an angle that guides the ultrasound beam just below the sternum.

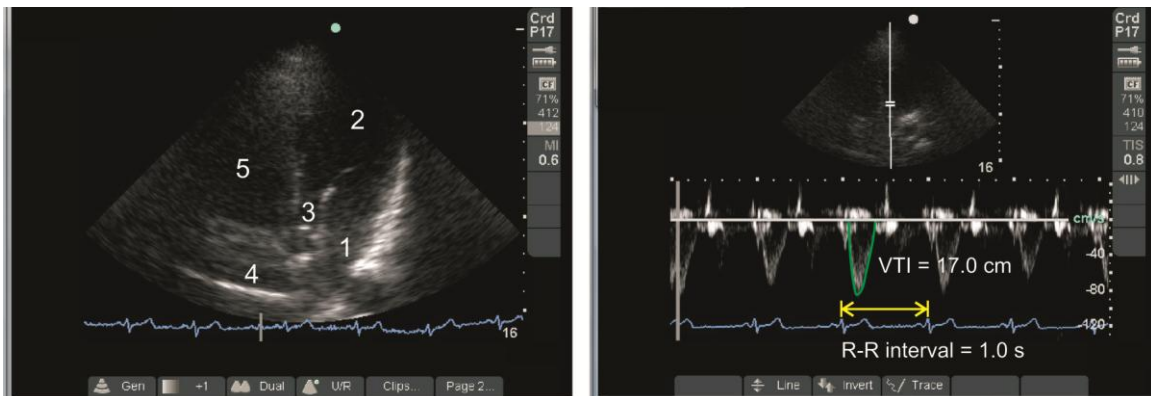


Figure 10 Apical 5 chamber image in a healthy pregnant woman – Transthoracic echocardiography image and velocity time integral and R-R interval

The view on the left is obtained by freezing the two dimensional video image. This image is during diastole with the mitral valve open. The image on the right is the pulse wave Doppler waveform of the left ventricular outflow tract during systole. Tracing the waveform gives the left ventricular outflow tract velocity time integral (green outline). In this case the left ventricular outflow tract velocity time integral is 17.0 cm. The calculation of the heart rate is made from the same image that the velocity time integral is measured. Heart rate = (1/R-R interval (sec)) × 60. The R-R interval is 1.0 s therefore the heart rate is 60 beats/minute. 1 = left atrium; 2 = left ventricle; 3 = left ventricular outflow tract; 4 = right atrium; 5 = right ventricle. Note that velocities moving away from the transducer are shown as velocities below the baseline.

Calculation of cardiac output

Cardiac output (CO) was calculated using the formula:

$$\text{CO (ml/min)} = \text{HR (beats/min)} \times ((\text{LVOT diameter}^2) \times \pi/4) (\text{cm}^2) \times \text{VTI (cm)}.$$

Where $(\text{LVOT diameter}^2) \times 0.785 = \pi \times \text{LVOT radius}^2 =$ cross sectional area of the LVOT. From Figure 8 (page 67) and Figure 10 (page 69) the cardiac output is 3202.8 ml/min.

Fractional shortening

Fractional shortening is well correlated with angiographically determined ejection fraction. The reference range is 27 - 45% for non-pregnant females (Lang, Bierig *et al.* 2005). It is based on the assumption that the shortening of the minor axis of the left ventricle is a reflection of the overall systolic function (Klimczak 2008). M-mode was recorded at the tips of the mitral valve in the PLAX image. The cursor was placed perpendicular to the long or short axis of the left ventricle just distal to the tips of the open mitral valve leaflets. Left ventricular end diastolic diameter (LVEDD) measurements were made at the onset of QRS complex inner edge to inner edge and the average of three measurements was taken. Left ventricular end systolic (LVESD) measurements were made at the point marking the peak posterior deflection of the interventricular septum inner edge to inner edge and the average of three measurements were taken.

$$\text{Fractional shortening (FS) (\%)} = (\text{LVEDD} - \text{LVESD})/\text{LVEDD} \times 100.$$

Left ventricular end diastolic diameter has the reference range of 3.9 – 5.3 cm in the non-pregnant female population (Lang, Bierig *et al.* 2005). M-mode was also recorded at the mid-papillary level in the PSAX image using the same technique as above (Figure 11 and Figure 12).

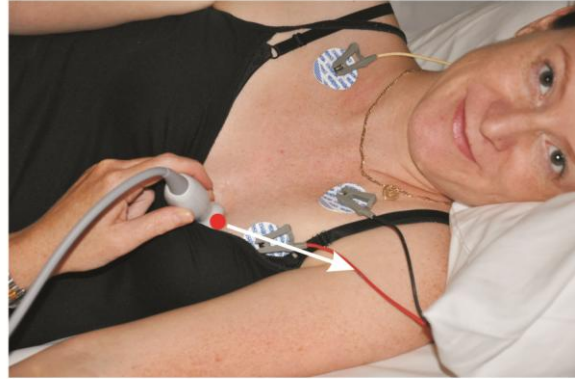


Figure 11 Parasternal short axis view in a healthy pregnant woman – Cardiac model and probe position

The image on the left shows the short axis of the left ventricle with the right ventricle at the lower part of the picture (the parasternal short axis image). The image on the right shows the position of the cardiac probe on the chest to acquire the parasternal short axis image. Note the index marker (red dot) on the probe is directed towards the left shoulder tip and the probe is held softly and perpendicular to the chest.

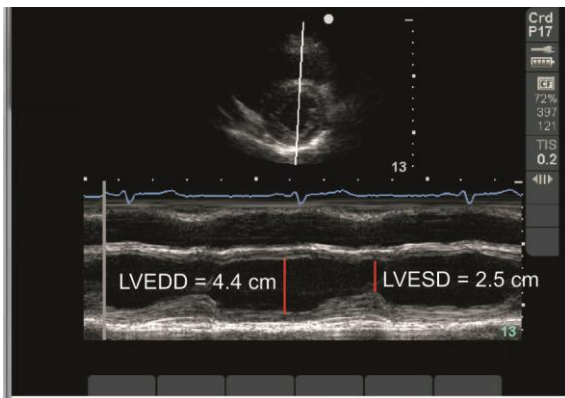


Figure 12 Parasternal short axis image in a healthy pregnant woman – Transthoracic echocardiography M-mode mid-papillary region left ventricle – Fractional shortening

The image shows the left ventricular end diastolic diameter (red line) (4.4 cm), the left ventricular end systolic diameter (red line) (2.5 cm). Fractional shortening $[(LVEDD-LVESD)/LVEDD] \times 100 = 43\%$. Note that for accuracy the diastolic measurement should precede the systolic measurement in each individual cardiac cycle because for each stroke volume the left ventricle fills (diastole) then empties (systole).

Fractional area change

Fractional area change (FAC) is the proportional change in area of the left ventricular short axis during systole (PSAX). Reference range is 36 – 64% (Schiller, Shah *et al.* 1989). Fractional area change and fractional shortening are well validated with ejection fraction and stroke volume (Figure 13).

$$\text{Fractional area change (FAC) (\%)} = (\text{LVEDA} - \text{LVESA}) / \text{LVEDA} \times 100$$

LVEDA = left ventricular end diastolic area

LVESA = left ventricular end systolic area

It was measured from the PSAX at the mid-papillary level. LVEDA (cm²) was calculated by freezing the largest image and manually tracing the endocardial surfaces excluding papillary muscles (Levitov, Mayo *et al.* 2009; Royse, Barrington *et al.* 2000; Schiller, Shah *et al.* 1989; Sidebottom, Merry *et al.* 2003). The average of three measurements was recorded. LVESA (cm²) was calculated by freezing smallest image trace endocardial surfaces excluding papillary muscles. The average of three measurements was recorded.

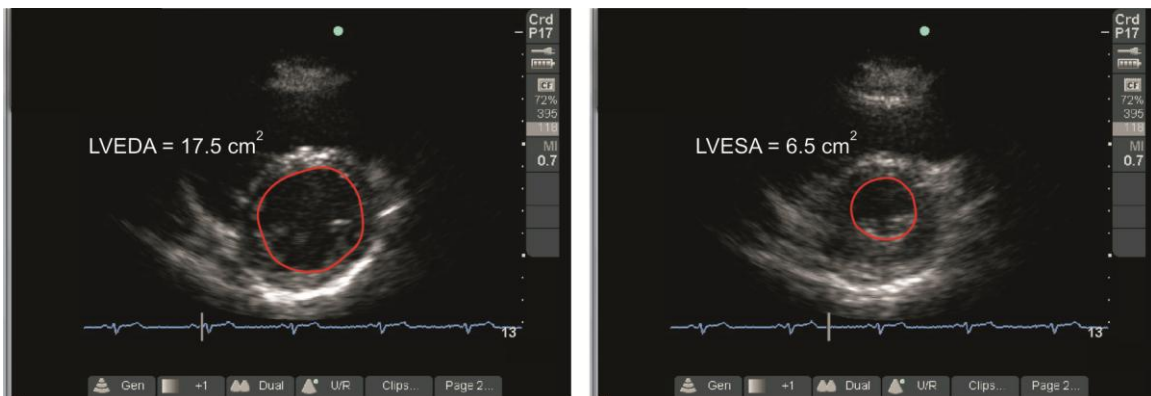


Figure 13 Parasternal short axis image in a healthy pregnant woman – Transthoracic echocardiography mid-papillary region left ventricle – Fractional area change

The image on the left is obtained by freezing the two dimensional video at end diastole and measuring the area (red circle) (17.5 cm²). The image on the right is obtained by freezing the two dimensional video during systole and measuring the area (red circle) (6.5 cm²). Fractional area change = [(LVEDA – LVESA)/LVEDA] × 100 = 63%.

Velocity of circumferential fibre shortening

The velocity of circumferential fibre shortening (Vcf) measure reflects the rate and amplitude of left ventricular contraction.

$$Vcf = (LVEDD - LVESD) / (LVEDD \times \text{ejection time})$$

$$Vcf > 1.1 \text{ circumferences/s}$$

Tissue Doppler systolic velocity

Mitral annular systolic velocity (s') is measured in the apical 4 chamber view (Figure 14). It is the velocity that the left ventricular wall moves at during systole (Figure 15). It has been shown to be related to ejection fraction and an s' velocity of greater than 7.5 cm/s has a sensitivity of 79% and a specificity of 88% in predicting normal left ventricular function in the non-pregnant population (Alam, Wardell *et al.* 2000).

Tissue Doppler imaging (TDI) records the velocities of the myocardium (tissue) rather than the velocities of red blood cells (as in pulse wave or continuous wave Doppler). The movement of the myocardial wall using TDI is measured in the apical 4 chamber view. The typical waveform is characterized by three distinctive velocities – the upward deflection (s'), a velocity occurring during systole, followed by a downward velocity (e') corresponding to the initial movement of the myocardium during diastole or early myocardial movement, and then a later diastolic movement relating to the movement of the myocardial caused by the atrial contraction. e' is related to the time constant of isovolumetric relaxation (tau) and elastic recoil. When the ventricular becomes stiffer or relaxation is prolonged, e' velocity is reduced. In addition to the three significant waveforms there are also an additional two waves - one that occurs between the a' and s' wave i.e. during the isovolumetric contraction period, and one that occurs between the s' and e' velocity waveforms i.e. during the isovolumetric relaxation period (Figure 15).

Haemodynamic and systolic data reference ranges, abbreviations and units are summarized in Table 14 (page 90).



Figure 14 Apical 4 chamber view in a healthy pregnant woman – Cardiac model and probe position

The image on the left shows the left atrium (1), left ventricle (2), right atrium (4) and right ventricle (5) in a model of the heart (the apical 4 chamber image). The image on the right shows the position of the cardiac probe on the chest to acquire the apical 4 chamber image. The index marker on the probe is directed towards the left hand side of the subject adjacent to the bed. The probe is held softly and directed under the left breast and along an imaginary line towards the subject's thoracic vertebrae. Note that "chamber 3" (the left ventricular outflow tract) which makes up the 5th chamber in the apical 5 chamber view is not seen in this view. Doppler alignment in the apical 4 chamber view ensures accuracy in measurement of the mitral valve inflow and septal tissue Doppler velocities.

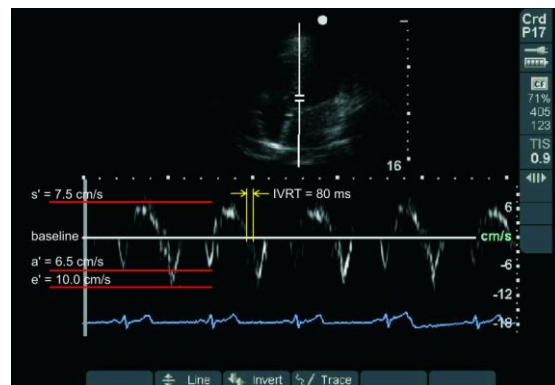
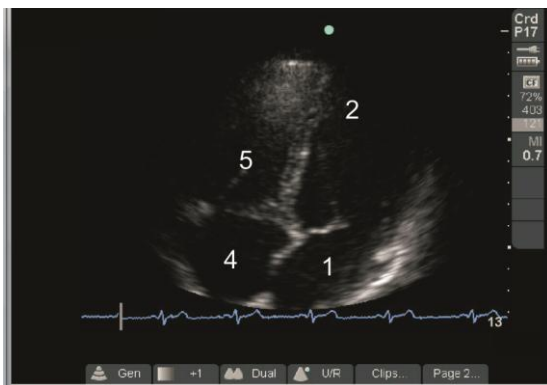


Figure 15 Apical 4 chamber image in a healthy pregnant woman – Transthoracic echocardiography image and septal tissue Doppler waveform

The view on the left is obtained by freezing the two dimensional video image. The image on the right is the septal tissue Doppler waveform of the left ventricle. The septal s' velocity is shown (7.5 cm/s) as well as the two diastolic velocities (e' 10.0 cm/s and a' 6.5 cm/s) and the isovolumetric relaxation time (IVRT) 80 ms. 1 = left atrium; 2 = left ventricle; 4 = right atrium; 5 = right ventricle. Note that systolic velocities move towards the probe and are shown as positive deflections; diastolic velocities move away from the probe and are shown as negative deflections.

3.10. Diastolic function

Diastolic function is the term applied to the ability of the heart to relax and fill efficiently during diastole. The process of diastole is an active process utilising energy and therefore subject to the usual requirements for efficiency. It is also defined as the time in the cardiac cycle from the end of aortic ejection (aortic valve closure) to the development of myocardial tension or isovolumetric contraction. Normal diastolic function of the heart allows adequate filling with minimal intracavity pressure rises. It is divided into early and late phases. Approximately 80% of ventricular filling occurs in the early phase with approximately 20% occurring late as a result of atrial contraction. As diastolic failure develops, the ventricle stiffens and becomes less compliant and, in order to maintain an adequate stroke volume, the left ventricular intracavity pressure increases.

It is important to correctly define the terms stiffness and relaxation. Stiffness refers to a reduction in compliance during the later phases of diastole whereby the intraventricular pressure rises markedly for little change in ventricular pressure. Relaxation is the initial process during diastole and reflects myocardial relaxation.

Doppler echocardiography is the primary method to assess diastolic function in the clinical setting as invasive monitoring devices are rarely used due to their complications. Echocardiographic methods of determining diastolic function have been compared with invasive methods and have been found to be reliable (Anderson 2007).

Early left ventricular diastolic dysfunction leads to a reduction in the initial flow from the left atrium into the left ventricle during rapid filling. This is because the pressure gradient between the left atrium and left ventricle in the rapid filling phase of diastole is reduced due to the increased left ventricular intracavity pressure resulting from delayed relaxation. This is detected by a reduction in mitral valve E and tissue Doppler e' velocities. The atrial component of filling is preserved with increased pressure from the left atrium into the left ventricle. This is demonstrated by an increase in the mitral valve A and tissue Doppler a' velocities. As diastolic failure develops, the left atrial pressure is increased, thereby

restoring the transmitral pressure gradient, but with the clinical consequence of this raised pressure translated back through the pulmonary circulation increasing the likelihood of pulmonary venous congestion and symptoms of heart failure.

3.10.1. Measurements of diastolic function

The combination of mitral valve inflow (left ventricular inflow) and tissue Doppler imaging (TDI) provide reliable, accurate, reproducible and clinically applicable information about the diastolic function of the heart (Bess, Khan *et al.* 2006), (Table 10 Table 11).

Left ventricular inflow velocities and times

From the apical 4 chamber (A4C) image a pulse wave Doppler sample volume (1 – 2 mm caliper separation) was positioned centrally at the tips of the open mitral valve leaflets on the left ventricular side of the valve (Bess, Khan *et al.* 2006; Nagueh, Appleton *et al.* 2009). These velocities are also known as transmitral inflow velocities and the mitral valve inflow Doppler velocities. The waveform consists of the E wave and the A wave. The E wave represents the peak velocity of blood in the early phase of diastole (Early diastolic filling = E wave). The A wave represents the late velocity of blood entering the left ventricular during diastole and is related to the atrial contraction in the later phase of diastole (Atrial contraction, late diastolic filling = A wave). Therefore the mitral inflow or left ventricular inflow waveform demonstrates five measures of diastolic function – the E wave velocity, the A wave velocity, the E/A ratio, and the deceleration time of E wave (DT) and the A wave duration. Simultaneous display of the LVOT pulse wave Doppler and left ventricular inflow (transmitral flow) velocities enables calculation of the isovolumetric relaxation time and isovolumetric contraction time. The average of three measurements was made for the isovolumetric relaxation time, the E wave, the A wave and the DT. The A wave duration was measured at the level of the mitral valve annulus. The average of three consecutive measurements was recorded (Figure 16).

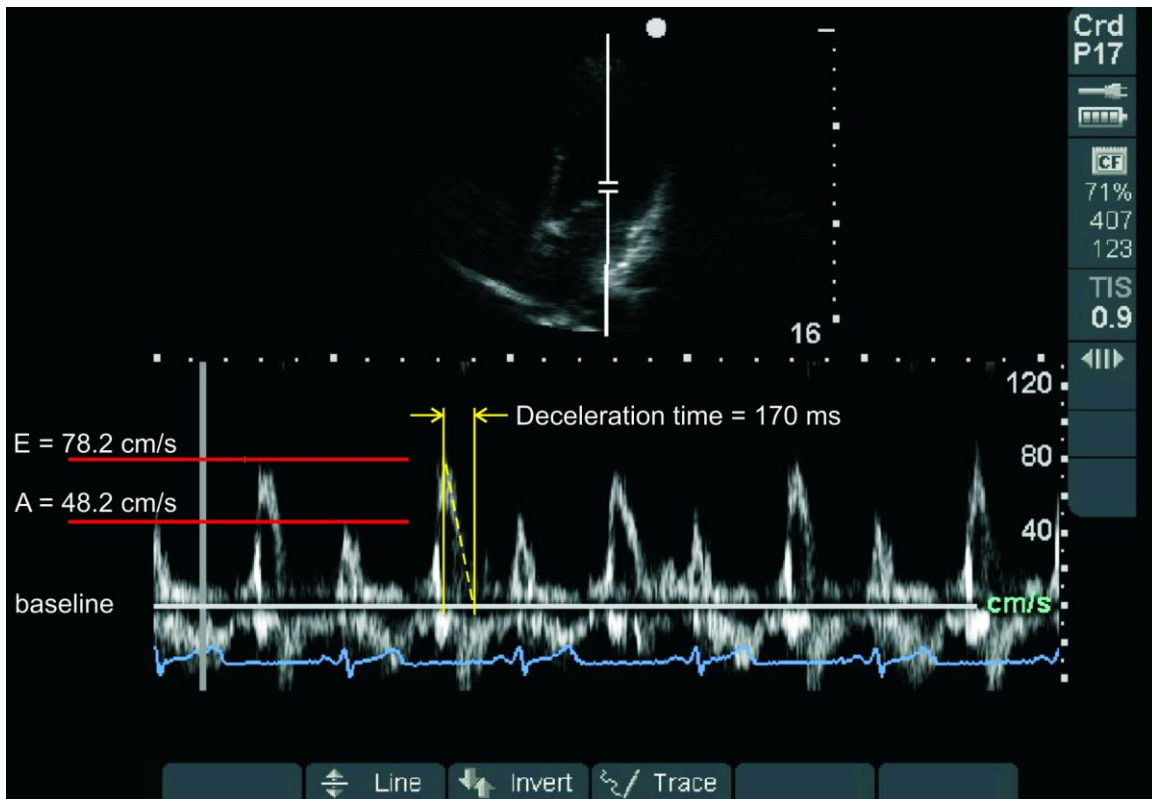


Figure 16 Apical 4 chamber view in a healthy pregnant woman – Mitral valve inflow Doppler waveform

The mitral valve E wave is 78.2 cm/s. The mitral valve A wave is 48.2 cm/s. Therefore mitral valve E/A ratio = 1.6. The mitral valve deceleration time is 170 ms. Velocities are shown as positive deflections as flow is towards the transducer during diastole.

Tissue Doppler diastolic velocities and time

Tissue Doppler is used to measure the motion of the interventricular septum at the level of the mitral valve annulus during diastole. The interventricular septum at the level of the mitral valve annulus moves in the long axis of the heart during systole and diastole (Figure 17). The TDI waveform has both systolic and diastolic components. The diastolic waveform is composed of two movements, an early (e') movement and a later (a') movement. The systolic movement is labelled s'. The pattern obtained in healthy people is that of the e' velocity being greater than the a' velocity.

When the left ventricle becomes stiffer the e' velocity decreases as this movement and velocity represent the rate of ventricular relaxation. The septal e' < 8 cm/s is generally considered abnormal and associated with diastolic dysfunction in non-pregnant

population. There is a clinically important and validated approximation of left ventricular filling pressure (left atrial pressure) using the ratio mitral valve E/septal e'. Using echocardiography it is possible to measure this marker of left ventricular filling pressure and also observe aetiological factors such as pericardial effusions or myocardial disease, and also to quantify the severity of the diastolic changes (Ng and Swanevelder 2010). The association is due to the fact that the blood flowing across the mitral valve during diastole is determined by the pressure gradient between the left atrium and the left ventricle (the left ventricular filling pressure), the movement of the ventricle during diastole (its relaxation kinetics) and age. This determines the mitral valve E wave velocity. The movement of the mitral annulus (the e' velocity) is determined only by the left ventricle relaxation kinetics and age. Therefore, when mitral valve E velocity is divided by the tissue Doppler e' velocity, only the left atrial driving pressure remains and therefore the left ventricular filling pressure (Ng and Swanevelder 2010). A mitral valve E/septal e' > 15 indicates increased left ventricular end diastolic pressure (> 20 mmHg) and a mitral valve E / septal e' < 8 indicates normal left ventricular end diastolic pressure (LVEDP) (< 15 mmHg) (Ommen, Nishimura *et al.* 2000). This relationship is maintained for patients with sinus tachycardia. Sohn and colleagues demonstrated that the relationship between mitral valve E/septal e' is load-independent (Sohn, Chai *et al.* 1997; Sohn, Song *et al.* 1999). From Figure 16 and Figure 17 the mitral valve E/septal e' ratio is 7.8.

Left atrial pressure can also be estimated by $E/e' \times 1.25 + 1.9$
(Nagueh, Middleton *et al.* 1997).

From the A4C a 5 mm sample volume was placed on the interventricular septum at the junction of the left ventricular wall with the fibrous mitral annulus. The average of three measurements of the e', a' and s' waves and the isovolumetric contraction time and isovolumetric relaxation time were recorded (Figure 17). Reference values of isovolumetric relaxation time are 70 – 90 ms with impaired relaxation associated with an increase in isovolumetric relaxation time > 90 ms. (Nagueh, Appleton *et al.* 2009; Nagueh, Kopelen *et al.* 1995; Roscoe 2007).

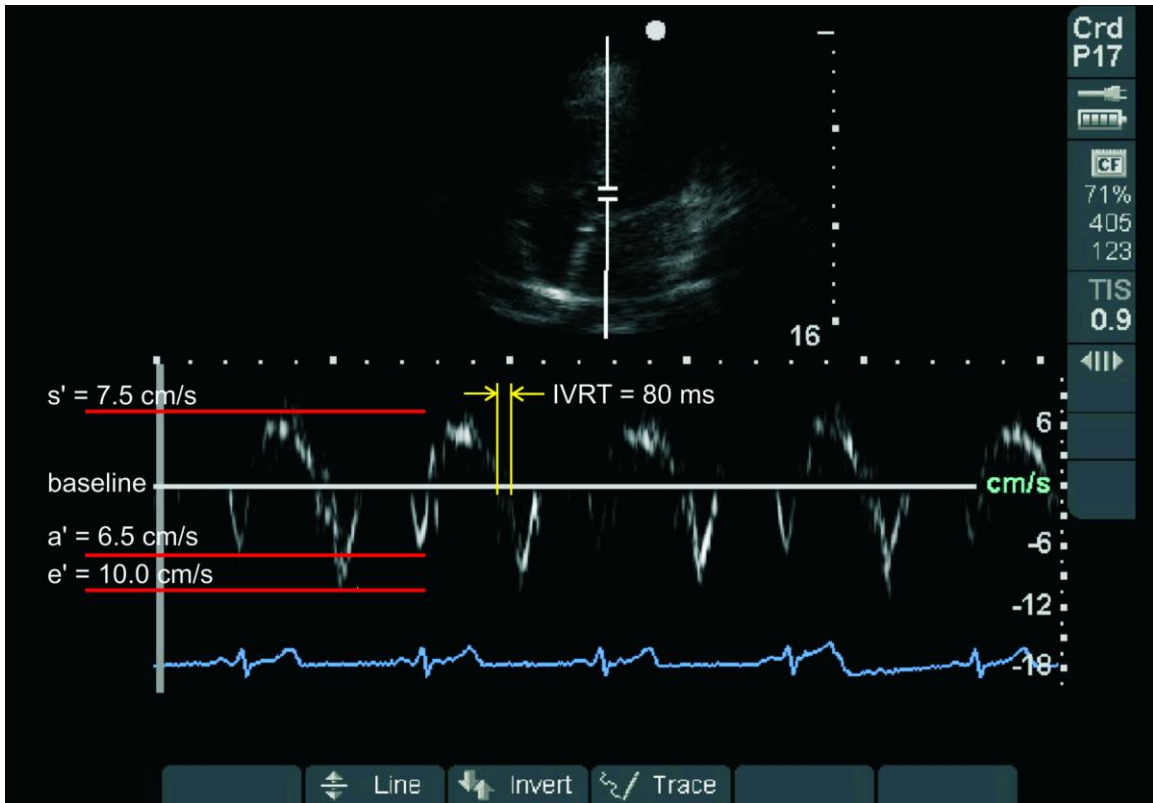


Figure 17 Apical 4 chamber view in a healthy pregnant woman – Septal tissue Doppler waveform

This is the appearance of the septal tissue Doppler waveform in a healthy pregnant woman. The waveform consists of three main deflections. The first deflection above the baseline (0 cm/s line) that occurs during systole is the s' wave. The peak velocity is recorded as the s' velocity. In this case the s' velocity is 7.5 cm/s. The first deflection below the baseline in diastole is the e' wave. The peak velocity of this wave is recorded as the e' velocity. In this example it is 10.0 cm/s. The time period between the end of the s' wave and the beginning of the e' wave is known as the isovolumetric relaxation time (IVRT). In this example it is 80 ms. The second downward deflection during diastole is the a' wave. The peak deflection is recorded as the a' velocity and in this case it is 6.5 cm/s. Therefore the septal e' /septal a' ratio is 1.5. Using Figure 16 (page 77) the mitral valve E/septal e' = 7.8.

Table 10 Reference values for Doppler derived diastolic parameters for age groups – non-pregnant population

Parameter	Age group (yr)			
	16 – 20	21 – 40	41 – 60	> 60
IVRT (ms)	50 ± 9 (32 – 68)	67 ± 8 (51 – 83)	74 ± 7 (60 – 88)	87 ± 7 (73 – 101)
E/A	1.88 ± 0.45 (0.98 – 2.78)	1.53 ± 0.4 (0.73 – 2.33)	1.28 ± 0.25 (0.78 – 1.78)	0.96 ± 0.18 (0.6 – 1.32)
DT (ms)	142 ± 19 (104 – 180)	166 ± 14 (138 – 194)	181 ± 19 (143 - 219)	200 ± 29 (142 – 258)
Septal e' velocity (cm/s)	14.9 ± 2.4 (10.1 - 19.7)	15.5 ± 2.7 (10.1 - 20.9)	12.2 ± 2.3 (7.6 - 16.8)	10.4 ± 2.1 (6.2 - 14.6)
Septal e'/a'	2.4*	1.6 ± 0.5 (0.6 - 2.6)	1.1 ± 0.3 (0.5 - 1.7)	0.85 ± 0.2 (0.45 - 1.25)

*calculated value; IVRT = isovolumetric relaxation time; DT = mitral valve deceleration time; E = Mitral valve E wave velocity; A = Mitral valve A wave velocity
Mean ± SD (95% confidence interval).

Adapted from (Nagueh, Appleton *et al.* 2009).

Table 11 Mitral valve E/Septal e' relationship to left ventricular filling pressure – non-pregnant population

Author	Participant number	Value	Filling pressure	Sensitivity	Specificity
(Ommen, Nishimura <i>et al.</i> 2000)	100	> 15	M – LVDP > 15 mmHg	NR	86
(Kim and Sohn 2000)	200	≥ 9	Pre-A > 12 mmHg	81	80
(Rivas-Gotz, Manolios <i>et al.</i> 2003)	54 55	≥ 20 ≥ 12	PCWP > 15 mmHg	59 70	88 60

M-LVDP = mean left ventricular diastolic pressure; Pre-A = left ventricular pressure prior to atrial contraction; PCWP = pulmonary capillary wedge pressure; NR = not recorded

Adapted from (Anderson 2007).

Left atrial diameter

In the PLAX view, the cursor was directed perpendicular to the long axis of the aorta and through the aortic root at the level of the aortic valve leaflets. Measurement occurred at the maximal left atrial size immediately prior to mitral valve opening i.e. inner edge (trailing) of the posterior wall of the aorta to the inner edge (leading) of the posterior wall of the left atrium, at the end of ventricular systole (end of T wave). The average of three measurements was taken. The reference range for non-pregnant females for left atrial diameter is 2.7 – 3.8 cm (Lang, Bierig *et al.* 2005).

3.11. Structure elements

3.11.1. Measurements of structural elements

Left ventricular mass calculation

Echocardiography is the method of choice for identifying and quantifying left ventricular hypertrophy.

A two dimensional (2D) image was obtained and was used to guide the M-mode cursor placement. The PLAX view was used and also the PSAX at the level of the papillary muscles. The American Society of Echocardiography (ASE) method was adopted, which uses a regression equation derived from the calculation of left ventricular mass. Uncorrected measures without regression scaling tend to overestimate the left ventricular mass by approximately 25% (Anderson 2007).

$$\text{Left ventricular mass} = [1.04 \times ([\text{LVID} + \text{PWT} + \text{IVST}]^3 - \text{LVID}^3)] \times 0.8 + 0.6$$

Where 1.04 = specific gravity of the myocardium (g/ml).

The measurements are in cm made at end diastole using M-mode in the PLAX image. End diastole is defined as the beginning of the QRS complex using the leading-edge to leading-edge technique. The left ventricular internal dimension (cm) (LVID) was measured from

the posterior endocardial surface of the interventricular septum to the endocardial surface of the posterior wall. The average of three measurements was recorded.

The posterior wall thickness (cm) (PWT) is measured from the endocardial surface to epicardial surface of the posterior wall of the left ventricle. The average of three measurements was recorded. The interventricular septal thickness (cm) (IVST) was measured at end-diastole between the anterior and posterior endocardial surfaces of the interventricular septum. The average of three measurements was recorded.

Diastolic and structural data reference ranges, abbreviations and units are summarized in Table 15 (page 91).

3.12. Systemic vascular system

3.12.1. Measurements of peripheral vascular system

Blood pressure measurement

Baseline systolic and diastolic blood pressure was obtained non-invasively using a calibrated sphygmomanometer on the left arm recording the diastolic value as Korotkoff V according to the American Heart Association (Pickering, Hall *et al.* 2005). The onset of flow was heard and systolic pressure was recorded as the first appearance of a clear tapping sound (phase 1 Korotkoff I) and the diastolic level at the point at which the sounds disappear (phase 5 Korotkoff V). The measurement was made to the closest 2 mmHg. The optimum bladder cuff size was used. This was where the width equals the arm circumference/2.5 and so that the cuff was able to be placed approximately 3 cm above the elbow crease. Accuracy of this type of non-invasive device is approximately within 10% of direct arterial blood pressure measurements in the non-pregnant population although accuracy may differ in the pregnant population. The mean arterial pressure (MAP) was calculated.

Mean arterial pressure

MAP = (Systolic blood pressure + (2 × diastolic blood pressure))/3

The vascular system (blood pressure) systolic pressure is predominantly dependent on stroke volume and aortic compliance. The diastolic pressure is predominantly dependent on peripheral vascular resistance.

Systemic vascular resistance (SVR)

SVR (dynes.s/cm⁵) = Calculated mean arterial pressure × 80/cardiac output (l/min)

Right atrial pressure not included as assumed to be small and not directly measured.

3.13. Overall myocardial performance

3.13.1. Measurements of overall myocardial performance

The end results of the combination of systolic, diastolic and the intrinsic and extrinsic structural aspects of the heart is the ability to efficiently generate a cardiac output for a given metabolic requirement at the lowest possible pressure. An overall summary with haemodynamic calculations is given in Table 16 (page 92).

Tei index

The Tei index is the ratio of combined isovolumetric contraction and relaxation time to ejection time. Referring to Figure 18,

$$\text{Tei index} = (a-b)/b$$

The reference values of the Tei index are 0.33 ± 0.09 . The Tei index is used as a measure of global myocardial performance.

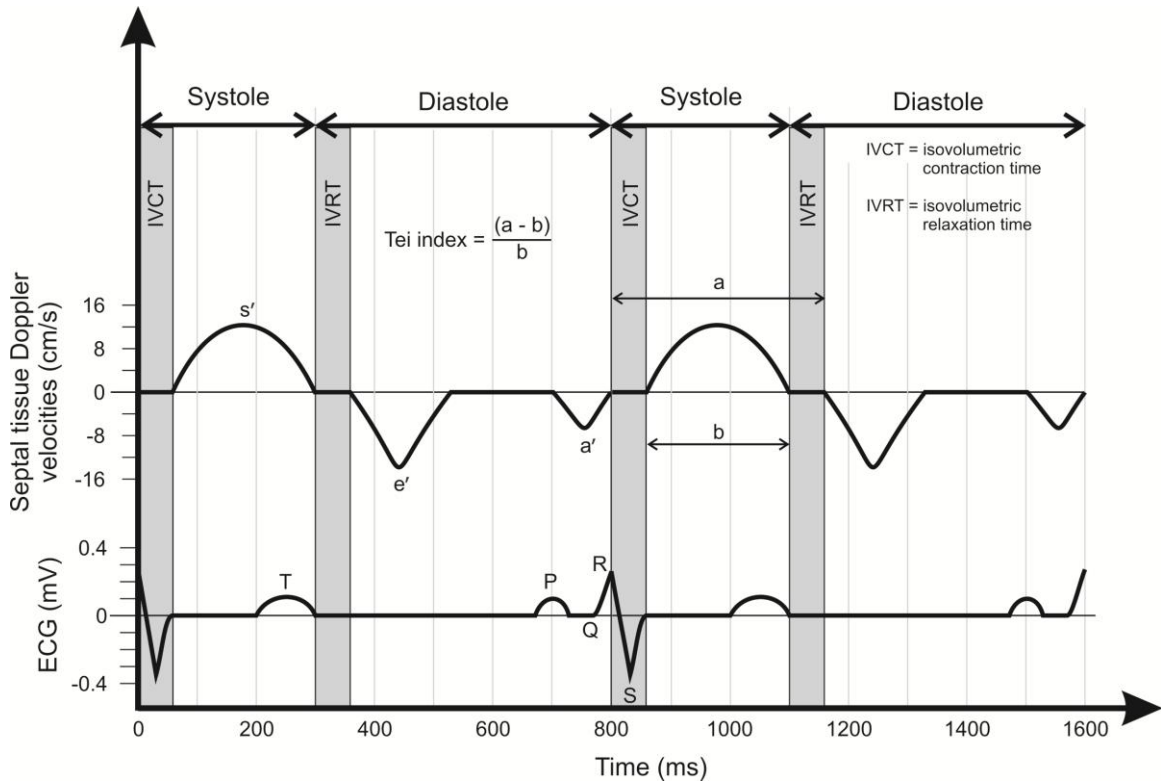


Figure 18 Tei index measurement from the tissue Doppler waveform

3.14. Body surface area calculation

DuBois and Dubois (1916) define body surface area (BSA) as

$$BSA (m^2) = 0.007184 \times \text{weight (kg)}^{0.425} \times \text{height (cm)}^{0.725} .$$

3.15. Statistical methods

Background

Human Study 1 was conducted with the following aims: establishing the technique of transthoracic echocardiography in the perioperative setting; establishing a research environment in the hospital and assessing the applicability of the technique in healthy term pregnant women; to define the range of normal cardiac output in this group of women, and to examine the effect of postural changes. Human Study 1 established the mean and range of data so that the sample size for the healthy comparison group and the women with preeclampsia group sample size could be calculated for Human Study 2.

Statistical methods Human Study 1

Human Study 1 had one group of women. A convenience sample of 30 women was chosen for Human Study 1 as it was representative of the larger population of healthy term pregnant women based on the assumption of a normal distribution of data. This was subsequently confirmed with the performance of F tests on the data. The postural changes were included in this study. The postural changes that were recorded did not use predetermined changes in velocity time integral (VTI), heart rate (HR), cardiac output and cardiac index due to paucity of data in the area and as the aim of this preliminary study was to observe what changes, if any, occurred with posture.

Demographic and obstetric data were displayed as mean and standard deviation, median with interquartile ranges, or number and percentage, as appropriate. Analysis of variance (ANOVA) and comparisons of systolic and diastolic variables, heart rate and blood pressure were performed using the General Linear Model with significant P value defined as < 0.05 . Once observed, ANOVA was used to compare cardiac output, cardiac index, VTI and HR in the four positions. The General Linear Model was used, with Dunnett's *post hoc* analysis comparing each position against position 1 (baseline, level position P1), with significant P value defined as < 0.05 .

Pearson's correlation coefficient (r) was used to test the linear association between cardiac output and fetal weight.

The analyses were performed using Minitab 15® Statistical software package.

Statistical methods Human Study 2

From the data from Human Study 1, a sample size of 40 healthy women compared with 40 women with untreated preeclampsia was estimated to be able to detect a 750 ml cardiac output difference between these two groups with a power of 0.8 and P value < 0.05 . Therefore, 40 women with untreated preeclampsia were gestationally matched with 40 healthy pregnant women in this study.

Human Study 2 had three groups of women:

1. non-pregnant women (NP),
2. healthy pregnant women (HP) gestationally matched to
3. women with untreated preeclampsia (UnRxP).

One-way ANOVA was used across the three groups. If $P > 0.05$, no further analysis was performed. If $P < 0.05$, the Tukey HSD (Tukey-Kramer) test was applied for all possible pairwise comparisons of means.

All the haemodynamic measurements from one woman were assumed to be a family of measurements, so the Ryan-Holm stepdown Bonferroni procedure was applied to the outcomes of the ANOVA in order to control the risk of Type 1 error. This generated P' values.

Correlation statistics were applied to the data to determine predictors of cardiac function. If the P value for the correlation statistic was < 0.05 the least product regression (LPR) was calculated (Ludbrook 1997; Ludbrook 1998; Ludbrook 2002).

Analyses were performed using SYSTAT version 12 (Systat Software Inc., Chicago IL).

Statistical methods Animal Study 1

Statistical analysis used the General Linear Model and unpaired t-test comparisons.

Inter- and intra observer reliability measurements - General issues

Inter- and intraobserver relationships (also referred to as inter-rater and intra-rater reliability) were analysed using the Bland-Altman methodology and determining the limits of agreement. A correlation coefficient was also calculated and P value attached. The null hypothesis was rejected if $P < 0.05$ for each of the P or P' variables.

Interobserver reliability measurements (Human and Animal studies)

Interobserver observations were defined as the average of three consecutive measurements of LVOT and VTI from the two independent observers. Reliability of interobserver (inter-

rater) and intraobserver measurements of LVOT and VTI were analysed using the Bland-Altman method.

The use of the Bland-Altman figure also shows the limits of agreement; these are the values that are two standard deviations away from the mean. The limits of agreement give an indication of how good or bad agreement between two measurements might be. For each particular variable it is the clinician or reader who judges whether the differences are acceptable or not.

As the limits of agreement are estimates based on a single sample, describing their precision using a confidence interval is frequently done.

Intraobserver reliability measurements (Human and Animal studies)

Intraobserver observations, defined as observations by the same operator when performing the real time TTE and used to analyse reliability, were the three consecutive measurements of both the LVOT and VTI. Bland-Altman plots were generated and show the difference between measurements of the raters (or occasions) against the mean of the measurements of the raters (or occasions). These are appropriate plots for examining the agreement between the two different raters or occasions. They allow examination of the average difference in agreement and the spread of the differences in agreement and to check for any systematic relationship between the level of measurement and the differences; ideally the differences between the measurements will not be related to the magnitude of the measurements taken (Bland and Altman 1986; Bland and Altman 1995).

The intraobserver reliability was measured from the repeated first measurement of LVOT diameter and LVOT VTI from each woman.

Table 12 Haemodynamic and systolic data - Acquisition and measurement method

Variable	Acquisition method	Method of measurement
Haemodynamic and systolic data		
Systolic blood pressure	Left arm, left lateral position after 10 minutes rest	Korotkoff I
Diastolic blood pressure	Left arm, left lateral position after 10 minutes rest	Korotkoff V
Left ventricular outflow tract (LVOT) diameter	Parasternal long axis (PLAX). A good quality image was defined as one in which the aortic valve could be seen and the structure looked tubular. A zoomed two-dimensional image in systole during quiet breathing was recorded.	Zoomed LVOT image was frozen during systole. End systole was defined as the frame preceding early diastolic mitral valve opening. End diastole was defined as the onset of the Q wave of the QRS complex. LVOT was measured three times perpendicular to the aortic root with the anterior caliper positioned at the junction of the anterior coronary cusp and the interventricular septum. The posterior caliper was positioned at the junction of the posterior non-coronary aortic cusp and the anterior mitral valve leaflet. The three measurements were averaged.
Left ventricular outflow tract velocity time integral (VTI)	Apical 5 Chamber (A5C) view, with the Doppler integration angle <20° to flow. An optimal image was assessed by maximal chamber size, a vertical long axis and maximal mitral valve opening size. Pulse wave Doppler was used with a 3 mm sample volume placed within the LVOT approximately 0.5 cm proximal to the aortic valve. At least three consecutive waveforms were recorded.	The leading edge of the velocity spectrum of three consecutive beats was traced and the three measurements were averaged.
Heart rate (HR)	The ECG recording from the LVOT VTI waveform image.	$(1/R-R \text{ interval (sec)}) \times 60$
Left ventricular end diastolic diameter (LVEDD)(left ventricular internal diameter)	An M-mode image was recorded with the cursor placed perpendicular to the long (PLAX) and short axis (PSAX mid-papillary level) of the LV just distal to the tips of the open mitral valve leaflets.	LVEDD measurements were made at the onset of QRS complex inner edge to inner edge and the average of three measurements was taken.
Left ventricular end systolic diameter (LVESD)	An M-mode image was recorded with the cursor placed perpendicular to the long (PLAX) and parasternal short axis (PSAX) mid-papillary level of the LV just distal to the tips of the open mitral valve leaflets.	LVESD measurements were made at the point marking the peak posterior deflection of the interventricular septum inner edge to inner edge and three measurements were recorded and averaged.
Left ventricular end diastolic area (LVEDA)	PSAX image was recorded at the mid-papillary level	LVEDA was calculated by freezing the largest image and tracing the endocardial surfaces excluding papillary muscles. Three measurements were recorded and averaged.
Left ventricular end systolic area (LVESA)	PSAX image was recorded at the mid-papillary level	LVESA was calculated by freezing smallest image trace endocardial surfaces excluding papillary muscles. Three measurements were recorded and averaged.
Isovolumetric contraction time (IVCT)	Apical 4 chamber (A4C) view with tissue Doppler Imaging at an interrogation angle <10° to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the LV wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The IVCT was the time measured from the end of the a' wave until the beginning of the s' wave. The time was measured for three consecutive waves and then averaged.
Velocity time integral (VTI) time	A5C view, with the Doppler integration angle <20° to flow. An optimal image was assessed by maximal chamber size, a vertical long axis and maximal mitral valve opening size. Pulse wave Doppler was used with a 3 mm sample volume placed within the LVOT approximately 0.5 cm proximal to the aortic valve. At least three consecutive waveforms were recorded.	The VTI time was the time measured from the start of the VTI wave until the end of the wave. The time was measured for three consecutive waves and then averaged.
Septal s' velocity	A4C view with Tissue Doppler Imaging at an interrogation angle <10° to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the LV wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The septal s' wave was the first upward (positive) waveform during systole of each beat. The peak velocity of the s' waveform was measured in three consecutive beats and the measurements were averaged.
Septal s' time	A4C view with Tissue Doppler Imaging at an interrogation angle <10° to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the LV wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The septal s' time was the time measured from the start of the s' wave until the end of the wave. The time was measured for three consecutive waves and then averaged.

All measurements performed in left lateral position for all study participants.

Table 13 Diastolic and structural data - Acquisition and measurement method

Variable	Acquisition method	Method of measurement
Diastolic data		
Left atrial (LA) diameter	In the parasternal long axis view (PLAX) the cursor was directed perpendicular to the long axis of the aorta and through the aortic root at the level of the aortic valve leaflets.	Measurement occurred at the maximal LA size immediately prior to mitral valve (MV) opening i.e. posterior aortic wall to the posterior wall of the left atrium at the end of ventricular systole (Isovolumetric relaxation time (IVRT)) i.e. end of T wave – inner edge (trailing) of the posterior wall of the aorta to the inner edge (leading) of the posterior wall of the left atrium. Three measurements were taken and then averaged.
Septal e' velocity	Apical 4 chamber (A4C) view with Tissue Doppler Imaging at an interrogation angle <10% to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the left ventricle (LV) wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The septal e' wave was the first downward (negative) waveform during diastole of each beat. The peak velocity of the e' waveform was measured in three consecutive beats and the measurements were averaged.
Septal a' velocity	A4C view with Tissue Doppler Imaging at an interrogation angle <10% to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the LV wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The septal a' wave was the second downward waveform during diastole of each beat. The peak velocity of the a' waveform was measured in three consecutive beats and the measurements were averaged.
Isovolumetric relaxation time	A4C view with Tissue Doppler Imaging at an interrogation angle <10% to flow. With a sample volume of 5 mm the cursor was placed on the septum at the junction of the left LV wall and the fibrous mitral annulus. At least three consecutive waveforms were recorded.	The IVRT was the time measured from the end of the s' wave until the beginning of the e' wave. The time was measured for three consecutive waves and then averaged.
Mitral valve E wave velocity	From the A4C image, pulse wave Doppler (PWD) sample volume of 1-2 mm was positioned centrally at the tips of the open mitral valve leaflets on the LV side of the valve. At least three consecutive waveforms were recorded.	The mitral valve E wave was the first upward (positive) waveform of each beat. The peak velocity of the E waveform was measured in three consecutive beats and the measurements were averaged.
Mitral valve A wave velocity	From the A4C image PWD sample volume of 1-2 mm was positioned centrally at the tips of the open mitral valve leaflets on the LV side of the valve. At least three consecutive waveforms were recorded.	The mitral valve A wave was the second upward waveform of each beat. The peak velocity of the A waveform was measured in three consecutive beats and the measurements were averaged.
Deceleration time	From the A4C image PWD sample volume of 1-2 mm was positioned centrally at the tips of the open mitral valve leaflets on the LV side of the valve. At least three consecutive waveforms were recorded.	The deceleration time was the time measured from the peak of the mitral valve E wave until the time when that waveform reached the zero velocity time. Three consecutive waveform times were measured and then averaged.
Structural data		
Posterior wall thickness	An M-mode image was recorded with the cursor placed perpendicular to the long (PLAX) and parasternal short axis (PSAX) mid-papillary level, of the LV just distal to the tips of the open mitral valve leaflets.	The posterior wall thickness (PWT) was measured from the endocardial surface to epicardial surface of the posterior wall of the left ventricle. Three consecutive measurements were recorded and then averaged.
Interventricular septum thickness	An M-mode image was recorded with the cursor placed perpendicular to the long (PLAX) and short axis (PSAX mid-papillary level) of the LV just distal to the tips of the open mitral valve leaflets.	The interventricular septal thickness (IVST) was measured at end-diastole between the anterior and posterior endocardial surfaces of the interventricular septum. Three consecutive measurements were recorded and then averaged

All measurements performed in left lateral position for all study participants.

Table 14 Haemodynamic and systolic data - Reference values, abbreviation and units

Variable	Reference range and/or average value	Abbreviation	Units	Key issues/references
Haemodynamic and systolic data				
Systolic blood pressure	90 -139 mmHg (P)	SBP	mmHg	Hypotension < 90 mmHg, gestational hypertension, preeclampsia ≥ 140 mmHg
Diastolic blood pressure	< 90 mmHg	DBP	mmHg	Preeclampsia ≥ 90 mmHg
Left ventricular outflow tract diameter	1.8 – 2.4 (NP) 2.2 (NP)	LVOTd	cm	(Kitzman, Scholz <i>et al.</i> 1988)
Left ventricular outflow tract velocity time integral		LVOT VTI	cm	
Heart rate	60 – 90 BPM	HR	Beats per minute	
Left ventricular end diastolic diameter (left ventricular internal diameter)	3.9 – 5.3 cm (NP female)	LVEDD	cm	(Lang, Bierig <i>et al.</i> 2005) Fractional shortening 27-45% NP females (Lang, Bierig <i>et al.</i> 2005)
Left ventricular end systolic diameter	-	LVESD	cm	
Left ventricular end diastolic area		LVEDA	cm	Fractional area change 36 – 64% (Schiller, Shah <i>et al.</i> 1989)
Left ventricular end systolic area	-	LVESA	cm	
Isovolumetric contraction time	-	IVCT	ms	
Velocity time integral time	> 330 ms (NP)	VTI time	ms	The velocity (VTI time) waveform time is also used in oesophageal Doppler and is known as the corrected flow time.
Septal s' velocity	9.5 ± 1.4 (NP)	ss'	cm/s	s' velocity of greater than 7.5cm/s has a sensitivity of 79% and a specificity of 88% in predicting normal LV function in the NP population (Alam, Wardell <i>et al.</i> 2000).
Septal s' time	-	ss' time	ms	

NP = non-pregnant; P = pregnant; LV = left ventricle

Table 15 Diastolic and structural data - Reference values, abbreviation and units

Variable	Reference range and/or average value	Abbreviation	Units	Key issues/references
Diastolic data				
Left atrial diameter	2.7 – 3.8 cm	LAD	cm	(Lang, Bierig <i>et al.</i> 2005).
Septal e' velocity	10.1 – 20.9 cm/s 15.5 ± 2.7 cm/s Age 21 -40 years	se'	cm/s	septal e' < 8 cm/s considered abnormal and associated with diastolic dysfunction in NP population.
Septal a' velocity	11.3 ± 2.3 cm/s	sa'	cm/s	
Isovolumetric relaxation time (IVRT)	51 – 83 ms 67 ± 8 ms Age 21 -40 years	IVRT	ms	Impaired relaxation is associated with an increase in IVRT > 90ms.
Mitral valve E wave velocity	-	MV E	cm/s	
Mitral valve A wave velocity	-	MV A	cm/s	
Deceleration time	166 ± 14 ms Age 21 -40 years	DT	ms	
Structural data				
Posterior wall thickness	0.6 – 0.9 cm	PWT	cm	(Lang, Bierig <i>et al.</i> 2005).
Interventricular wall thickness	0.6 – 0.9 cm	IVST	cm	(Lang, Bierig <i>et al.</i> 2005).

All non-pregnant (NP) values; Values are mean ± standard deviation.

Table 16 Haemodynamic calculations

Calculations and derived variables	Reference range and/or average value	Abbreviation	Units	Key Issues
Body mass index = weight (kg)/height (m) ²	20 -25 kg/m ² (NP)	BMI	kg/m ²	
Mean arterial pressure = (Systolic blood pressure + 2×Diastolic blood pressure)/3	80 mmHg (NP)	MAP	mmHg	
Body surface area = 0.007184 × weight (kg) ^{0.425} × height (cm) ^{0.725} .		BSA	m ²	
Cardiac output = Stroke volume × Heart Rate (HR)	4 - 8 l/min (NP)	CO	ml/min	
Cardiac index = CO / BSA	2.8 – 4.2 L/min/m ² (NP)	CI	ml/min/m ²	
Systemic vascular resistance = (MAP × 80)/CO(ml) × 1000		SVR	dyne.s/cm ⁵	Precisely MAP – Right atrial pressure (RAP) as pressure gradient. RAP assumed to be close to zero.
Stroke volume = (Left Ventricular Outflow Tract diameter) ² × 0.785 × Velocity Time Integral	30 – 65 ml (NP)	SV	ml (= cm ³)	
Stroke work index = (SV × MAP)/BSA		SWI	mmHg.ml/m ²	
Cardiac work index = SWI × HR × 0.001		CWI	mmHg.L/m ²	
Left ventricular mass = [1.04((LVEDD cm + PWT cm + IVST cm) ³ – LVEDD ³)] × 0.8 + 0.6	80.9 ± 24.7 g (NP)*	LV Mass	g	American Society of Echocardiography Method.
Fractional shortening = (LVEDD – LVESD)/LVEDD × 100	Fractional shortening 27-45% NP females (Lang, Bierig <i>et al.</i> 2005).	FS	%	FS is well correlated with angiographically determined ejection fraction. It is based on the assumption that the shortening of the minor axis of the LV is a reflection of the overall systolic function. It is however a one-dimensional measurement (Klimczak 2008). FS is a load dependent measurement as is effected by heart rate, preload and afterload and myocardial contractility.
Fractional area change = (LVEDA-LVESA)/LVEDA × 100	36 – 64% (Schiller, Shah <i>et al.</i> 1989) (NP)	FAC	%	FAC is the proportional change in area of the LV short axis during systole (Schiller, Shah <i>et al.</i> 1989) (Schiller, Shah <i>et al.</i> 1989).
Septal e' wave velocity/ septal a' wave velocity	1.6 ± 0.5 (NP) Range 0.6 – 2.6 (NP) Age range 21-40 years)	s'e/sa'		
Mitral valve E wave velocity/mitral valve A wave velocity	1.53 ± 0.4 (NP)	MV E/A		MV E/A considered abnormal in if < 1.4
Mitral valve E wave velocity/septal e' wave velocity	< 8 (NP)	MV E/se'		LV filling pressure correlates with MV E/septal e'. MVE/septal e' > 15 indicating increased LVEDP, MVE/septal e' < 8 normal LVEDP.
Velocity of fibre shortening = (LVEDD-LVESD)/LVEDD × VTI time (ejection time)	1.12 – 1.24 (mean 1.18) (NP) > 1.1 (NP)	Vcf	Circumference/s	This measure reflects the rate and amplitude of left ventricular contraction
Rate corrected velocity of fibre shortening = Vcf/√RR interval		Vcfc		
Tei index (tissue Doppler) = [(ss' time +IVRT + IVCT) – ss' time] / ss' time	0.42 ± 0.09 (NP)	Tei index		

*The upper limit of normal is defined as two standard deviations above the mean values of the healthy volunteers in the Framingham Heart Study. The upper limit derived from that study for women is 100 g/m² (McKee, Castelli *et al.* 1971). NP = non-pregnant; LVEDD = left ventricular end diastolic diameter; LVESD = left ventricular end systolic diameter; PWT = posterior wall thickness; IVST = interventricular septum thickness; LVEDA = left ventricular end diastolic area; LVESA = left ventricular end systolic area; IVRT = isovolumetric relaxation time; IVCT = isovolumetric contraction time

Chapter 4. Human Study 1 Cardiac output in healthy pregnant women

4.1. Introduction

The aims of this study were to

1. determine cardiac output using TTE in the baseline resting position and in three additional positions in healthy term pregnant women having elective caesarean birth,
2. assess the acceptability and applicability of TTE in this setting, to establish a research protocol,
3. develop a minimum data set for subsequent studies, and
4. generate baseline mean and variation data for the assessment of sample size for the later comparison study in women with preeclampsia.

4.2. Methods

This study was conducted with Institutional Ethics Committee approval and written informed consent was obtained from all patients (Appendix A). The women recruited to this study were all healthy term pregnant women undergoing elective caesarean birth and were recruited before the day of surgery.

The method for Human Study 1 is summarized in Figure 19.

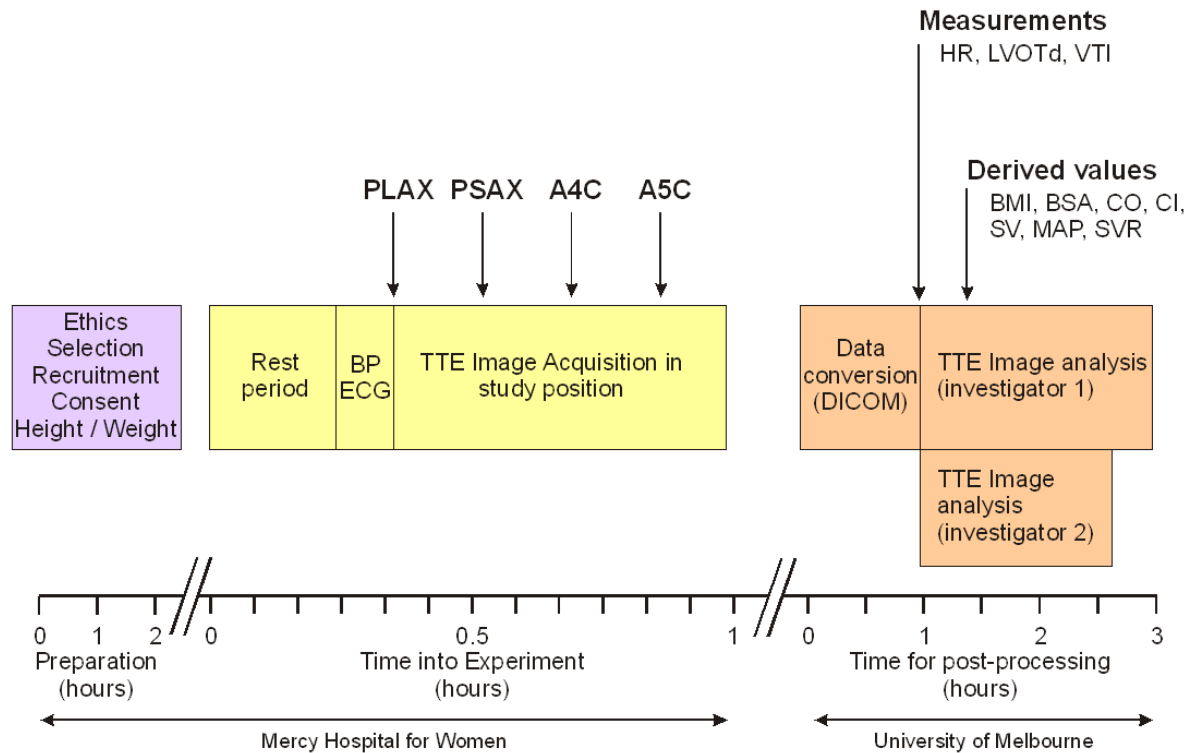


Figure 19 Human Study 1 Experimental procedure and timeline

This figure is divided into three sections – the preparation time during which patient selection, recruitment, consent and weight and weight measurements are recorded (purple section), the experimental procedure (yellow section) and the post-processing procedure where the data are converted and measured by two independent observers (orange section).

TTE = transthoracic echocardiography; BP = blood pressure; ECG = electrocardiograph; PLAX = parasternal long axis view; PSAX = parasternal short axis view; A4C = apical 4 chamber view; A5C = apical 5 chamber view; HR = heart rate; LVOTd = left ventricular outflow tract diameter; VTI = left ventricular outflow tract velocity time integral; BMI = body mass index; BSA = body surface area; CO = cardiac output; CI = cardiac index; SV = stroke volume; MAP = mean arterial pressure; SVR = systemic vascular resistance

Study group

Thirty fasted healthy term women were enrolled and the study was performed on the day of elective caesarean delivery. Fetal heart rate assessment was performed immediately beforehand. Inclusion criteria: age 18-39 years, body mass index (BMI) <33 kg/m², gestation ≥ 37 weeks, singleton pregnancy, fundal height equivalent to dates and American Society of Anesthesiologists (ASA) physical status I or II. Exclusion criteria: patient refusal, after hours surgery, current administration of vasoactive drugs including salbutamol and thyroxine, pre-existing or gestational diabetes, smoking, pre-existing or gestational hypertension or preeclampsia, and known uterine abnormality.

Anaesthesia for the caesarean delivery, use of medications and intravenous fluids were at the discretion of the individual consultant anaesthetists. The study investigators were not involved in the delivery of anaesthesia.

Experimental conditions and techniques and statistical methods

These were conducted as outlined in Chapter 3.

The four positions in which the TTE examination was performed were:

P1 left lateral level baseline, P2 left lateral 20° head-up, P3 left lateral 10° head-down. TTE examinations in these three positions were performed preoperatively. P4 was supine postoperatively in the post anaesthesia care unit. Based on clinical experience with positioning of pregnant women, tilts for P2 and P3 were chosen as maximal values that still ensured patient comfort.

Postoperative review:

During the postoperative review, satisfaction with echocardiography was assessed as unsatisfied, satisfied, or very satisfied with being involved in the study.

4.3. Results

Thirty women were recruited to the study and agreed to participate. In two women apical images could not be obtained in the P1 position due to discomfort. Apical images could

not be obtained in three women in the P2 and P3 positions due to discomfort. One woman developed haemodynamically stable rapid atrial fibrillation in the PACU and was excluded from the P4 data analysis. PLAX images were obtained from all 30 women and data were complete for all four positions in 26 of the 30 women. Demographic and obstetric characteristics of the women are shown in Table 17.

Five women stated they fell asleep and eight women found the head-down position uncomfortable due to dizziness (5), headache (1) and increased abdominal pressure (2). The average time to perform the three preoperative examinations was 22 min. All women underwent neuraxial anaesthesia, with a resultant Bromage 3 motor block (inability to move feet or knees) and bilateral sensory blockade to the T4 dermatome before the surgery and in the PACU at the time of TTE measurements.

Cardiac output, cardiac index, velocity time integral, and heart rate data are shown in Table 18, Table 19 and

Figure 20. Cardiac output in the P1 position varied widely among patients (2518-7486 ml/min). There was a significant decrease in cardiac output in the P3 position which returned to baseline following delivery. Compared to the P1 position, the velocity time integral (which is proportional to stroke volume) decreased in the P3 and increased in the P4 positions, and was associated with reduced heart rate in the P4 position.

All the women were either satisfied or very satisfied at participating in the study and all would consider participating again. All women were sent a copy of the results in the form of the publication arising from the work (Dennis, Arhangelschi *et al.* 2010).

Table 17 Human Study 1 Demographic and obstetric data

Variable	n = 28
Age (years)	32 ± 3.5
ASA I	26 (93%)
Number Caucasian	25 (89.3%)
Body mass index (kg/m ²)	28.3 ± 3.5
Systolic blood pressure (mmHg)	119 ± 13.0
Diastolic blood pressure (mmHg)	71.8 ± 11.6
Heart rate (beats/min)	81.5 ± 9.3
Haemoglobin (g/dl)	117 ± 9.7
Parity	1 [1]
Gravida	3 [2]
Gestation (weeks)	39 [1]
Birth weight (g)	3424 ± 362

Values are mean ± standard deviation; median [Interquartile range] or number (%) as appropriate; ASA = American Society of Anesthesiologists.

Table 18 Human Study 1 Cardiac output, velocity time integral, cardiac index and heart rate in four positions - Term healthy women

Position	n	Cardiac output		Cardiac index		Velocity time integral (cm)		Heart rate (Beats/min)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
P1	28	4407	1109	2390	570	18.8	3.2	77.6	9.8
P2	27	4182	825	2270	450	17.4	2.5	80.0	10.3
P3	27	4031♦	798	2190	440	17.1♦	3.3	79.4	10.7
P4	26	4641	1064	2520	540	22.8♦	3.4	67.8♦	10.5

n is sample size, SD is standard deviation. P1 left lateral level baseline; P2 left lateral 20° head-up position; P3 left lateral 10° head-down position; P4 supine postoperatively.

♦ *P* value < 0.05 for comparison against baseline P1 values (Analysis of variance (ANOVA) with Dunnett's *post hoc* Analysis).

Table 19 Human Study 1 Analysis of variance and comparisons of measures of cardiac output in four positions

Measure			
Cardiac output		$F_{(3, 77)} = 4.48, P = 0.006$	
Comparisons	Mean difference	95% confidence interval	<i>P</i> value
Position 2 – Position 1	-248.6	-657.1, 160.0	0.30
Position 3 – Position 1	-410.7	-819.3, -2.1	0.05
Position 4 – Position 1	176.8	-237.1, 590.6	0.60
Velocity time integral		$F_{(3, 77)} = 30.8, P < 0.001$	
Comparisons	Mean difference	95% confidence interval	<i>P</i> value
Position 2 – Position 1	-1.44	-2.96, 0.07	0.07
Position 3 – Position 1	-1.74	-3.26, -0.23	0.02
Position 4 – Position 1	3.82	2.29, 5.36	< 0.001
Cardiac index		$F_{(3, 77)} = 4.37, P = 0.007$	
Comparisons	Mean difference	95% confidence interval	<i>P</i> value
Position 2 – Position 1	-0.13	-0.35, 0.09	0.40
Position 3 – Position 1	-0.21	-0.43, 0.00	0.06
Position 4 – Position 1	0.10	-0.12, 0.32	0.60
Heart rate		$F_{(3, 77)} = 20.7, P < 0.001$	
Comparisons	Mean difference	95% confidence interval	<i>P</i> value
Position 2 – Position 1	2.36	-1.82, 6.54	0.40
Position 3 – Position 1	1.16	-3.02, 5.34	0.80
Position 4 – Position 1	-10.22	-14.45, -5.99	< 0.001

For each measure, an analysis of variance comparing the four conditions is reported in this table (Table 19). Table 19 also gives the mean difference between each of positions 2, 3 and 4, and position 1. It provides a 95% confidence interval and *P* value for these comparisons with position 1. These are based on Dunnett's procedure.

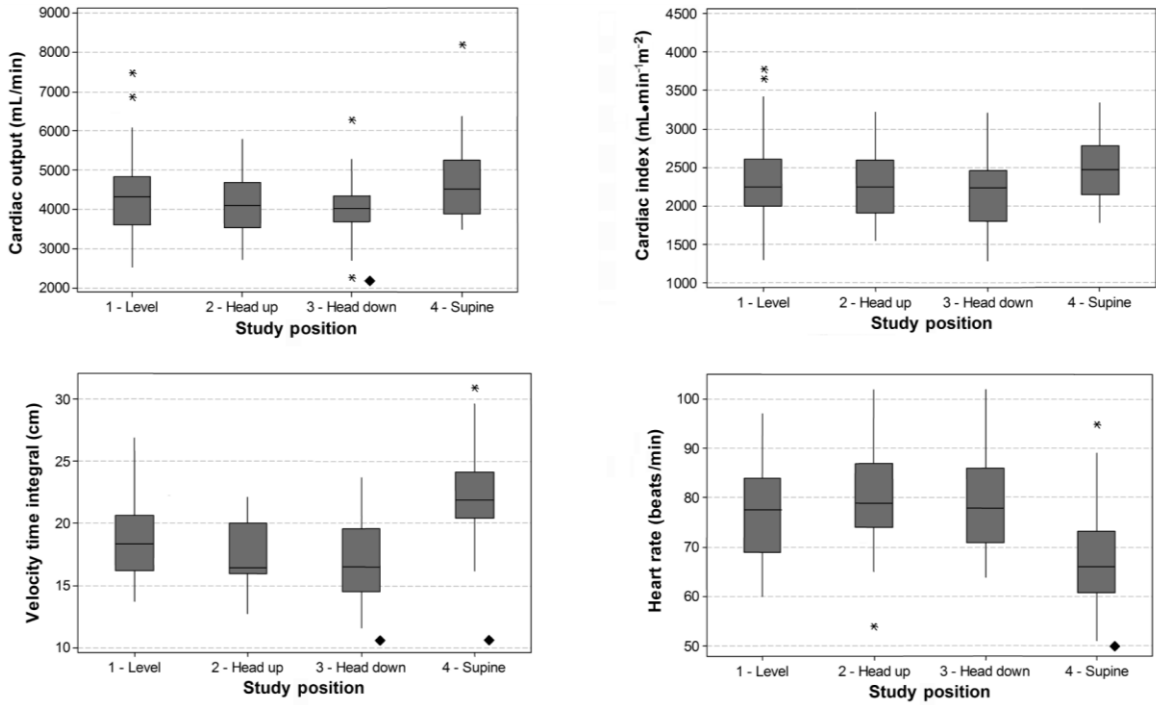


Figure 20 Human Study 1 Box plots for cardiac output, cardiac index, velocity time integral and heart rate, measured at each position

The horizontal line within the box is the median value and the upper and lower box limits are the interquartile range Q1 – Q3. The vertical whiskers extend to the upper and lower values defined by $1.5 \times$ interquartile range. Outliers are indicated by an *. ♦ is P value < 0.05 for comparison against Study Position 1 level values (Analysis of variance (ANOVA) with Dunnett's *post hoc* Analysis).

Analysis of groups according to initial cardiac output

The 28 women were divided into three groups, according to their cardiac output in position 1 (P1) (Figure 21, Figure 22 and Figure 23). The cut-offs for the groups were 3500 and 5000 ml/min.

There were six women with position 1 cardiac output less than 3500 ml/min and five with a value more than 5000 ml/min. Seventeen women had results between 3500 ml/min and 5000 ml/min. The small numbers in the groups makes formal comparisons between the groups relatively imprecise and due to the small *post hoc* subgroup numbers, these groups were not formally compared.

Figure 21, Figure 22 and Figure 23 show the mean for the outcome of interest with a 95% confidence interval for a particular group (defined by position 1 cardiac output) and in different positions. The confidence intervals provide a guide to comparisons between groups, but not between positions.

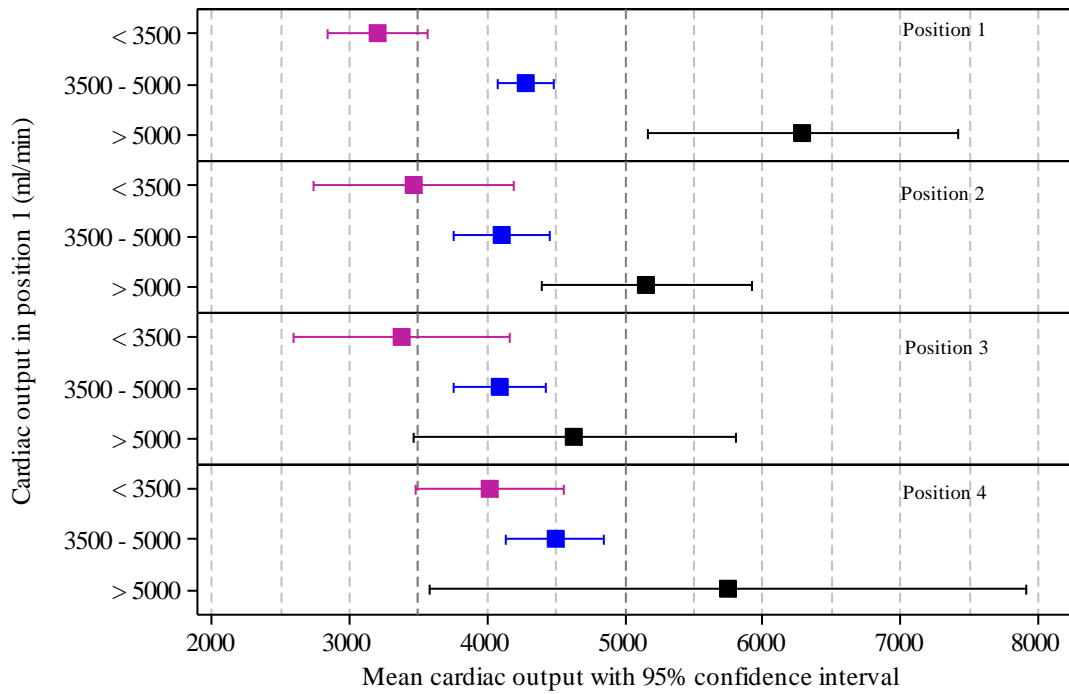


Figure 21 Human Study 1 Cardiac output changes with posture according to baseline cardiac output

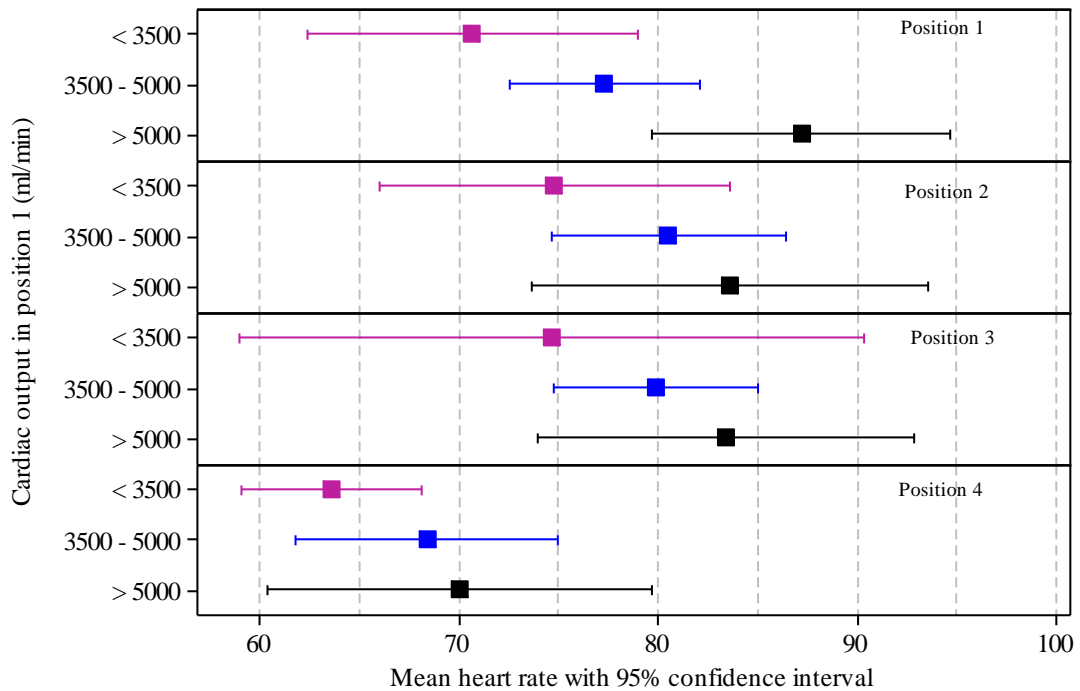


Figure 22 Human Study 1 Heart rate changes with posture according to baseline heart rate

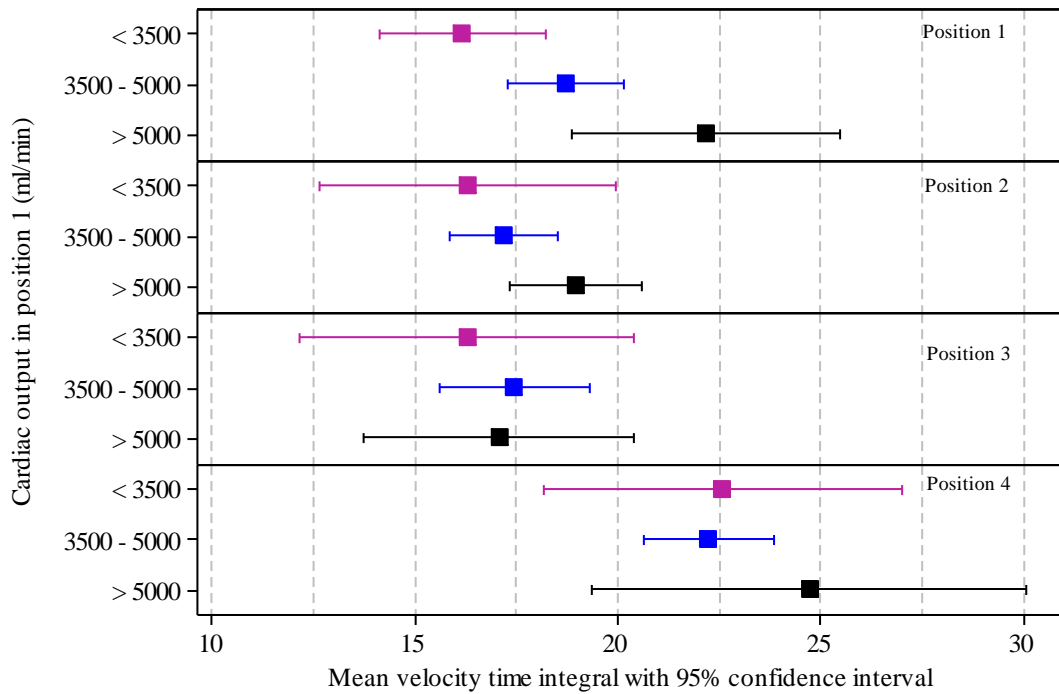


Figure 23 Human Study 1 Velocity time integral changes with posture according to baseline velocity time integral

Relationship between cardiac output and fetal weight

Figure 24 shows the relationships between cardiac output in the four positions and fetal weight. Table 20 provides Pearson’s correlation with a 95% confidence interval, and a *P* value for a test of no underlying correlation between fetal weight and cardiac output.

The Pearson’s correlations (*r*) between fetal weight and cardiac output in each position were small with wide confidence (CI) intervals, indicating that the linear relationship was not strong (all $r \leq 0.25$, CI -0.27 to 0.58, $P \geq 0.200$) (Figure 24 and Table 20).

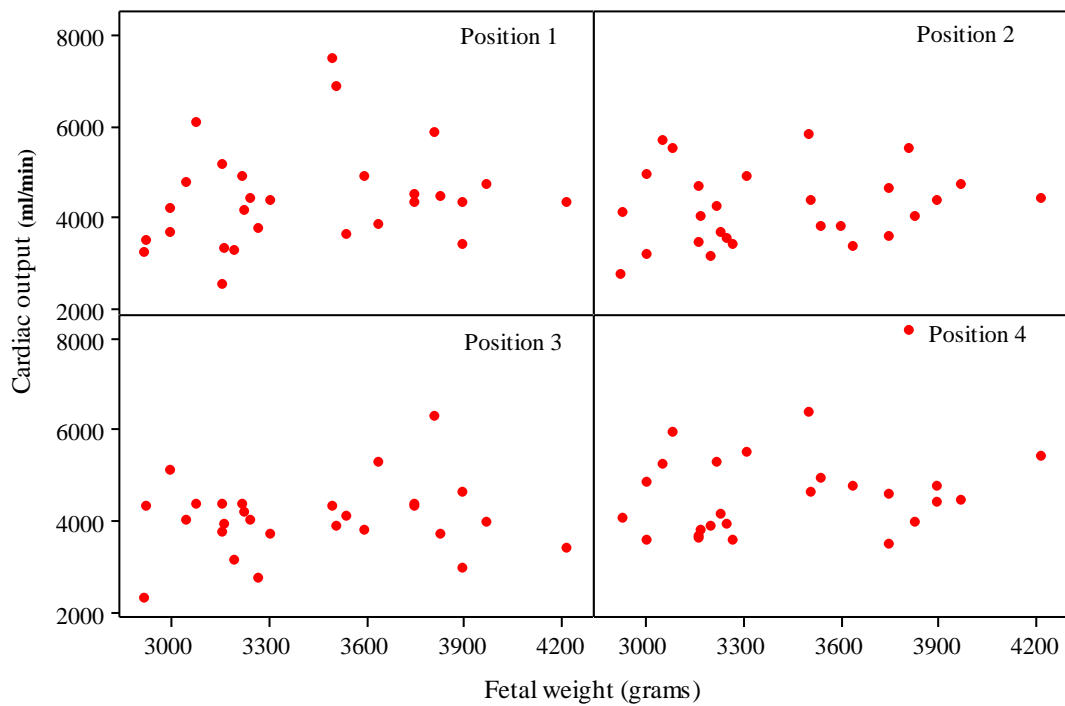


Figure 24 Human Study 1 Cardiac output and fetal weight according to position

Table 20 Human Study 1 Correlations between cardiac output and fetal weight

	Position			
	1	2	3	4
Pearson's <i>r</i>	0.19	0.17	0.13	0.25
95% confidence interval	-0.20, 0.53	-0.23, 0.51	-0.27, 0.48	-0.15, 0.58
<i>P</i> value	0.3	0.4	0.5	0.2

Interobserver and intraobserver reliability are shown in Table 21 and Figure 25, Figure 26, Figure 27 and Figure 28. The differences were small and the limits of agreement acceptable.

Table 21 Human Study 1 Interobserver and intraobserver differences

	Mean value, Mean difference (cm)		Lower limit of agreement (cm)		Upper limit of agreement (cm)	
		95% CI		95% CI		95% CI
Interobserver						
LVOT	1.96, 0.02	-0.02, 0.04	-0.26	-0.31, -0.21	0.29	0.24, 0.34
VTI	19.27, -1.06	-1.38, 0.74	-4.44	-5.00, -3.88	2.23	1.77, 2.88
Intraobserver						
LVOT	1.96, 0.00	-0.02, 0.02	-0.14	-0.17, -0.12	0.15	0.12, 0.17
VTI	18.33, -0.22	-0.42, -0.03	-2.26	-2.60, -1.93	1.82	1.48, 2.15

LVOT = left ventricular outflow tract; VTI = velocity time integral. CI is confidence interval. Mean value is average of LVOT or VTI from the two observer means or the two occasion means. Estimated mean value and mean difference between the two observers (or the two occasions) and limits of agreement with 95% confidence intervals.

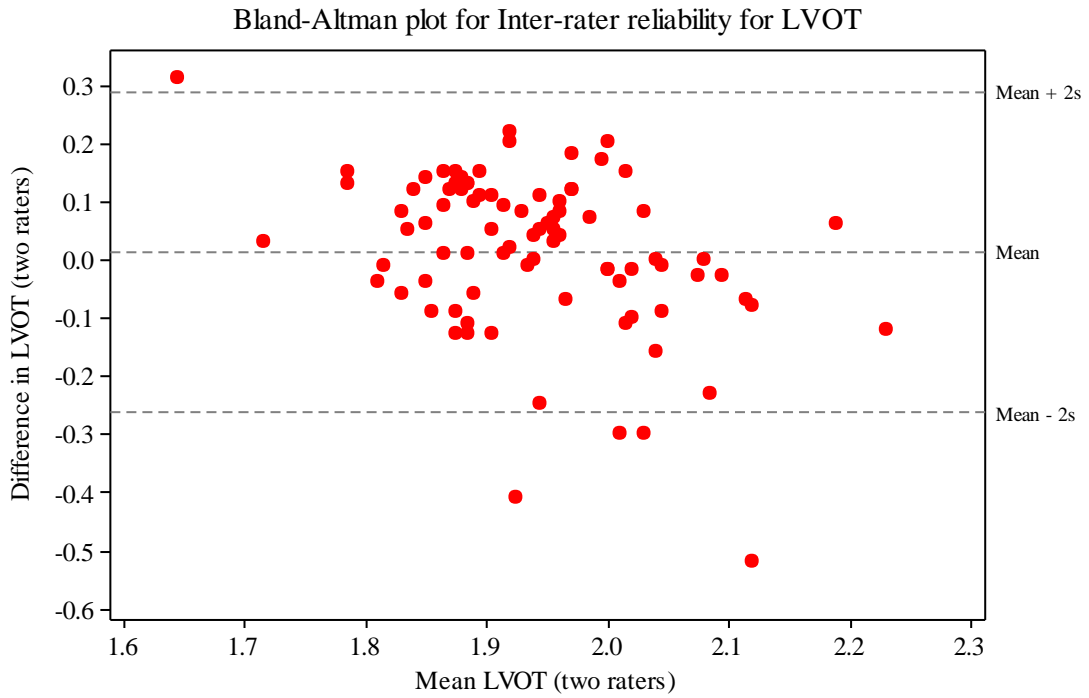


Figure 25 Human Study 1 Bland-Altman plot of inter-rater reliability for left ventricular outflow tract diameter

LVOT = left ventricular outflow tract diameter.

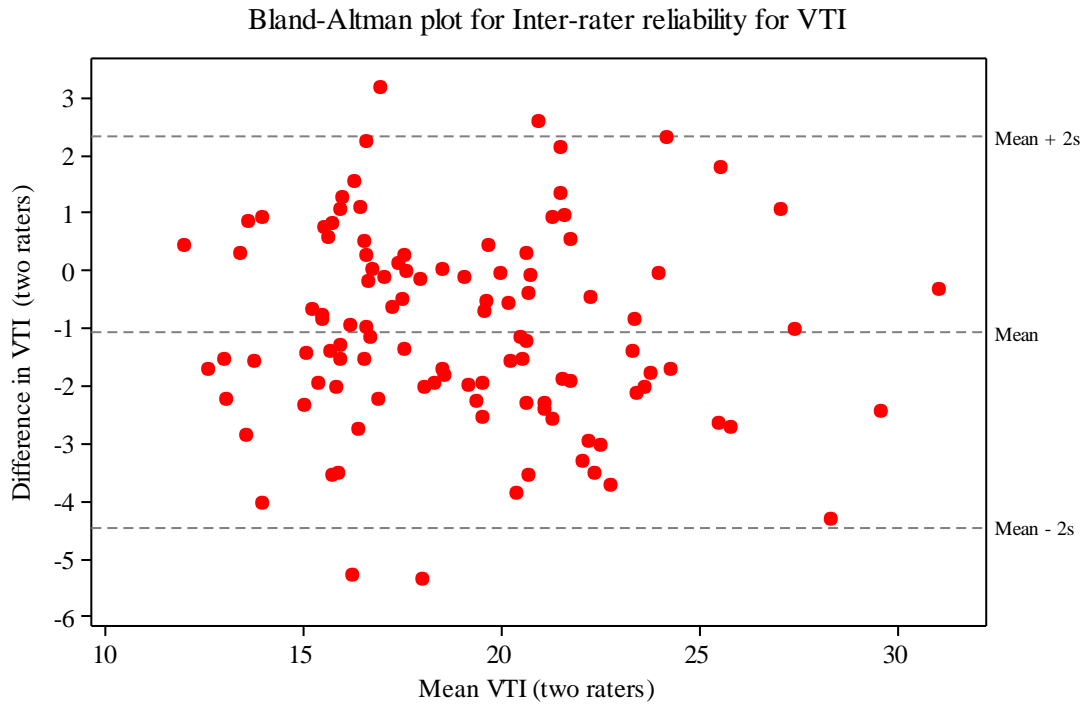


Figure 26 Human Study 1 Bland-Altman plot of inter-rater reliability for left ventricular outflow tract velocity time integral

VTI = velocity time integral.

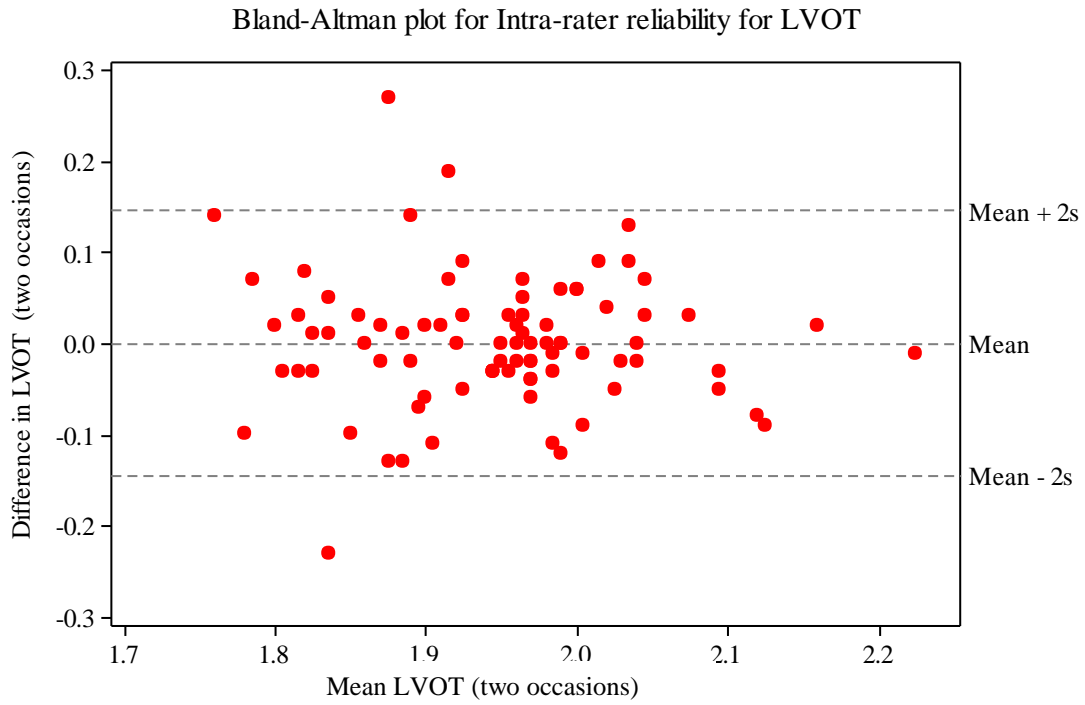


Figure 27 Human Study 1 Bland-Altman plot of intra-rater reliability for left ventricular outflow tract diameter

LVOT = left ventricular outflow tract diameter.

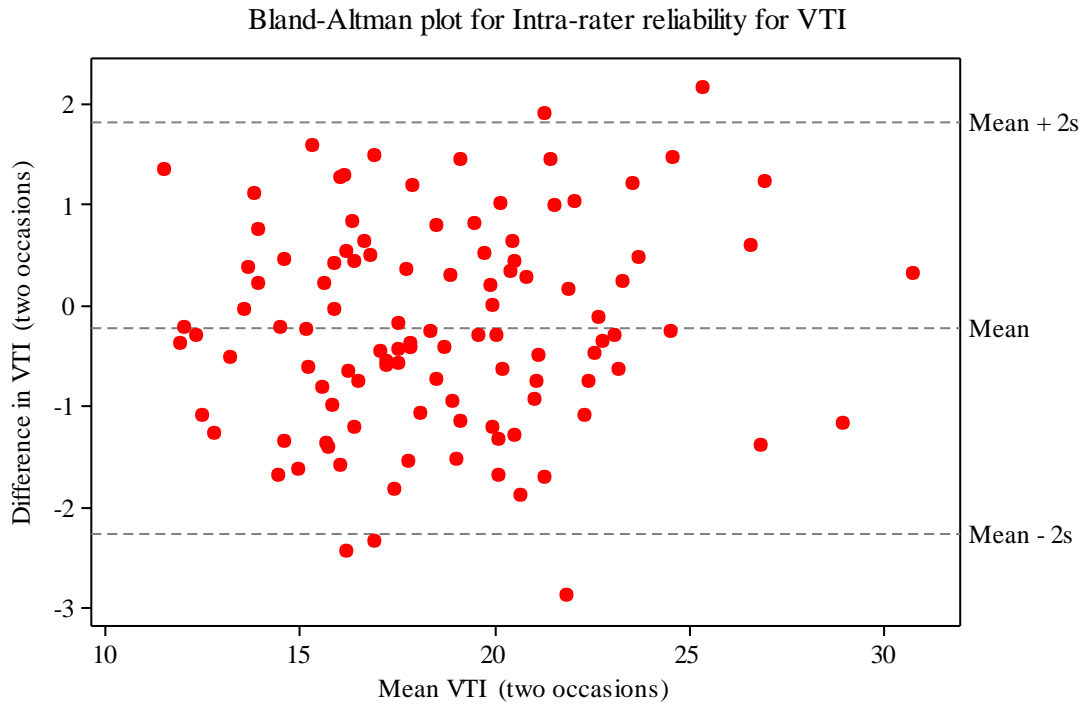


Figure 28 Human Study 1 Bland-Altman plot of intra-rater reliability for left ventricular outflow tract velocity time integral

VTI = velocity time integral.

4.4. Discussion

This study showed that transthoracic echocardiography can be used to determine cardiac output in women during the peripartum period, is well tolerated and applicable. Resting cardiac output in the left lateral level position in healthy pregnant women at term was lower than previously reported. Achievement of basal resting conditions was achieved as evidence by lower heart rates than many previous studies. Large variability, with women demonstrating both very high and very low cardiac output at rest, and elevated resting heart rate at term compared with non-pregnant women, was also demonstrated, which is a common feature in previously published data (Desai, Moodley et al. 2004; Simmons, Gillin et al. 2002; van Oppen, Stigter et al. 1996). Cardiac output was shown to decrease in the left lateral 10° head-down position, primarily due to a reduction in stroke volume. In the postoperative period, cardiac output returned to baseline values primarily from an increase in stroke volume with a concomitant decrease in heart rate.

In determining cardiac output in pregnancy, various invasive and non-invasive techniques can be used (Berkowitz and Rafferty 1980; Cholley and Payen 2005; Desai, Moodley et al. 2004; Dyer and James 2008; Easterling, Benedetti et al. 1989a; Mabie, DiSessa et al. 1994; Masaki, Greenspoon et al. 1989; Robson, Dunlop et al. 1987c; Simmons, Gillin et al. 2002; van Oppen, Stigter et al. 1996; Zentner 2006). When examining the literature regarding cardiac output changes in pregnancy, the strengths and weaknesses of the various techniques and characteristics of the study group should be considered. Cardiac output may be artificially elevated in healthy women by invasive techniques, even though validated against non-invasive techniques.

Limitations in studies of non-invasive techniques include small study numbers, lack of study group homogeneity, questionable accuracy of the measurement device and technique, and a general absence of reporting of inter- and intraobserver reliability. Human Study 1 controlled for these important confounding factors. Homogeneity of the group was achieved by limiting age and weight extremes, including only healthy fasted women and limiting the gestation to ≥ 37 weeks. Cardiac output was measured in a

uniform environmental temperature, after an adequate period of rest, in a quiet and calm setting and, for the preoperative examinations, in the absence of intravenous access or medications, anaesthesia or intravenous fluids. Cardiac output was measured with TTE using the LVOT diameter and LVOT VTI which is a robust and reliable method (Gottdiener, Bednarz *et al.* 2004; Lang, Bierig *et al.* 2005).

Postural changes during pregnancy have demonstrated hypotension due to compression of the aorta and vena cava by the gravid uterus (Bieniarz, Yoshida *et al.* 1969; Kerr, Scott *et al.* 1964; Kinsella 2003). Right or left lateral positions compared to supine positions have demonstrated variability in subjective and objective responses (Clark, Cotton *et al.* 1991; Danilenko-Dixon, Tefft *et al.* 1996). Hypotension in the presence of neuraxial anaesthesia may be particularly marked and prevention and treatment of this may involve postural changes although studies specifically examining this intervention are small (Bamber and Dresner 2003; Cyna, Andrew *et al.* 2006). One study of sixteen women assessed with TTE during the mid-third trimester demonstrated no significant changes in cardiac output between supine and standing positions (Del Bene, Barletta *et al.* 2001). Other small studies have compared the supine and lateral positions or the left lateral and erect positions (Dyer, Anthony *et al.* 2004). Though variable, the magnitude and direction of cardiac output can be altered by posture; therefore vigilance is required by both anaesthetists and pregnant women to change posture with the onset of symptoms or changes in blood pressure or heart rate.

No studies have been identified that examine the effects of changing posture serially from left lateral level to left lateral 20° head-up, then left lateral 10° head-down positions on cardiac output in healthy pregnant women. This study showed that term pregnant women placed in the left lateral head-down position might have a lower cardiac output with a lower VTI but no significant change in heart rate. This was associated with symptoms of dizziness, headache and abdominal tension in some women. This is different from the non-pregnant state, where the head-down position is commonly employed to improve blood pressure and cardiac output by increased venous return. The common clinical practice of raising the legs or adopting a head-down position to increase venous return and cardiac output in the hypotensive pregnant woman or placing the hypotensive

pregnant women in the head-down position, instead of the left lateral position, requires further investigation given this study's findings (AHA 2005).

The findings indicate a lack of predictability in resting cardiac output in seemingly healthy women, which may explain the large variability and unpredictability in the occurrence of supine hypotensive syndrome. In addition, the direction of cardiac output alterations with postural changes cannot be predicted by conventional means of clinical history and examination. These alterations appear to be independent of fetal weight. Further studies are required to evaluate cardiac output responses in other settings, such as hypovolaemia or significant sympathetic blockade.

The unpredictable haemodynamic responses that can occur with neuraxial anaesthesia for caesarean delivery in healthy women may be related to a lower than expected resting cardiac output in some women. The lower cardiac output in the head-down position may be due to the mechanical and compressive effects of the fetus, or other underlying cardiac function perturbations such as diastolic dysfunction. Such dysfunction has been indicated in healthy term pregnant women by alterations in pulmonary vein or mitral valve inflow (Mesa, Jessurun *et al.* 1999) or reduced septal and lateral wall early diastolic myocardial velocities (Zentner, du Plessis *et al.* 2009). This dysfunction may be intrinsic to the myocardium or extrinsic via pressure of the gravid uterus; further investigation is necessary.

This study was designed as an observational study and thus was not powered to analyse intraoperative interventions. Moreover, the anaesthetic technique and the use of vasopressors were not standardized, nor was fluid input or output controlled. However, the anaesthesia was conducted by experienced consultant obstetric anaesthetists who managed the haemodynamics and anaesthesia within accepted practice. There were no intraoperative complications requiring conversion to general anaesthesia or blood product transfusions. Thus the post anaesthetic care unit measurements most likely represent the range of haemodynamic variables that would be expected in this healthy group of patients undergoing elective caesarean delivery anaesthesia in the hospital. It is not possible to make any valid inferences about the key factors that may have influenced the

postoperative TTE findings of an elevated stroke volume and reduced heart rate. Future studies can be designed to evaluate potential contributing factors and examine the effect of the head-down position on stroke volume in the immediate postpartum period.

The key aim of this study, fundamental to this thesis, was to determine the reference range for cardiac output in healthy pregnant women which was achieved through analysis of the cardiac output in position 1 (P1). Regarding other elements of this study, there are limitations. The study cohort was healthy and homogeneous, and the range of cardiac output may be different in women who have very high or very low BMIs, or who have medical conditions including preeclampsia. There is controversy regarding indexing of cardiac output to body surface area, including which equation to use and the applicability of such equations during pregnancy (De Paco, Kametas *et al.* 2008; Desai, Moodley *et al.* 2004; van Oppen, van der Tweel *et al.* 1995). The lack of significant changes in cardiac index in this study may reflect this issue. In addition, this study was limited to women at term gestation, so inferences cannot be made regarding cardiac output before term. Finally, this study was designed to examine cardiac output changes only in three preoperative positions and one postoperative position before and after neuraxial anaesthesia, respectively. In normal clinical obstetric anaesthesia practice, a range of different positions, such as left lateral, right lateral, head-up, sitting, head-down and pelvic tilt, are employed during establishment and maintenance of neuraxial anaesthesia (Dresner 2008; Paech 2008; Russell 1996; Russell 2008; Zhou, Shao *et al.* 2008).

Large longitudinal studies incorporating pre-pregnancy, pregnancy and post-partum TTE measurements until at least six months post partum should be designed to evaluate serial changes in cardiac output during pregnancy. Further studies should also specifically examine the effects of posture and anaesthesia in subgroups of women based on initial preoperative cardiac output as well as the effect of posture on cardiac output in the presence of neuraxial and general anaesthesia.

From this first study it can be concluded that transthoracic echocardiography is applicable and acceptable in healthy pregnant women. Cardiac output has a wide baseline range, decreases in the left lateral 10° head-down position due to a reduction in stroke volume,

and is maintained in the immediate postoperative period primarily by an increase in stroke volume. The method of cardiac output measurement utilising two acoustic winders and 2D and Doppler echocardiography was demonstrated to be a robust and reliable method of measurement in this group of women.

4.5. Key findings

1. Cardiac output is lower than previously reported but consistent with achievement of the basal resting state low heart rate and behavioural features of sleeping during the study.
2. Cardiac output reduces in the 10° head-down position primarily due to a reduction in stroke volume.
3. Transthoracic echocardiography is an acceptable technique in this setting from both a system hospital environment perspective and from the woman's perspective.
4. From the mean and standard deviation in this study, and considering a clinically relevant cardiac output difference of 750 ml, a power of 0.8 and a *P* value of 0.05, the sample size for the subsequent study was determined to be 80 women – 40 women with untreated preeclampsia, gestationally matched with 40 healthy pregnant women.

Chapter 5. Human Study 2 Cardiac function in women with untreated preeclampsia

5.1. Introduction

In this core study of the PhD, the aim was to determine left ventricular function and haemodynamic state using transthoracic echocardiography (TTE) in women with untreated preeclampsia to define the native disease state, characterized by the following measurements: left ventricular systolic function (cardiac output (CO), fractional shortening (FS), fractional area change (FAC), septal s' velocity, septal s' time, VTI time, isovolumetric contraction time (IVCT), velocity of circumferential fibre shortening (Vcfc), Tei index, stroke work index (SWI), cardiac work index (CWI)), and diastolic function (mitral valve (MV) E and A wave velocities, mitral valve deceleration time (MV DT), septal e' and a' velocities, isovolumetric relaxation time (IVRT), left atrial (LA) size), and left ventricular mass and systemic vascular resistance (SVR)).

The background to this study was that preeclampsia is responsible for a significant amount of maternal and neonatal morbidity and mortality both in the short term and long term (Lewis 2007; Maron, Towbin *et al.* 2006; Mosca, Banka *et al.* 2007; Singhal, Kimberly *et al.* 2009; Sullivan, Hall *et al.* 2007; WHO 2007a). There is a lack of knowledge regarding cardiac function in women with preeclampsia and historically blunt non-specific diagnostic tools have been used to investigate women with preeclampsia (Easterling 1992; Sibai, Mabie *et al.* 1987; Sibai and Mabie 1991; Visser and Wallenburg 1991). There are also complications related to therapeutic interventions which may be attributed to this lack of cardiovascular system knowledge (Altman, Carroli *et al.* 2002; Benedetti, Kates *et al.* 1985; Christiansen and Collins 2006; Duley, Henderson-Smart *et al.* 2006; Goldenberg, Culhane *et al.* 2008).

Invasive monitoring devices are rarely used in the clinical or research settings due to their risks (Harvey, Young *et al.* 2006). Transthoracic echocardiography however, is widely used in other areas of medicine and in clinical research. It is non-invasive, precise, validated in healthy pregnant women and in women with preeclampsia and is frequently

considered the reference standard for cardiovascular system monitoring (Charron, Caille *et al.* 2006; Gottdiener, Bednarz *et al.* 2004; Nagueh, Appleton *et al.* 2009).

5.2. Methods

Human Study 1 was used to determine the sample size and after institutional ethics approval (Appendix A) and informed consent, 40 women with untreated preeclampsia and 40 gestationally matched healthy pregnant women and 20 non-pregnant non-smoking healthy women were enrolled as control subjects (Appendix B).

The method for Human Study 2 is summarized in Figure 29.

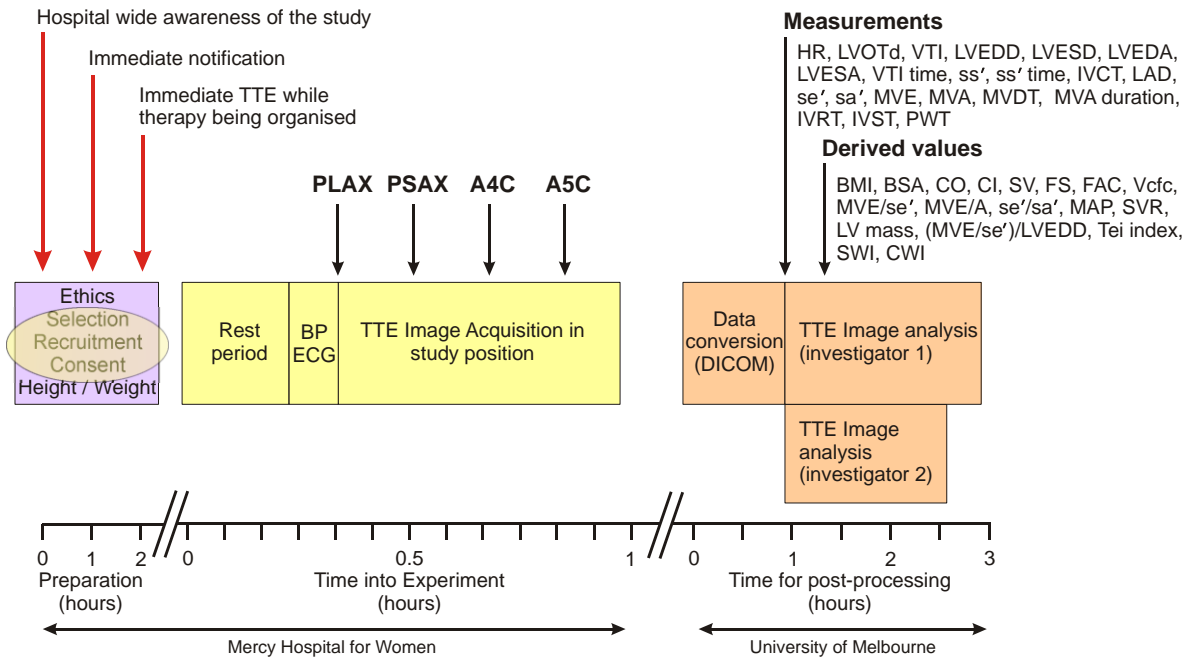


Figure 29 Human Study 2 Experimental procedure and timeline

This figure is divided into three sections – the preparation time in which there was immediate notification and transthoracic echocardiography at the time of patient diagnosis (purple section), the experimental procedure (yellow section) and the post-processing procedure where the data are converted and measured by two independent observers (orange section).

TTE = transthoracic echocardiography; BP = blood pressure; ECG = electrocardiograph; PLAX = parasternal long axis view; PSAX = parasternal short axis view; A4C = apical 4 chamber view; A5C = apical 5 chamber view; HR = heart rate; LVOTd = left ventricular outflow tract diameter; VTI = left ventricular outflow tract velocity time integral; LV = left ventricular; EDD = end diastolic diameter; ESD = end systolic diameter; EDA = end diastolic area; ESA = end systolic area; s = septal, IVCT = isovolumetric contraction time; LAD = left atrial diameter; MV = mitral valve; DT = deceleration time; IVRT = isovolumetric relaxation time; IVST = interventricular septum thickness; PWT = posterior wall thickness; BMI = body mass index; BSA = body surface area; CO = cardiac output; CI = cardiac index; SV = stroke volume; FS = fractional shortening; Vcfc = circumferential fibre length shortening; MAP = mean arterial pressure; SVR = systemic vascular resistance; SWI = stroke work index; CWI = cardiac work index

Study group

Forty women with preeclampsia were recruited at the time of diagnosis according to accepted definitions of preeclampsia (ACOG 2002; Brown, Hague *et al.* 2000; RCOG 2006; Sibai, Taslimi *et al.* 1986). The diagnosis was based on Table 1 (page 9). Preeclampsia was classified as mild or severe with severe disease defined as marked elevation of blood pressure (SBP \geq 160 mmHg, DBP \geq 110mmHg) and/or extreme derangements of organ function in the presence of hypertension. These included central nervous system problems of seizures (eclampsia), impaired conscious state and visual disturbances, renal dysfunction (urinary protein \geq 5 g protein/24 hours) and haematological complications or Haemolysis Elevated Liver enzymes Low Platelets (HELLP) syndrome.

The most important characteristic influencing the observations of what occurs in the native disease state with respect to cardiac function in women with preeclampsia was deemed to be whether they had received treatment for the disease or not. In addition they had to have no underlying cardiovascular system disease, no pre-existing hypertension and were functionally fit and healthy and on no cardiovascular system medications prior to pregnancy or during any interpregnancy time periods. Age, gestation, parity, weight, height, body mass index limits were not placed on the inclusion criteria. Therefore recruited women were untreated which meant that they had received no medication oral or intravenous including antihypertensive agents or magnesium sulphate, for preeclampsia at any time prior to the study. They did not have intravenous access and were not receiving intravenous fluids or epidural analgesia. They were not in labour. Haematological, biochemical and urinary analysis were performed on the day of diagnosis (i.e. same day as the TTE examination) in women with preeclampsia. Treatment interventions used after TTE were recorded for women with preeclampsia. Exclusion criteria for women with preeclampsia were inability to consent to the study, pre-existing hypertension, women in labour, and prior treatment interventions for preeclampsia or vasoactive medications of any kind for the treatment of other diseases.

The healthy pregnant women were gestationally matched with the women with preeclampsia. They were ASA I or II women without medical problems, not receiving medications, non-smokers. A full blood examination was performed as part of routine antenatal care in the healthy pregnant women.

The 40 healthy pregnant women and the 40 women with preeclampsia were followed during their hospital stay and data were collected regarding maternal and neonatal complications, and hospital length of stay.

The twenty healthy non-pregnant controls were ASA I or II, non-smokers, with no history of cardiovascular disease or not receiving any vasoactive medication.

Resting haemodynamics

Women rested in the left lateral position on a comfortable bed in a quiet, temperature-controlled environment with partners present for a minimum of ten min before the measurements. Baseline systolic and diastolic blood pressure, using a manual calibrated sphygmomanometer, was obtained non-invasively using the left arm recording the diastolic value as Korotkoff V and using the appropriately sized cuff. An electrocardiograph (ECG) was attached.

Patient position and image acquisition

All TTE studies were performed by one observer, the principal investigator (ATD) using a MicroMaxx P17 5 – 1 MHz transducer SonoSite®. All women had measurements performed in the left lateral position with the degree of tilt governed by participant comfort. The same method as was employed in Human Study 1 was used in this study and additional measurements of systolic, diastolic and structural elements (Figure 29) were recorded according to the methods described in Chapter 3.

Image analysis

Images were converted to *Digital images and communications in medicine* (DICOM) format and analysed off-line using ProSolv® software. Two investigators (ATD, JC) independently reviewed and measured the stored images. The second investigator (JC),

reviewed the images blinded to the disease status of the woman and haemodynamic measurements, in a randomly allocated order and at a remote time and location to ATD.

Statistical methods

The sample size was determined from Human Study 1. One-way ANOVA was used across the three groups (Non-pregnant (NP), Healthy pregnant, (HP), Untreated preeclampsia (UnRxP)). If $P < 0.05$ the Tukey HSD (Tukey-Kramer) test was applied for all possible pairwise comparison of means.

Haemodynamic measurements from one patient were considered as a family of measurements, therefore a Ryan-Holm stepdown Bonferroni procedure applied to the outcomes of the ANOVA resulting in P' values. Fisher's exact test was used to compare proportions.

Correlation statistics were applied to the data to determine predictors of cardiac function. If the P value was < 0.05 the least product regression (LPR) was calculated (Ludbrook 1997; Ludbrook 1998; Ludbrook 2002).

Inter- and intraobserver relationships were analysed using the Bland-Altman methodology. In addition, the correlation coefficient was calculated and a P value attached. If the P value was < 0.05 calculation using the least product regression (LPR) was performed.

The null hypothesis was rejected if $P < 0.05$ for each of the P or P' variables.

5.3. Results

5.3.1. Demographic and obstetric data

100 women were recruited to the study. Systolic, diastolic and structural information was able to be obtained in all women. Demographic and obstetric characteristics healthy pregnant (HP) and women with untreated preeclampsia (UnRxP) groups of women are shown in Table 22. Healthy non-pregnant women (NP) (n=20) were of similar age and ethnicity to women with preeclampsia. They had lower body mass index ($23 \pm 0.4 \text{ kg/m}^2$) compared with the healthy pregnant women ($P = 0.003$). All participants were satisfied with their participation in the study, and there were no complications relating to study procedures.

Table 22 Human Study 2 Demographic and obstetric data

Characteristic	Healthy pregnant women (control) HP n = 40	Women with untreated preeclampsia UnRxP n = 40	<i>P</i> value
Age (yr)	32 ± 4	31 ± 5	0.347
Gestation (wks)	36 ± 5	36 ± 4	0.763
Caucasian	34 (85)	33 (83)	1.00
Nulliparous	10 (25%)	26 (65%)	< 0.001
Multiparous	30 (75%)	14 (35%)	< 0.001
BMI (kg/m ²)	28 ± 4	32 ± 7	< 0.001
Haematocrit	0.34 ± 0.03	0.35 ± 0.03	0.153
Haemoglobin	119 ± 10	123 ± 11	0.073
Platelet count	291 ± 81	270 ± 76	0.264
Proteinuria*	0	31 (78)	N/A
Number with severe preeclampsia*	0	19 (48)	N/A

Values are mean ± Standard Deviation, number (%) as appropriate.

BMI = body mass index.

* As defined Table 1.

Women with preeclampsia were recruited in the following locations – Pregnancy assessment day centre 19 (48%), ward 12 (30%), emergency department 5 (13%), birthing suite 3 (8%). Three of the women with untreated preeclampsia were multiple pregnancies (twins). Analysis of the data with these three women excluded demonstrated no change in any of the systolic, diastolic or structural data.

5.3.2. Haemodynamic and systolic data

The systolic and haemodynamic data are shown in Table 23.

Mean \pm standard deviation systolic blood pressure was 108.7 ± 10.5 mmHg (HP) versus 147.0 ± 7.9 mmHg (UnRxP) $P < 0.001$. The mean \pm standard deviation diastolic blood pressure was 66.9 ± 9.0 mmHg (HP) versus 92.7 ± 6.5 mmHg (UnRxP) $P < 0.001$.

There are differences in all the variables between the groups. The cardiac output is increased in women with untreated preeclampsia due to an increase in stroke volume. Whilst differences in heart rate are shown between healthy non-pregnant and healthy pregnant women, with healthy pregnant women having a higher heart rate, women with untreated preeclampsia do not have a statistically or clinically significant difference in their heart rate from healthy pregnant women. The systemic vascular resistance (SVR) was decreased in the healthy pregnant women compared to non-pregnant women; however in untreated preeclampsia SVR is increased. The mean septal s' velocity in all groups remains above 8 cm/s as the threshold value for healthy systolic function.

Figure 30 and Table 23 show the very important novel finding of increased systolic function, increased inotropy and increased fractional area change in women with untreated preeclampsia compared to healthy pregnant women. This occurred in the presence of preserved or increased systemic vascular resistance. There was increased fractional shortening (FS) % mean \pm SD (34.8 ± 7.1 (HP) vs 40.9 ± 7.6 (UnRxP) $P' < 0.002$), with the similar and statistically insignificant left ventricular end diastolic diameter (LVEDD) (cm) mean \pm SD (4.6 ± 0.4 (HP) vs 4.6 ± 0.3 (UnRxP) $P = 0.95$).

This was also reflected in the results for fractional area change (FAC) % mean \pm SD (57.12 ± 7.16 (NP) vs 57.06 ± 9.21 (HP) vs 64.49 ± 9.3 (UnRxP) $P' = 0.002$) (Table 23), with similar and statistically insignificant left ventricular end diastolic area (LVEDA) (cm^2) in the non-pregnant, healthy pregnant and untreated preeclampsia groups (16.89 ± 1.7 (NP), 16.62 ± 2.7 (HP) and 16.57 ± 2.6 (UnRxP) $P' = 0.900$).

Systolic ejection time, the VTI time, was the same between the groups and thus in the presence of an increased FAC and FS demonstrates increased inotropy.

Table 23 Human Study 2 Haemodynamic and systolic data

Variable	Non-Pregnant (NP) n = 20 mean ± SD	Healthy Pregnant (HP) n = 40 mean ± SD	Untreated Preeclampsia (UnRxP) n = 40 mean ± SD	One-way ANOVA <i>P</i>	NP vs HP <i>P</i>	NP vs UnRxP <i>P</i>	HP vs UnRxP <i>P</i>	Corrected one-way ANOVA <i>P'</i>
MAP (mmHg)	86.5 ± 8.6	80.8 ± 8.3	110.8 ± 5.1	< 0.0001	0.0153	< 0.0001	< 0.0001	< 0.002
CO (ml/min)	3400 ± 752	4109 ± 595	4789 ± 1419	< 0.0001	0.0363	< 0.0001	< 0.0110	< 0.002
HR (BPM)	64.2 ± 18.4	77.8 ± 9.6	80.8 ± 12.7	< 0.0001	< 0.0001	< 0.0001	0.4396	< 0.002
SV (ml)	53.1 ± 9.5	53.2 ± 7.9	58.9 ± 12.8	0.0305	0.9992	0.1102	0.0432	0.044
SVR (dyne.cm/sec ⁵)	2116.1 ± 457.0	1612.5 ± 315.4	2015.7 ± 624.7	0.0001	0.0008	0.7335	0.0011	< 0.002
FAC (%)	57.12 ± 7.16	57.06 ± 9.21	64.49 ± 9.3	0.0006	0.9997	0.01213	0.0011	0.002
Septal s' (cm/s)	8.89 ± 0.73	8.89 ± 1.22	8.14 ± 1.58	0.0223	0.9998	0.09879	0.0306	0.044
VTI time	322 ± 21.8	303 ± 23.4	292 ± 30.7	0.0002	0.0191	0.0001	0.1522	< 0.002
Septal s' time	340.5 ± 31.8	327.5 ± 34.8	293.8 ± 33.4	< 0.0001	0.3665	< 0.0001	< 0.0001	< 0.002

MAP = mean arterial pressure; CO = cardiac output; HR = heart rate; SV = stroke volume; SVR = systemic vascular resistance; FAC = Fractional area change; VTI = velocity time integral; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

ANOVA = analysis of variance; n = number of women; SD = standard deviation

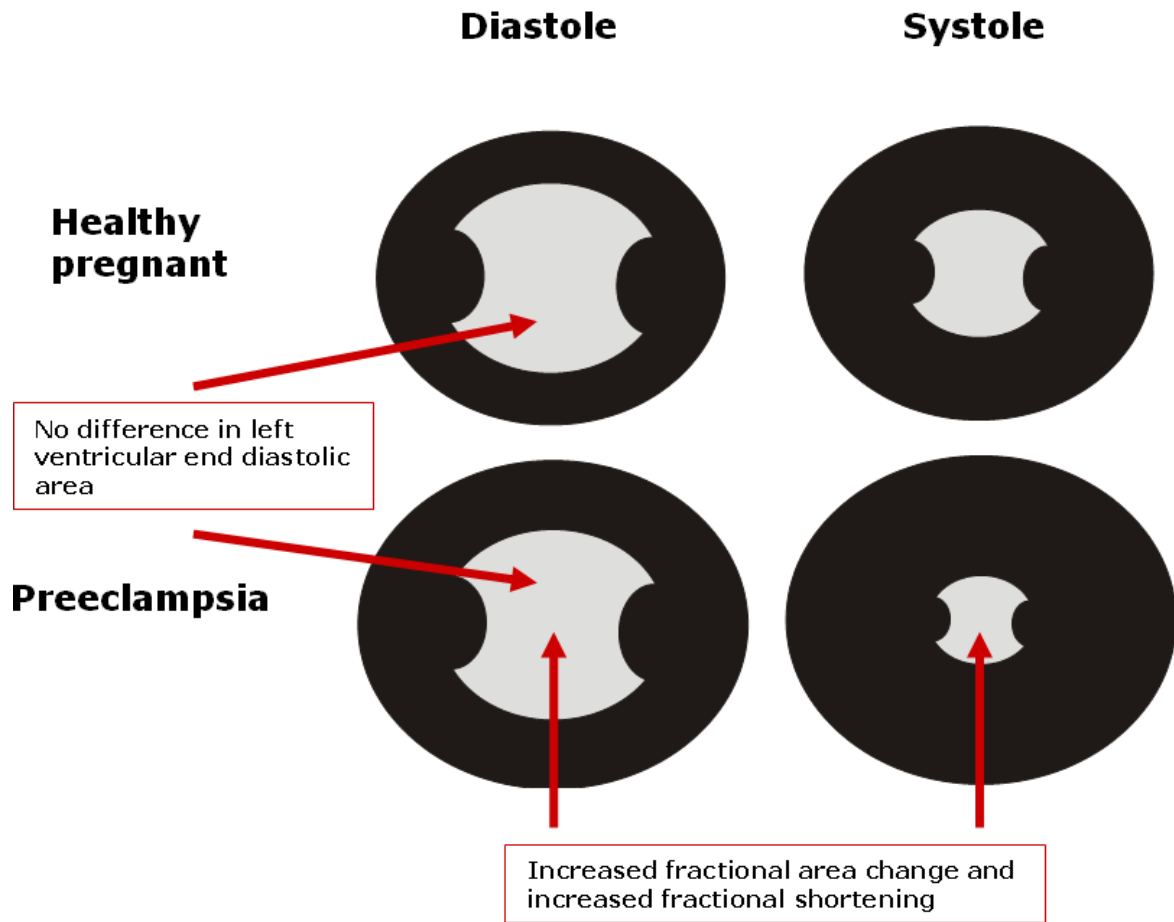


Figure 30 Human Study 2 Illustration of increased fractional area change in women with untreated preeclampsia

The upper panel shows the situation occurring in healthy pregnant women. Diastole is shown on the left and systole shown on the right. Left ventricular end diastolic area is represented in the left picture. The ventricle then contracts and reduces in size to the left ventricular end systolic area represented in the right hand side picture. This is the fractional area change and is correlated with ejection fraction.

The lower panel shows the situation occurring in women with untreated preeclampsia. Left ventricular end diastolic area is again represented in the left picture. This is the same end diastolic area as the healthy pregnant women. The ventricle then contracts, expelling the stroke volume, and reduces in size to the left ventricular end systolic area represented in the lower right picture. This is a smaller area than the area in the healthy women. The fractional area change shown in the women with untreated preeclampsia is therefore greater than the fractional area change in the healthy women. This occurs during the same ventricular ejection time and therefore represents an increase in inotropy in women with untreated preeclampsia.

5.3.3. Diastolic data

Diastolic data are shown in Table 24 and structural data are shown in Table 25.

The septal e' velocity is different between the three groups with healthy non-pregnant women having normal reference range e' velocities, however the healthy pregnant women show a reduced e' velocity and the women with untreated preeclampsia have a markedly reduced e' velocity with a mean value of 8.66 cm/s. The septal a' velocity demonstrates the opposite changes between the groups with healthy non-pregnant women having a low a' velocity and women with untreated preeclampsia showing a high a' velocity. In fact, 20 of the women (50%) with untreated preeclampsia were found to have a' velocities higher than e' velocities ($e'/a' < 1.0$), whereas none of the healthy pregnant women had this reversal (Table 26 page 134).

The isovolumetric relaxation time and the mitral valve deceleration time are prolonged in women with untreated preeclampsia. In addition, the mitral valve E/A ratio is reduced from the non-pregnant group but there was no difference shown when compared to healthy pregnant women.

The mitral valve E/septal e' ratio, an important correlate of left atrial pressure and left ventricular end diastolic pressure, was elevated to an abnormal non-pregnant level in women with untreated preeclampsia. A mean value of 10.37 was recorded in women with untreated preeclampsia.

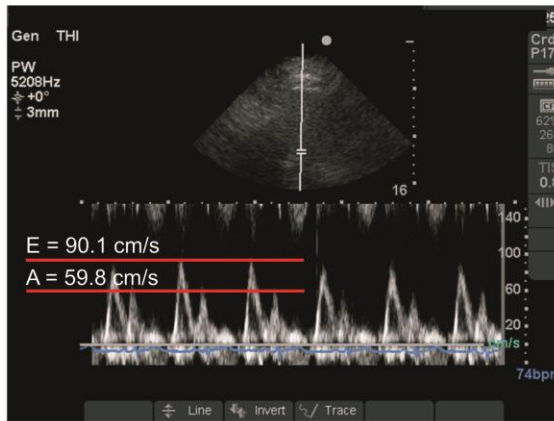
The mitral valve E and mitral valve A Doppler waveforms and the septal tissue Doppler e' and a' waveforms are shown in Figure 31.

Table 24 Human Study 2 Diastolic data

Variable	Non-Pregnant (NP) n = 20 mean ± SD	Healthy Pregnant (HP) n = 40 mean ± SD	Untreated Preeclampsia (UnRxP) n = 40 mean ± SD	One-way ANOVA <i>P</i>	NP vs HP <i>P</i>	NP vs UnRxP <i>P</i>	HP vs UnRxP <i>P</i>	Corrected one-way ANOVA <i>P'</i>
Septal e' velocity (cm/s)	14.20 ± 1.5	11.50 ± 2.3	8.66 ± 2.3	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.002
Septal a' velocity (cm/s)	6.4 ± 1.4	7.2 ± 1.2	8.4 ± 2.0	< 0.0001	0.1263	< 0.0001	0.0076	< 0.002
IVRT (ms)	56.8 ± 17.9	70.2 ± 17.4	90.5 ± 23.2	< 0.0001	0.0443	< 0.0001	< 0.0001	< 0.002
MV DT (ms)	172.8 ± 18.7	174.4 ± 22.4	202.3 ± 31.6	< 0.0001	0.973	0.0002	< 0.0001	< 0.002
MV E/A velocity	1.86 ± 0.44	1.45 ± 0.24	1.29 ± 0.34	< 0.0001	< 0.0001	< 0.0001	0.096	< 0.002
MV E/septal e'	6.0 ± 1.1	6.7 ± 1.3	10.37 ± 2.4	< 0.0001	0.3348	< 0.0001	< 0.0001	< 0.002

IVRT = isovolumetric relaxation time; MV = mitral valve; DT = deceleration time; MV E = E wave velocity; MV A = A wave velocity; e' = e' wave velocity; a' = a' wave velocity; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia
ANOVA = analysis of variance; n = number of women; SD = standard deviation

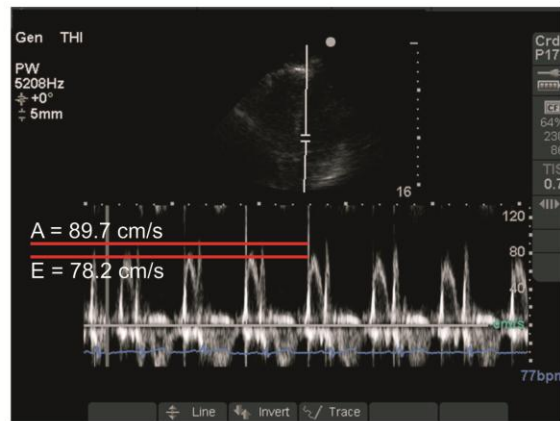
Healthy pregnancy



Measurements and derived data - healthy pregnancy

MV E/A = 1.5
 Septal e' velocity = 13.7 cm/s
 Septal e'/a' = 2.1
 MV E/septal e' = 6.6
 Septal s' velocity = 8.9 cm/s
 Diastolic function = Normal
 Systolic function = Normal

Preeclampsia



Measurements and derived data - preeclampsia

MV E/A = 0.9
 Septal e' velocity = 6.7 cm/s
 Septal e'/a' = 0.8
 MV E/septal e' = 11.7
 Septal s' velocity = 6.3 cm/s
 Diastolic function = Abnormal
 Systolic function = Abnormal

MV = mitral valve

Figure 31 Human Study 2 Mitral valve inflow Doppler waveform and septal tissue Doppler waveform comparison - healthy pregnant woman and a woman with untreated preeclampsia

Healthy pregnancy: The left hand side images illustrate the relationship in healthy pregnant women. The upper image is the mitral valve pulse wave Doppler waveform showing a healthy pattern of $E > A$. The lower image is the septal tissue Doppler image illustrating the healthy s' , e' and a' relationship.

Preeclampsia: The right hand side images illustrate the relationship that can occur in women with untreated preeclampsia. The upper image is the mitral valve pulse wave Doppler waveform showing an abnormal pattern of $E < A$. The lower image is the septal tissue Doppler image illustrating the abnormal pattern of reduced s' , and a reversed e' and a' relationship.

5.3.4. Cardiac structural and performance data

Cardiac structural and performance data are shown in Table 25. Left ventricular mass is increased in healthy pregnant women compared to non-pregnant women. Left ventricular mass is increased in women with untreated preeclampsia compared to healthy pregnant women. Both cardiac work index (CWI) and stroke work index (SWI) are increased in women with untreated preeclampsia. Overall myocardial performance is decreased in women with untreated preeclampsia indicated by a mean Tei index value of 0.55 which is significantly different from healthy pregnant women (non-pregnant reference value for Tei index is 0.33 ± 0.09).

There were no pericardial effusions in non-pregnant women. Nine healthy pregnant women had pericardial effusions (Figure 32) with all the pericardial effusions in this group measuring < 1.1 cm. 36 women with untreated preeclampsia had pericardial effusions ($P < 0.001$).

Left atrial size was increased in women with untreated preeclampsia compared with healthy pregnant women however the mean value was within the reference range for left atrial size in centimeters (3.75 ± 0.38 (HP) vs 3.99 ± 0.35 (UnRxP) $P = 0.005$).

Table 25 Human Study 2 Cardiac structure and performance data

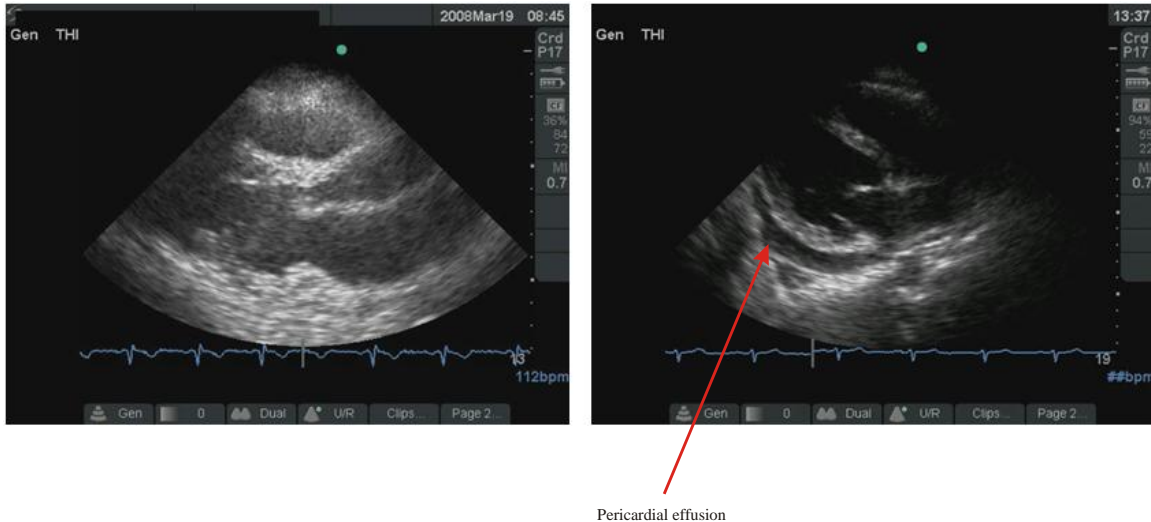
Variable	Non-Pregnant (NP) n = 20 mean ± SD	Healthy Pregnant (HP) n = 40 mean ± SD	Untreated Preeclampsia (UnRxP) n = 40 mean ± SD	One-way ANOVA <i>P</i>	NP vs HP <i>P</i>	NP vs UnRxP <i>P</i>	HP vs UnRxP <i>P</i>	Corrected one-way ANOVA <i>P'</i>
SWI (mmHg.ml/m ²)	2733.5 ± 556.5	2393.7 ± 339.8	3361.2 ± 619.9	< 0.0001	0.0435	< 0.0001	< 0.0001	< 0.002
CWI (mmHg.l/m ²)	175.6 ± 46.6	185.6 ± 31.7	272.1 ± 69.0	< 0.0001	0.7676	< 0.0001	< 0.0001	< 0.002
LV mass (g)	97.0 ± 24.7	130.8 ± 21.0	189.1 ± 40.1	< 0.0001	0.0004	< 0.0001	< 0.0001	< 0.002
Tei index	0.37 ± 0.08	0.42 ± 0.09	0.55 ± 0.13	< 0.0001	0.1699	< 0.0001	< 0.0001	< 0.002

SWI = stroke work index; CWI = cardiac work index; LV = left ventricular; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

ANOVA = analysis of variance; n = number of women; SD = standard deviation

Healthy pregnant woman

Woman with untreated preeclampsia



Pericardial effusion

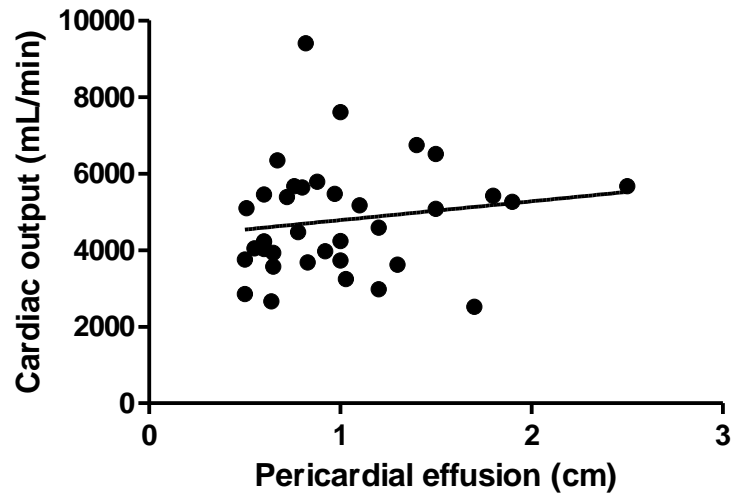
Figure 32 Human Study 2 Pericardial effusion – Parasternal long axis view

The cardiac image on the left is of a healthy pregnant woman. There is no obvious pericardial effusion. The cardiac image on the right is of a woman with untreated preeclampsia. The red arrow points to a moderately large pericardial effusion measuring approximately 1.5 cm.

5.3.5. Correlation data - Pericardial effusion

The relationship between pericardial fluid and cardiac output is shown in Figure 33. The size of the pericardial effusion did not predict the cardiac output as shown in Figure 33. The size of the pericardial effusion did not predict left ventricular mass as shown in Figure 34.

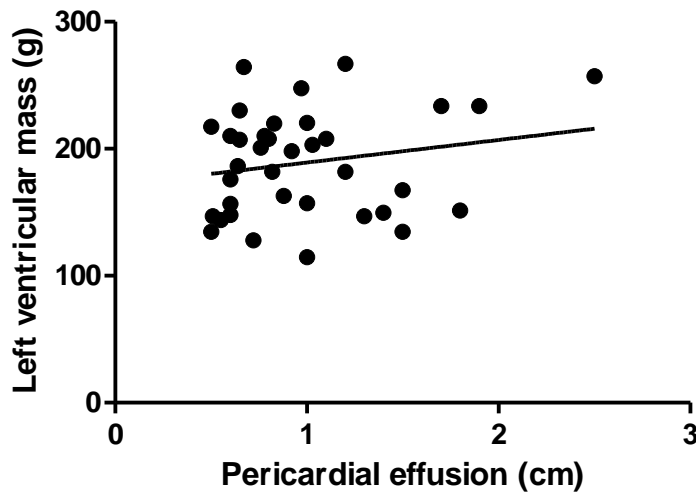
Does size of pericardial effusion predict cardiac output?



F statistic = 0.85
p = 0.362

Figure 33 Human Study 2 Correlation between pericardial effusion and cardiac output

Does size of pericardial effusion predict left ventricular mass?



F statistic = 1.39
p = 0.246

Figure 34 Human Study 2 Correlation between pericardial effusion and left ventricular mass

A summary of the diastolic and structural changes is given in Figure 35 and threshold values for abnormal cardiac function are shown in Table 26 (page 134).

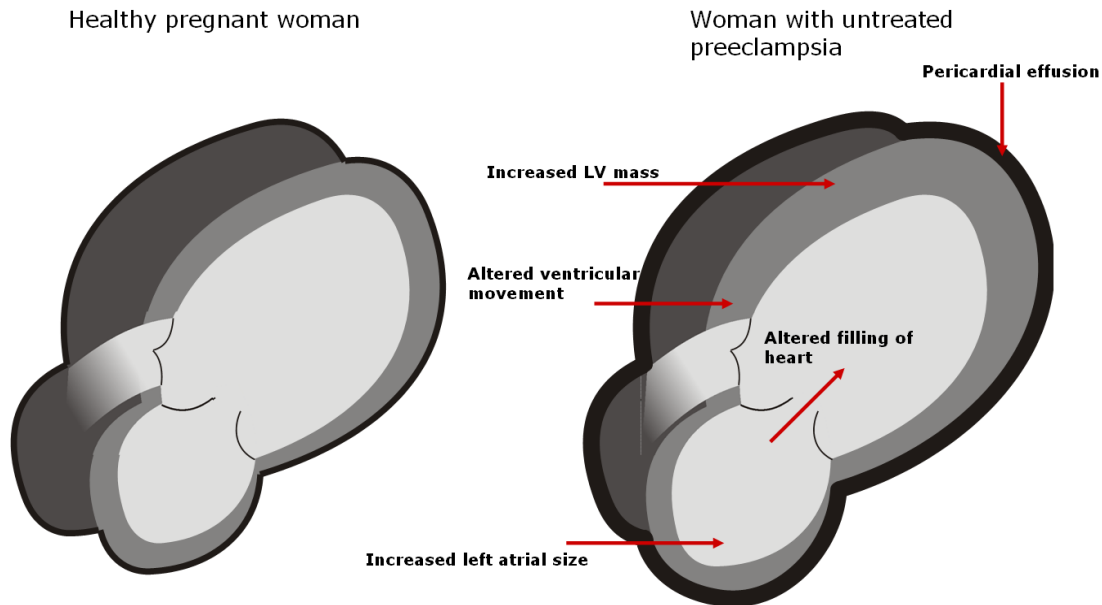


Figure 35 Human Study 2 Diastolic changes in women with untreated preeclampsia

The diagram on the left shows a healthy pregnant woman's heart. On the right the diastolic and structural changes that occur in women with untreated preeclampsia are illustrated. These are increased left atrial size, altered left ventricular filling (alterations in the mitral valve Doppler inflow pattern), altered ventricular wall movements (alterations in the septal myocardial tissue Doppler velocities), increased left ventricular (LV) mass and the presence of pericardial effusions.

5.3.6. Threshold values for abnormal cardiac function

The examination of threshold values, or actual values that correspond to abnormal function, enables an assessment of extent and severity of the condition. This is done by comparing the number of women who had abnormal findings in each group rather than comparing differences between mean values in the groups. Threshold values for abnormal cardiac function are based on accepted definitions as described in Table 14 (page 90) and Table 16 (page 92).

In Table 26 comparing healthy pregnant women with women with untreated preeclampsia, the healthy pregnant women showed abnormalities in systolic and diastolic

function. 45% of women had mitral valve E/A < 1.4, 8% had septal e' velocity < 8 cm/s and 15% demonstrated mitral valve E/septal e' > 8.

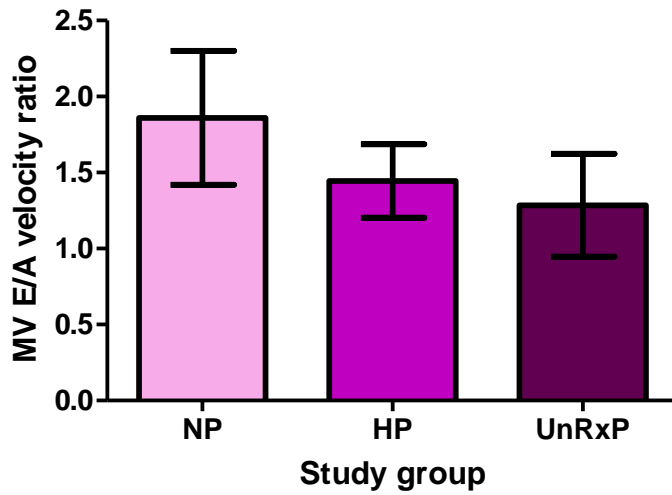
Regarding systolic measurements in the healthy pregnant women, 25% had septal s' velocities of < 8 cm/s and 23% had reduced circumferential fibre shortening. The reference ranges for the variables have not been established in pregnancy however in the non-pregnant population these changes would be considered significant markers of cardiac dysfunction.

The observations in the women with untreated preeclampsia show that more women have abnormal findings. The systolic changes are similar between the groups, however the diastolic changes occurred in more women with untreated preeclampsia and there were significant differences between the groups regarding diastolic changes. 73% of women had mitral valve E/A < 1.4, 50% demonstrated septal e' velocities of < 8 cm/s, 85% had values of mitral valve E/septal e' > 8 cm/s, and 50% of women had a septal a' velocity > than septal e' velocity. These relationships are shown in Figure 36, Figure 37, Figure 38 and Figure 39 which visually represents the range of values for each group.

Table 26 Human Study 2 Number of women with threshold values for abnormal cardiac function - Healthy pregnant women compared to women with untreated preeclampsia

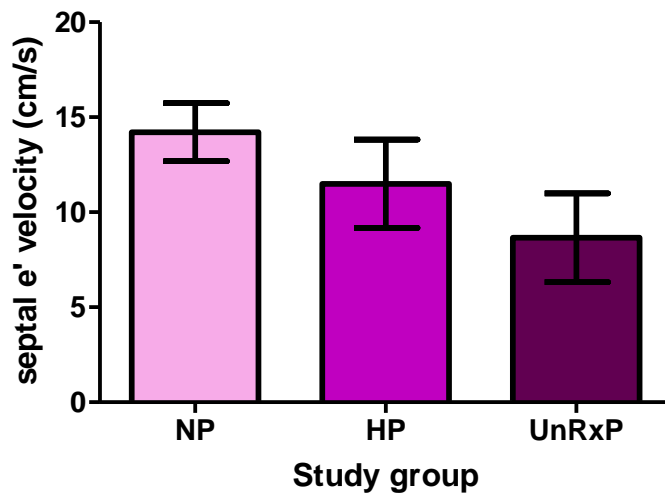
Value	Healthy pregnant women (HP) n = 40	Women with untreated preeclampsia (UnRxP) n = 40	P value
MV E/A < 1.4	18 (45)	29 (73)	0.023
Septal e' velocity < 8 cm/s	3 (8)	20 (50)	0.006
Septal s' velocity < 8 cm/s	10 (25)	19 (48)	0.062
Vcf ≤ 1.1	9 (23)	7 (18)	0.781
MV E/se' > 8	6 (15)	34 (85)	< 0.001
Septal a' velocity > Septal e' velocity	0	20 (50)	< 0.001

MV = mitral valve; Vcf = velocity of circumferential fibre shortening; Data are expressed as number of women and (%). Some women had > 1 abnormal value.



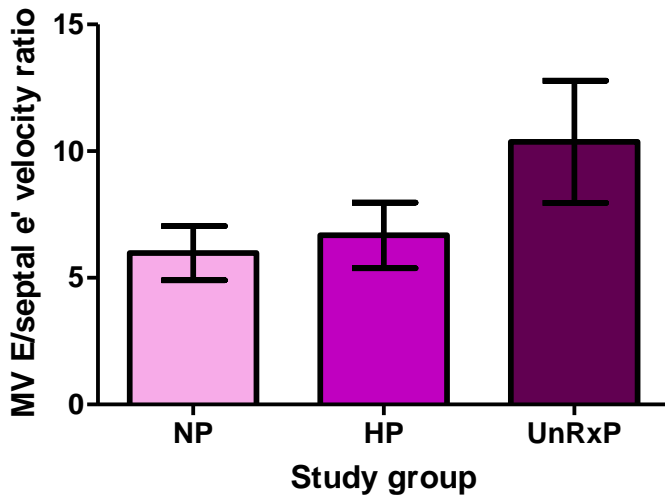
Mean ± Standard deviation; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

Figure 36 Human Study 2 Mitral valve E/A velocity ratios in the three groups



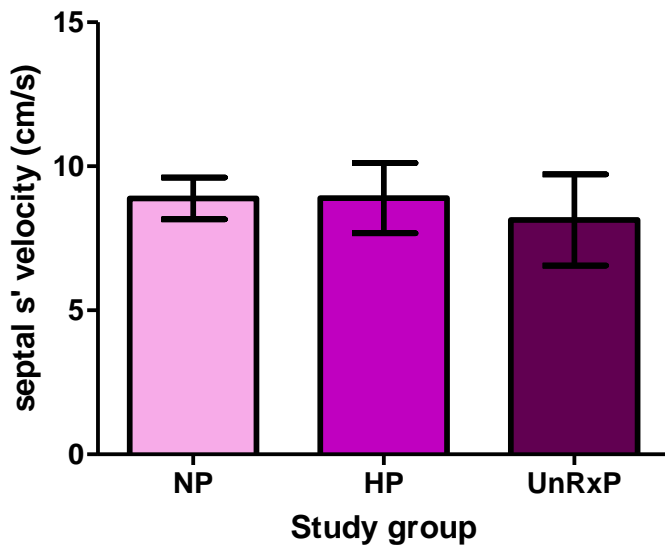
Mean ± Standard deviation; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

Figure 37 Human Study 2 Septal e' velocity in the three groups



Mean ± Standard deviation; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

Figure 38 Human Study 2 Mitral valve E/septal e' velocity ratio in the three groups



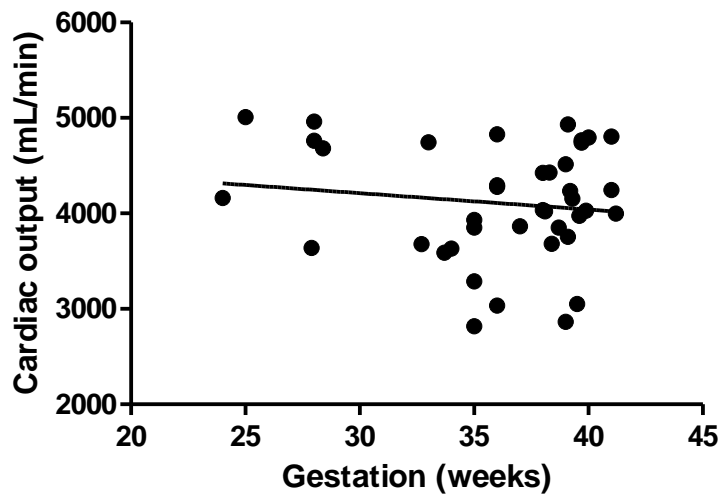
Mean ± Standard deviation; NP = non-pregnant; HP = healthy pregnant; UnRxP = untreated preeclampsia

Figure 39 Human Study 2 Septal s' velocity in the three study groups

5.3.7. Correlation data – Gestation and Blood Pressure

Gestation versus cardiac output for healthy women and for women with preeclampsia is shown in Figure 40 and Figure 41. Gestation did not predict cardiac output in healthy pregnant women or in women with preeclampsia. There was also no significant relationship demonstrated between blood pressure level and cardiac output as shown in Figure 42.

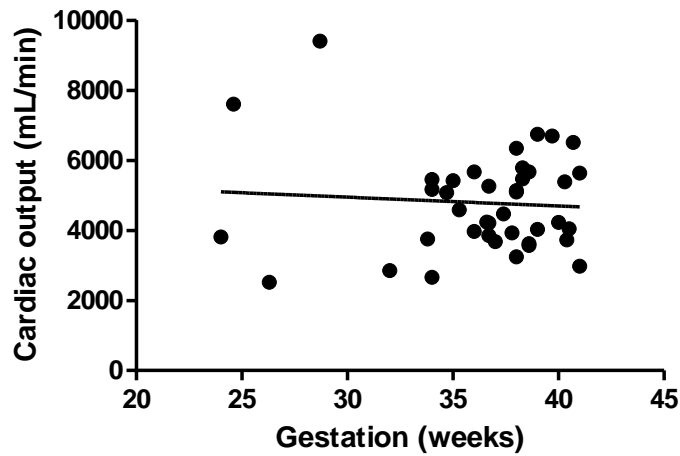
Does gestation predict cardiac output in healthy women?



F statistic = 0.66
 $p = 0.422$

Figure 40 Human Study 2 Correlation between gestation and cardiac output in healthy pregnant women

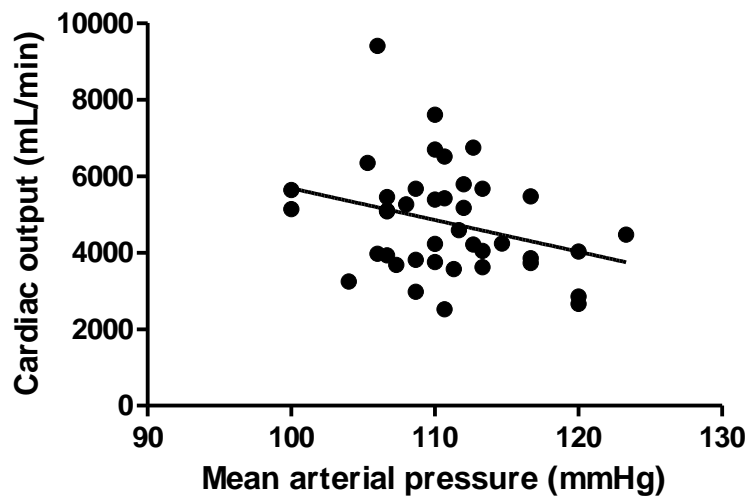
Does gestation predict cardiac output in women with preeclampsia?



F statistic 0.213
p = 0.647

Figure 41 Human Study 2 Correlation between gestation and cardiac output in women with untreated preeclampsia

Does blood pressure predict cardiac output in preeclampsia?



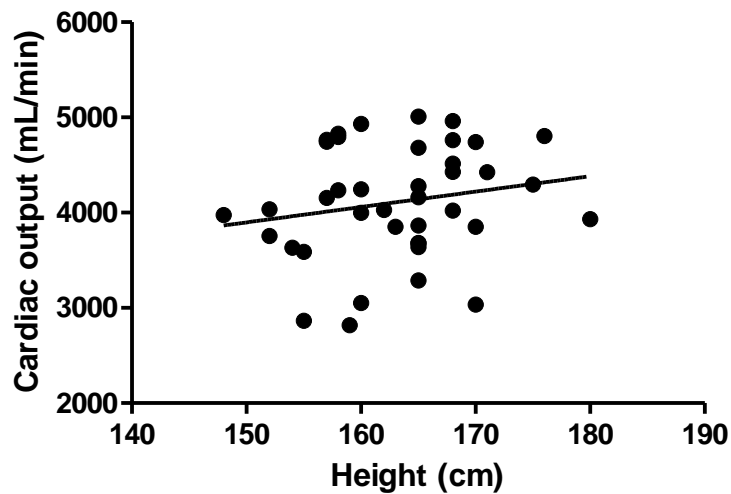
F statistic = 3.727
p = 0.061

Figure 42 Human Study 2 Correlation between blood pressure and cardiac output in women with untreated preeclampsia

5.3.8. Correlation data – Body mass index

The relationships between height, weight and body mass index (BMI) in healthy pregnant women and in women with preeclampsia are shown in Figure 43, Figure 44, Figure 45, Figure 46, Figure 47 and Figure 48. In healthy pregnant women there was no significant relationship demonstrated, however in women with untreated preeclampsia both weight and BMI predicted cardiac output.

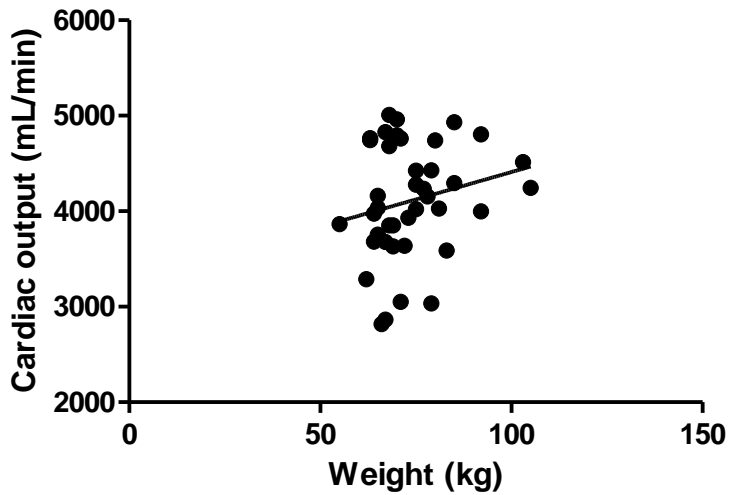
Does height predict cardiac output in healthy women?



F statistic = 1.411
p = 0.242

Figure 43 Human Study 2 Correlation between height and cardiac output in healthy pregnant women

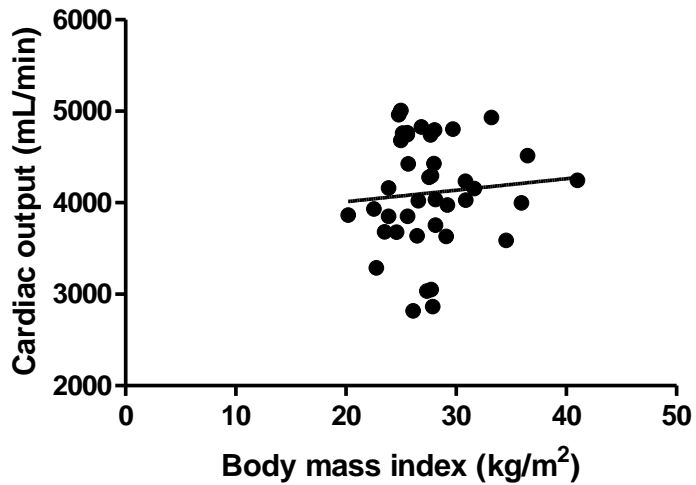
Does weight predict cardiac output in healthy women?



F statistic = 1.724
 $p = 0.197$

Figure 44 Human Study 2 Correlation between weight and cardiac output in healthy pregnant women

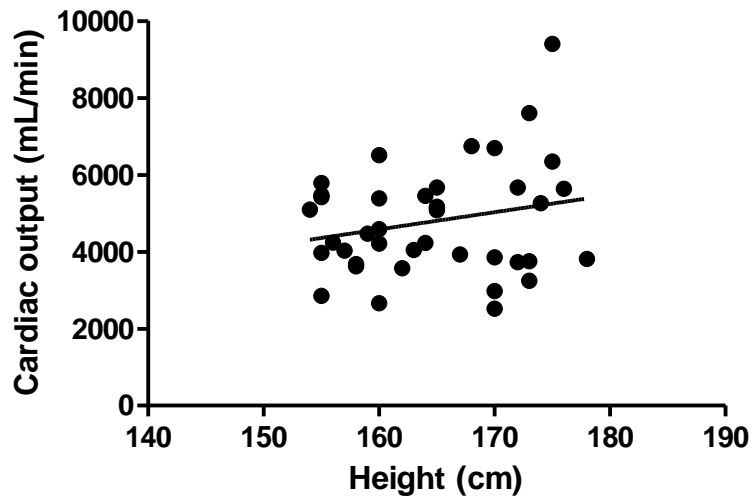
Does body mass index predict cardiac output in healthy women?



F statistic = 0.290
 $p = 0.593$

Figure 45 Human Study 2 Correlation between body mass index and cardiac output in healthy pregnant women

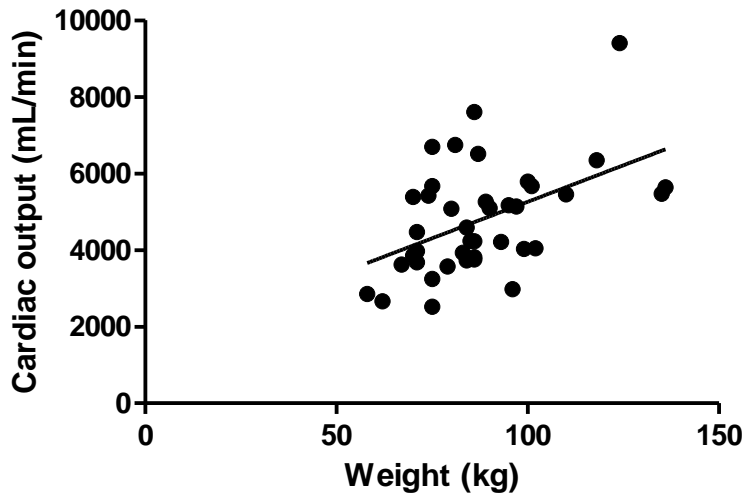
Does height predict cardiac output in preeclampsia?



F statistic = 2.05
p = 0.161

Figure 46 Human Study 2 Correlation between height and cardiac output in women with untreated preeclampsia

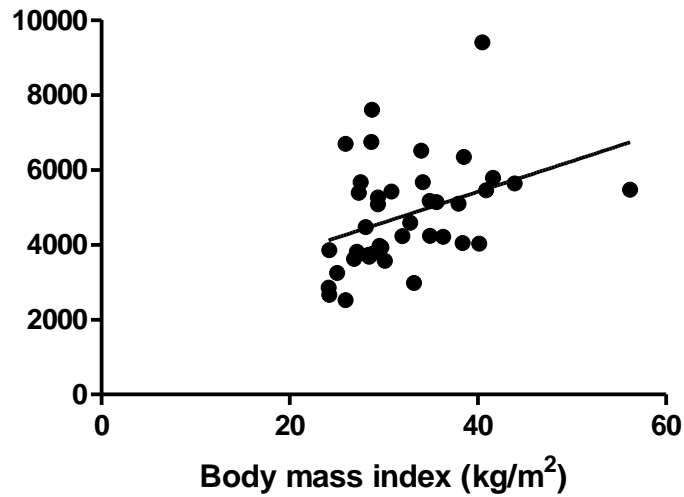
Does weight predict cardiac output in preeclampsia?



F statistic= 11.7
p = 0.002

Figure 47 Human Study 2 Correlation between weight and cardiac output in women with untreated preeclampsia

Does body mass index predict cardiac output in preeclampsia?



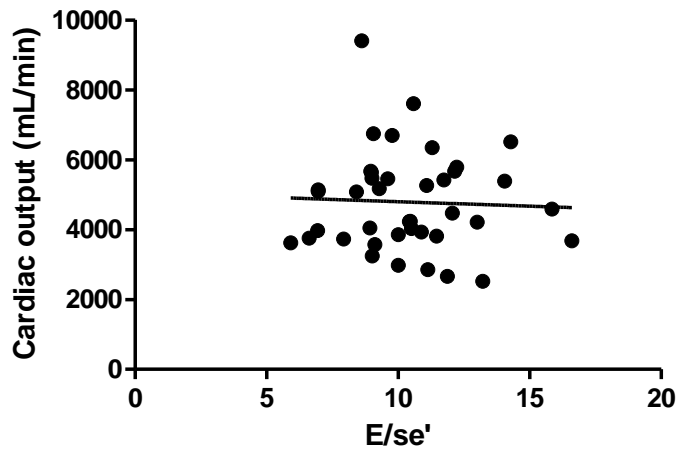
F statistic = 6.48
 $p = 0.015$

Figure 48 Human Study 2 Correlation between body mass index and cardiac output in women with untreated preeclampsia

5.3.9. Correlation data – Diastolic changes

The relationship between diastolic changes and cardiac output is shown in Figure 49 and Figure 50 . The diastolic estimates of MV E/septal e' and septal e'/septal a' do not predict cardiac output in women with untreated preeclampsia.

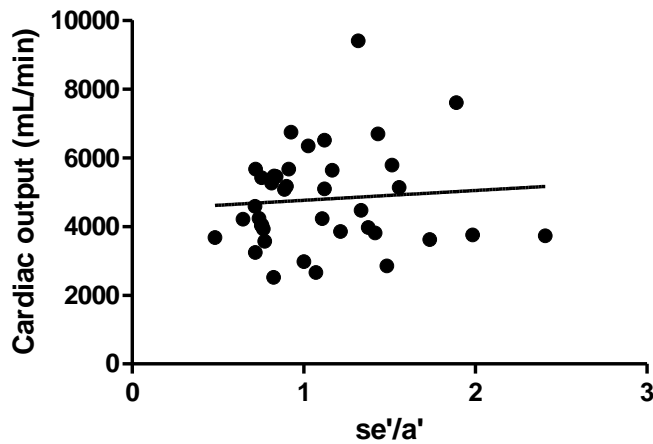
Do diastolic changes (E/se') predict cardiac output in preeclampsia?



F statistic = 0.072
 $\rho = 0.789$

Figure 49 Human Study 2 Correlation between mitral valve E/septal e' ratio and cardiac output in women with untreated preeclampsia

Do diastolic changes (se'/sa') predict cardiac output in preeclampsia?



F statistic 0.270
 $\rho = 0.606$

Figure 50 Human Study 2 Correlation between septal e'/septal a' ratio and cardiac output in women with untreated preeclampsia

5.3.10. Maternal and neonatal complications

Maternal and neonatal complications for the healthy pregnant women and women with preeclampsia are shown in Table 27 and Table 28. *P* values for differences between these groups were not calculated as the groups were not selected to be compared on these outcomes.

Table 27 Human Study 2 Maternal complications

	Healthy pregnant women (NP)	Women with untreated preeclampsia (UnRxP)
	n = 40	n = 40
Emergency caesarean birth	6 (15) #	17 (43)
Elective caesarean birth	14 (35)	7 (18)
Regional analgesia for vaginal birth	N/A	9 (23)
Anaesthetic intervention	N/A	34 (85)
Birth within 24 hours of TTE examination	14 (35)	22 (55)
Maternal complications	11 (28)	21 (53)
PPH	10 (25)	12 (30)
Number requiring blood transfusions	1 (3)	1 (3)
Hysterectomy for PPH	1 (3)	0
Eclampsia	0	1 (3)
Renal failure	0	0
ICU admission	0	1 (3) +
HDU admission	1 (3)	11 (28)
Wound infection/sepsis	0	3 (8)
Maternal length of stay (days)	3.4 ± 1.5	6.5 ± 9.6
Normotensive at 6 weeks postpartum	40 (100)	38 (95)*
Mortality	0	0

Results are mean ± SD, or count (%) as appropriate; TTE = transthoracic echocardiography; PPH = postpartum haemorrhage (estimated blood loss at vaginal birth of 500 ml, estimated blood loss at caesarean birth of 750 ml); ICU = intensive care unit (level 3 capabilities with renal replacement therapy, long term ventilation); HDU = high dependency unit (level 1 capabilities, advanced nursing observations, management of arterial line. No inotropes, no non-invasive or invasive ventilation). *100% normotensive at three months postpartum. + Refractory hypertension requiring sodium nitroprusside; # 1 case of undiagnosed breech.

Table 28 Human Study 2 Neonatal complications

	Healthy pregnant women (NP) n = 40	Women with untreated preeclampsia (UnRxP) n = 40
Gestation at birth (weeks)	40.1 ± 1.0	37.6 ± 3.4
Birth weight (g)	3607 ± 467	2976 ± 873
Neonatal complications	3 (8)	17 (43)
Apgar 1 minute	8.5 ± 1.4	8.0 ± 1.5
Apgar 5 minutes	9.2 ± 0.4	9.0 ± 0.7
NICU/SCN admission	1 (3)	16 (40)
Length of stay NICU/SCN (days)	4	18.9 ± 32.6
Quaternary centre transfer	0	2 (5)
NEC	0	2 (5)
Hypoglycaemia	0	3 (8)
Sepsis	1 (3)	4 (10)
RDS	2 (5)	6 (15)
Breastfeeding at discharge	N/A	38 (95)
Mortality	0	0

Results are mean ± SD, or count (%) as appropriate

NICU/SCN = neonatal intensive care/special care nursery; quaternary centre defined as NICU + neonatal surgery performed; NEC = necrotizing enterocolitis; RDS = respiratory distress syndrome.

5.3.11. Treatment interventions

Treatment interventions after TTE examination and subsequent pharmacological management for women with untreated preeclampsia are shown in Table 29. 33 women with preeclampsia were given pharmacological treatment interventions after TTE examination.

Table 29 Human Study 2 Treatment interventions after transthoracic echocardiography in women with preeclampsia

Pharmacological interventions	Women with untreated preeclampsia n = 40
Magnesium sulphate	10 (25)
Oral labetalol	13 (33)
Oral alpha methyldopa	1 (3)
Intravenous hydralazine	2 (5)
Oral nifedipine	4 (10)
Other*	17 (43)
1 medication	19 (48)
2 medications	4 (10)
3 medications	8 (20)
4 medications	1 (3)
5 medications	1 (3)

* Combination of induction of labour, urgent caesarean section, epidural analgesia. Data are expressed as number of women and (%).

5.3.12. Mild versus severe disease data

Subgroup analysis was undertaken to determine if there were differences in biochemical, haematological and urinary measurements and cardiac function measurements in women with mild preeclampsia versus women with severe preeclampsia (Table 30 and Table 31). The definition of severe preeclampsia was from Table 1 (page 9).

Demographic, haematological, biochemical and urinary data are shown in Table 30.

Women with severe untreated preeclampsia were of earlier gestations than women with mild untreated preeclampsia. Serum urea and creatinine levels were higher in women with severe disease. Serum urate levels were higher in women with severe disease.

Cardiac function data are shown in Table 31. MAP is higher in women with severe preeclampsia. The only differences observed between the women with mild disease compared to women with severe disease were in the size of their pericardial effusions. 95% of women with severe preeclampsia demonstrated pericardial effusions with 63% of these women having a significant pericardial effusion of ≥ 1.0 cm size. Two women with severe preeclampsia had TTE findings of significant fluid effects evidenced by M-mode and 2D changes of diastolic compression of the right heart chambers.

Threshold values of abnormal cardiac function in women with mild and severe preeclampsia are shown in Table 32. Thresholds for abnormal cardiac function are based on accepted definitions as described in Table 14 and Table 16. There were no observed differences in these threshold values between these groups.

Table 30 Human Study 2 Women with mild versus severe preeclampsia – Haematological, Biochemical and Urinary data

Variable	Untreated Preeclampsia (UnRxP) Mild n = 21	Untreated Preeclampsia (UnRxP) Severe n = 19	<i>P</i> value
Age (years)	31.2 ± 4.4	29.8 ± 6.1	0.429
Gestation at TTE (weeks)	37.6 ± 2.9	34.9 ± 4.9	0.043
BMI (kg/m ²)	33.0 ± 5.9	31.7 ± 7.4	0.563
Number nulliparous	13 (62)	13 (68)	0.748
Number with previous history of preeclampsia	0	2 (11)	0.165
Hct	0.35 ± 0.03	0.35 ± 0.03	0.810
Hb (g/l)	122.2 ± 9.6	124.8 ± 12.7	0.466
Pt (× 10 ⁹ /l)	280.6 ± 73.3	258 ± 79.4	0.372
Na (mmol/l)	135.5 ± 1.6	134.9 ± 3.9	0.560
K (mmol/l)	4.1 ± 0.4	4.3 ± 0.4	0.054
Urea (mmol/l)	2.76 ± 0.8	3.8 ± 1.4	0.006
Creatinine (µmol/l)	52.2 ± 9.4	61.5 ± 16.2	0.038
Cl (mmol/l)	105.3 ± 1.8	105.6 ± 2.9	0.660
HCO ₃ (mmol/l)	22.3 ± 1.9	21.6 ± 1.7	0.192
Urate (mmol/l)	0.29 ± 0.06	0.37 ± 0.08	0.001
Total protein level (g/l)	60.7 ± 3.03	59.4 ± 5.4	0.366
Albumin (g/l)	28.6 ± 2.04	28.7 ± 3.36	0.896
Total bilirubin (µmol/l)	10.57 ± 3.5	11.95 ± 6.0	0.388
Gamma GT (U/l)	11.95 ± 4.9	27.2 ± 43.01	0.142
ALT (U/l)	15.19 ± 7.8	15.47 ± 10.17	0.922
ALP (U/l)	135.4 ± 45.5	156.1 ± 56.1	0.212
Spot protein:creatinine ratio (g/mmol)	0.05 ± 0.03	0.15 ± 0.22	0.060

Data are mean ± standard deviation (SD) or count (%) as appropriate.

BMI = body mass index; Hct = haematocrit; Hb = haemoglobin; Pt = platelet; Na = sodium; K = potassium; Cl = chloride; HCO₃ = bicarbonate; gamma GT = gamma glutamyl transferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; Data are mean ± SD; TTE = transthoracic echocardiography

Table 31 Human Study 2 Women with mild versus severe preeclampsia - Cardiac function data

Variable	Untreated Preeclampsia (UnRxP) Mild n = 21	Untreated Preeclampsia (UnRxP) Severe n = 19	<i>P</i> value
MAP (mmHg)	108.7 ± 4.4	113.1 ± 4.95	0.006
CO (ml/min)	4737 ± 1457	4847 ± 1413	0.810
HR (BPM)	80.7 ± 11.7	80.8 ± 14.1	0.990
SV (ml)	58.15 ± 12.1	60.32 ± 14.02	0.612
SVR (dyne.s/cm ⁵)	1976 ± 512.6	2060 ± 741.5	0.684
FAC (%)	65.59 ± 7.0	63.27 ± 11.33	0.448
Septal e' (cm/s)	9.11 ± 2.42	8.15 ± 2.19	0.195
Septal a' (cm/s)	8.65 ± 2.08	8.01 ± 1.93	0.322
IVRT (ms)	86.03 ± 21.87	95.44 ± 24.27	0.208
MV DT (ms)	201.3 ± 34.77	203.3 ± 28.67	0.838
MV E/A	1.29 ± 0.34	1.28 ± 0.34	0.928
MV E/ septal e'	9.8 ± 2.5	11.0 ± 2.2	0.106
Size of effusion (cm)	0.76 ± 0.20	1.2 ± 0.53	0.002
Number of effusions	18 (86)	18 (95)	0.607
Number ≥ 1.0 cm	3 (14)	12 (63)	0.007
LV mass (g)	186.5 ± 38.35	191.9 ± 42.71	0.675
Tei index	0.54 ± 0.14	0.55 ± 0.11	0.808

Data are mean ± standard deviation; or count (%) as appropriate.

MAP = mean arterial pressure; CO = cardiac output; HR = heart rate; SV = stroke volume; SVR = systemic vascular resistance; FAC = fractional area change; e' = septal e' tissue Doppler wave velocity; a' = septal a' tissue Doppler wave velocity; IVRT = isovolumetric relaxation time; DT = deceleration time; E = mitral valve E wave velocity; A = mitral valve A wave velocity; LV = left ventricular; data are mean ± SD (standard deviation)

Table 32 Human Study 2 Number of women with threshold values for abnormal cardiac function – Women with mild versus severe preeclampsia

Value	Untreated Preeclampsia	Untreated Preeclampsia	<i>P</i> value
	(UnRxP)	(UnRxP)	
	Mild n = 21	Severe n = 19	
MV E/A < 1.4	16 (76)	13 (68)	0.506
Septal e' velocity < 8 cm/s	9 (43)	11 (58)	0.527
Septal s' velocity < 8 cm/s	10 (48)	9 (47)	1.000
Vcf ≤ 1.1	2 (10)	5 (26)	0.226
MV E/septal e' > 8	16 (76)	18 (95)	0.186
Septal a' velocity > septal e' velocity	9 (43)	11 (58)	0.341

MV = mitral valve; Vcf = velocity of circumferential fibre shortening; Data are expressed as number of women and (%). Some women had > 1 abnormal value.

Given the observation that many women with preeclampsia had pericardial effusions, and also that the women with severe preeclampsia demonstrated larger pericardial effusions, it can be conceived that there may be a relationship between serum protein levels. This is supported by the mechanism of fluid shifts throughout the body and colloid osmotic pressure. There were no differences shown however, between the women with mild preeclampsia and the women with severe preeclampsia regarding total protein levels or albumin levels.

Six women developed preeclampsia at gestations less than 34 weeks (15%) with 34 women developing preeclampsia at or after 34 weeks gestation. The study was not designed to test the hypothesis of differences between preterm and term preeclampsia and due to small numbers of women in the < 34 weeks gestation group statistical analysis was not performed on these data.

5.3.13. Interobserver and intraobserver reliability

Interobserver reliability using Bland-Altman plots for LVOT VTI is shown in Figure 51 and Figure 52. Interobserver reliability for LVOT diameter is shown in Figure 53 and Figure 54.

The mean (SD) differences for the LVOT diameter and LVOT VTI between two observers (ATD, JC) were 0.07 (0.09) and 0.18 (1.2) respectively.

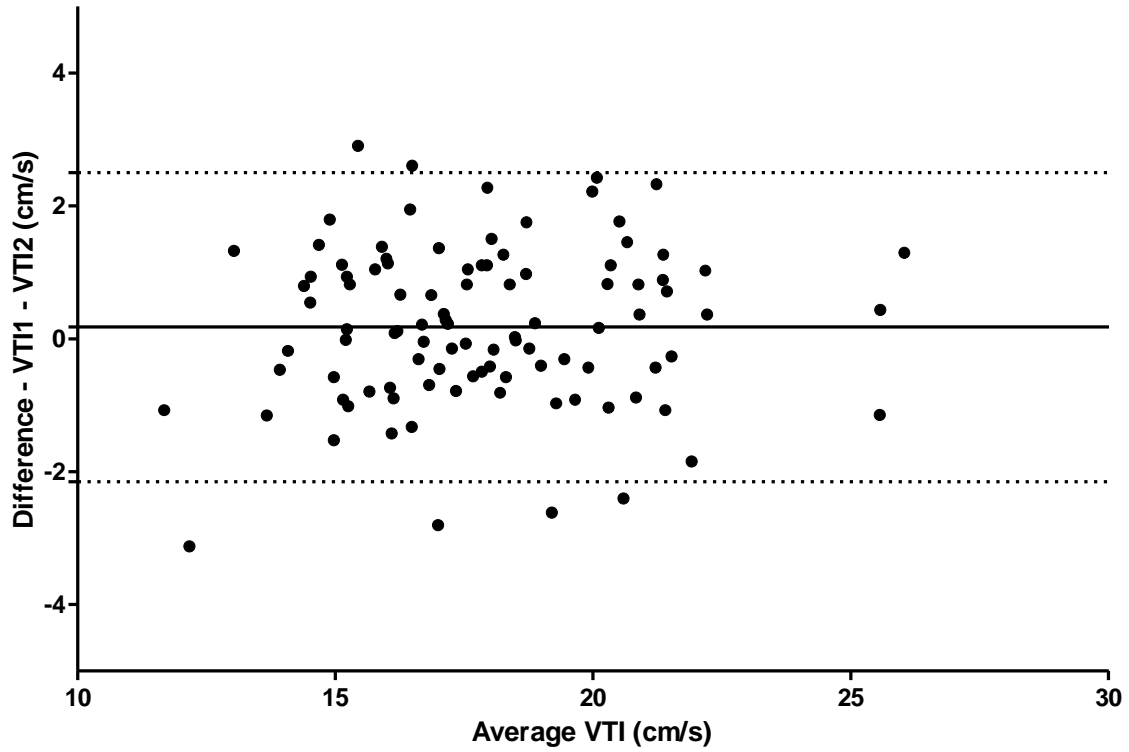
For the LVOT diameter this equates to 95% of the interobserver measurements being within 2.4 mm (13%) of the mean LVOT value for the two observers of 1.92 cm. For the LVOT VTI this equates to 95% of the interobserver measurements being within 2.5 cm/s (14%) of the mean value for the two observers of 17.88 cm/s.

The mean (SD) differences for the LVOT VTI (Figure 55 and Figure 56) and LVOT diameter for the single observer (ATD) (Figure 57 and Figure 58) were 0.01 (0.06) and 0.04 (0.15) respectively.

For the LVOT diameter this equates to 95% of the intraobserver measurements being within 1.2 mm (6%) of the mean LVOT value for the single observer of 1.95 cm. For the LVOT VTI this equates to 95% of the intraobserver measurements being within 0.29 cm/s (2%) of the mean value for the single observer of 17.97 cm/s.

These results show that for inter- and intraobserver reliability there is very good agreement and very good repeatability and reliability.

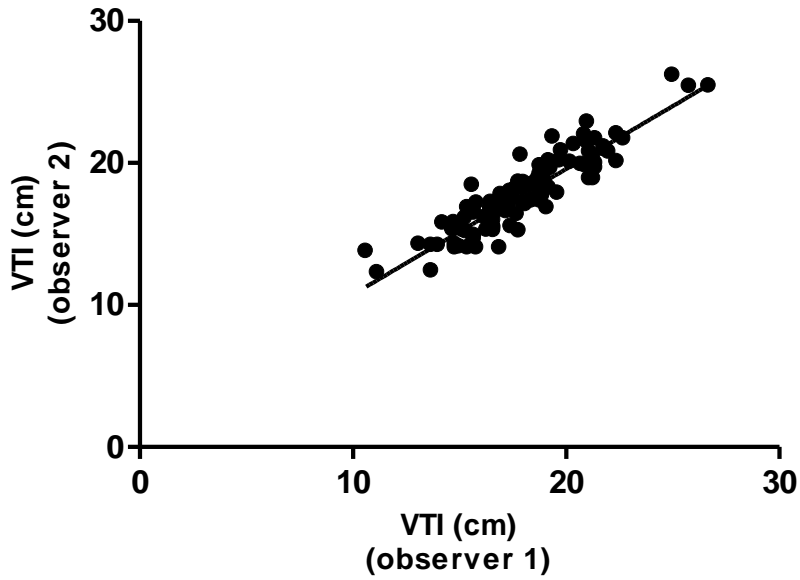
**Interobserver variability
Left ventricular outflow tract velocity time integral
n=100**



VTI = velocity time integral
mean difference = 0.18 cm/s
standard deviation (SD) = 1.2 cm/s
upper limit = mean + 1.96SD = 2.5 cm/s
lower limit = mean - 1.96SD = - 2.2 cm/s

Figure 51 Human Study 2 Bland-Altman plot of interobserver reliability left ventricular outflow tract velocity time integral

Correlation between two observers
(left ventricular outflow tract velocity time integral)



VTI = velocity time integral
Pearson $r = 0.911$
 $P < 0.0001$

Figure 52 Human Study 2 Correlation between two observers left ventricular outflow tract velocity time integral

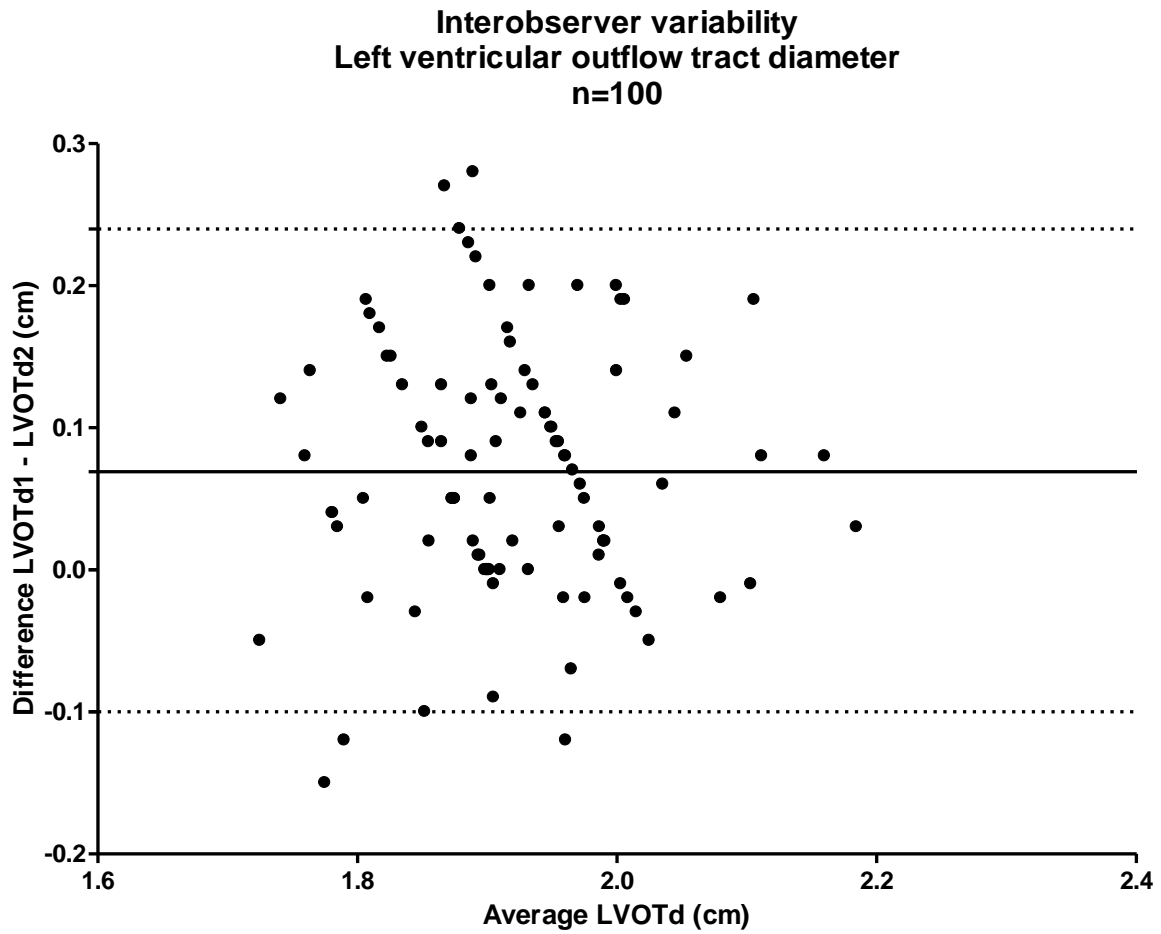
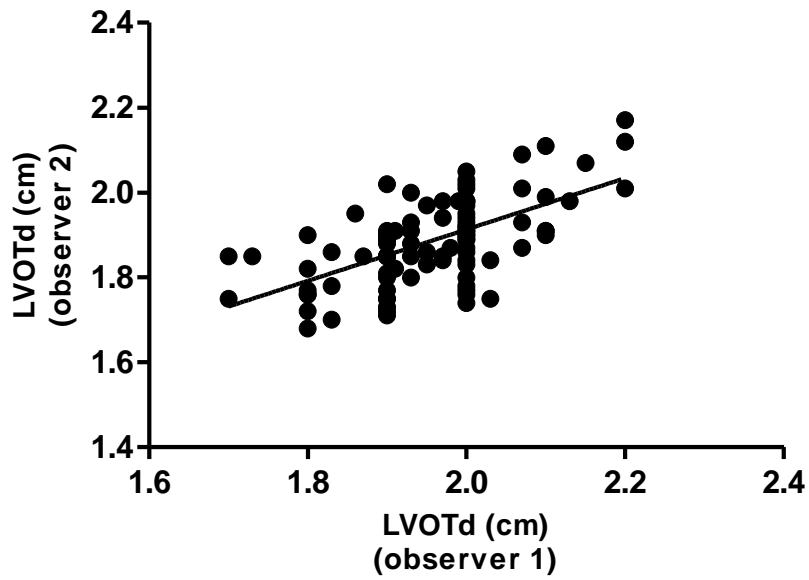


Figure 53 Human Study 2 Bland-Altman plot of interobserver reliability left ventricular outflow tract diameter

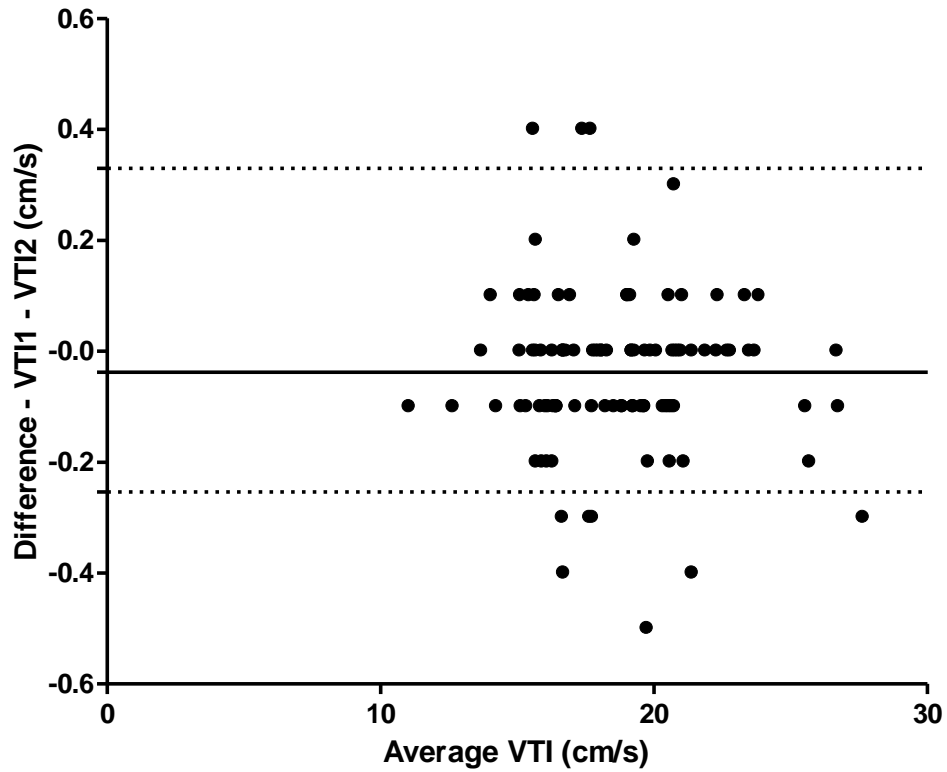
Correlation between two observers (left ventricular outflow tract diameter)



LVOTd = left ventricular outflow tract diameter
Pearson $r = 0.6056$
 $P < 0.0001$

Figure 54 Human Study 2 Correlation between two observers left ventricular outflow tract diameter

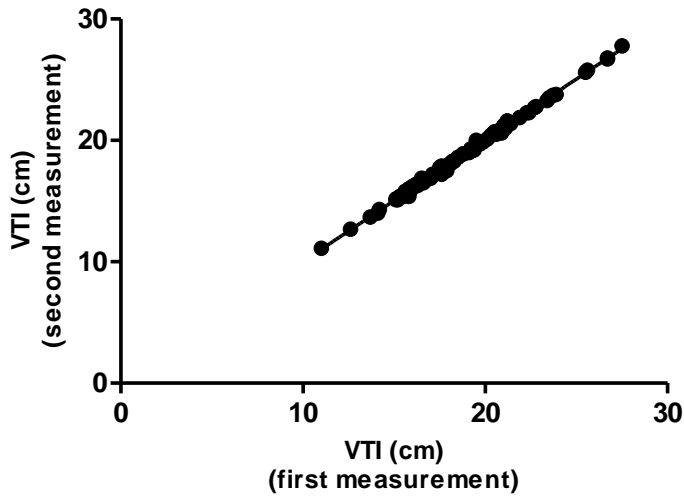
Intraobserver variability
Left ventricular outflow tract velocity time integral
n=100



VTI = velocity time integral
mean difference = 0.038 cm/s
standard deviation (SD) = 0.1489 cm/s
upper limit = mean + 1.96SD = 0.292 cm/s
lower limit = mean - 1.96SD = - 0.2538 cm/s

Figure 55 Human Study 2 Bland-Altman plot of intraobserver reliability left ventricular outflow tract velocity time integral

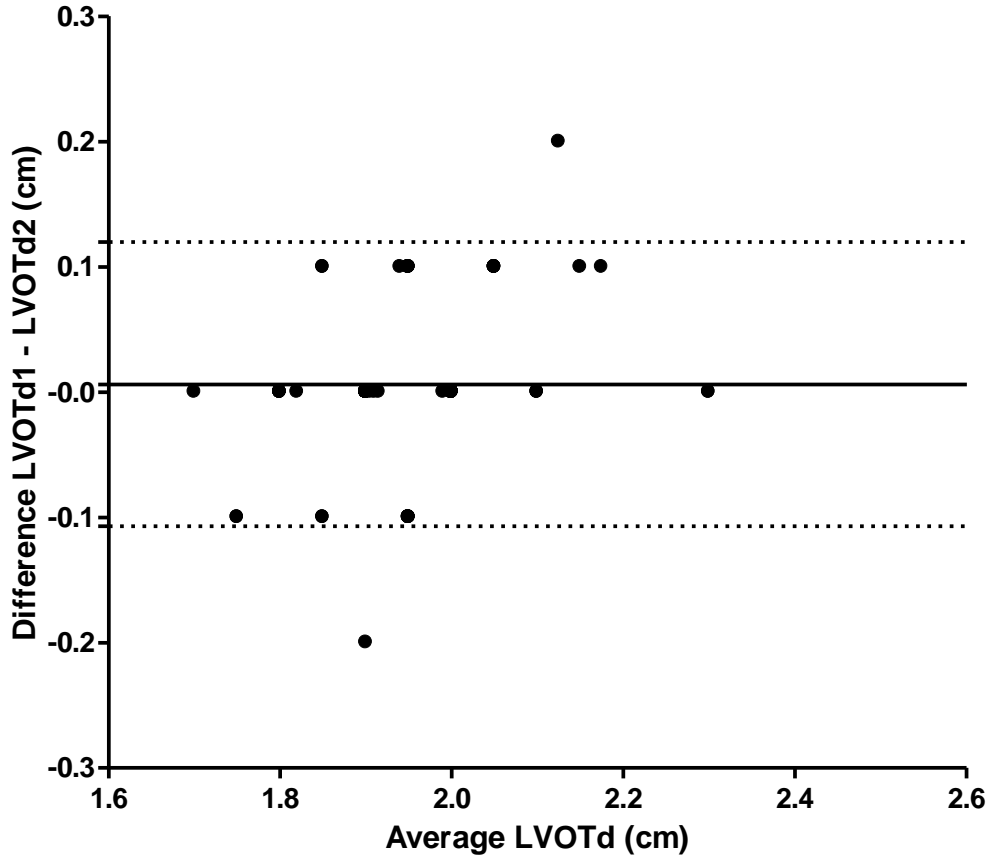
Correlation between two measurements
(left ventricular outflow tract velocity time integral single observer)



VTI = velocity time integral
Pearson $r = 0.999$
 $P < 0.0001$

Figure 56 Human Study 2 Correlation between two measurements left ventricular outflow tract velocity time integral

Intraobserver variability
Left ventricular outflow tract diameter
n=100

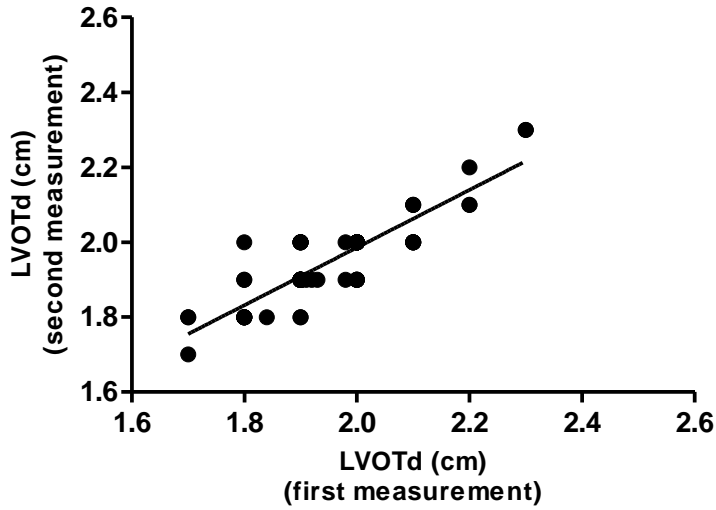


LVOTd= left ventricular outflow tract diameter
 mean difference = 0.006 cm
 standard deviation (SD) = 0.058 cm
 upper limit = mean + 1.96SD = 0.120 cm
 lower limit = mean - 1.96SD = - 0.107 cm

Sixty eight of the measurements showed no difference between the first and second measurements. Therefore a single appearing dot on the above graph represents many measurements resulting in the same data point on the graph.

Figure 57 Human Study 2 Bland-Altman plot of intraobserver variability left ventricular outflow tract diameter

Correlation between two measurements
(left ventricular outflow tract diameter single observer)



LVOTd = Left ventricular outflow tract diameter
Pearson $r = 0.873$
 $P < 0.0001$

Figure 58 Human Study 2 Correlation between two measurements left ventricular outflow tract diameter

5.4. Discussion

Human Study 2 of this thesis represents the first comprehensive study involving quantification of left ventricular systolic and diastolic function and left ventricular structural assessment of pregnant women compared with women with untreated preeclampsia. It is also the largest study examining systolic and diastolic function in women with untreated preeclampsia.

The key and novel finding is that of an ino vasoconstrictive state with diastolic impairment in women with untreated preeclampsia. The following sections discuss this finding in detail.

5.4.1. Haemodynamic and systolic changes

Increased cardiac output and inotropy

The cardiac output was increased in women with untreated preeclampsia compared with healthy pregnant women. This was due to an increase in stroke volume in women with untreated preeclampsia because there was no difference between the two groups regarding heart rate.

The end diastolic area between the two pregnant groups was similar. The end systolic area was smaller in the women with preeclampsia. This accounts for the significant difference in fractional area change (FAC) between the two groups as $FAC = ((LVEDA - LVESA)/LVEDA) \times 100$. Therefore the volume of blood expelled from the left ventricle is greater in women with untreated preeclampsia than in healthy pregnant women despite starting at the same left ventricular end diastolic area.

The similarity in the velocity time integral time between the two groups means that the time it takes for this increased amount of blood to be expelled from the left ventricle in women with untreated preeclampsia is the same as the time it takes to expel a smaller amount of blood from the left ventricle in healthy pregnant women. This would imply that the rate of change of left ventricular contraction is greater in women with untreated

preeclampsia than in healthy pregnant women. There is also a significant decrease in the septal s' time which indicates that the rate of change of septal myocardial wall movement is greater in women with untreated preeclampsia than in healthy pregnant women.

Additional information supports preserved systolic function as evidenced by mean septal s' velocity of > 7.5 cm/sec in women with untreated preeclampsia.

The following observations obtained in Human Study 2 in women with untreated preeclampsia demonstrate that women with untreated preeclampsia have increased inotropy compared with healthy pregnant women:

1. Significantly increased cardiac output
2. Significantly increased stroke volume
3. Similar left ventricular end diastolic area
4. Significantly smaller left ventricular end systolic area leading to increased fractional area change and increased fractional shortening
5. Similar velocity time integral waveform time
6. Significantly shorter septal s' velocity waveform time

The finding of increased inotropy has not previously been reported and is a novel finding from this work.

5.4.2. Aetiological implications

The inovoconstrictor concept

There is increased inotropy demonstrated in women with untreated preeclampsia occurring in the presence of increased systemic vascular resistance. This implies that the left ventricular systolic function is increased in the presence of an elevation in systemic vascular resistance leading to the concept of a circulating inovoconstrictor. A current substance of interest regarding the aetiology of preeclampsia is soluble FMS-like tyrosine kinase (sFlt1). This is a soluble vascular endothelial growth factor (VEGF) receptor. This

substance, however, does not have an effect on the heart consistent with what has been demonstrated in Human Study 2.

Other substances investigated that are known to have cardiac effects have produced conflicting results in the literature. Catecholamines and the sympathetic nervous system (SNS) have been extensively investigated. Most studies investigating catecholamines report increase in heart rate in association with increased cardiac output which was not demonstrated in this study.

These findings of increased cardiac output in women with preeclampsia are consistent with the work of Easterling and colleagues who have proposed a hyperdynamic model of preeclampsia (Easterling and Benedetti 1989). Easterling and colleagues have performed investigations assessing interventions based on haemodynamic assessment in early pregnancy and to assess whether this influences the development of gestational hypertension and preeclampsia as the pregnancy progresses. They have specifically investigated atenolol, a β_1 -adrenoceptor antagonist, because of their previous work which found that the nine women out of 179 women who developed preeclampsia had elevated cardiac outputs (> 7.4 l/min) prior to development of the clinical disease. Their recent results retrospectively comparing sFlt1 levels in women who have undergone haemodynamically directed atenolol therapy (high risk women) versus no atenolol therapy (low risk women) indicate that there is a reduction in sFlt1 levels and their conclusion was that haemodynamically directed therapy may alter the antiangiogenic state (Carr, Tran *et al.* 2009). The use of oral β -adrenoceptor antagonists has been reviewed by Magee and Duley in a Cochrane Systemic review. This work concluded that whilst these agents appear safe in comparison to other agents, they are associated with an increase in small for gestational age (SGA) infants and clear benefits to the woman and the baby have not been demonstrated in the group of women with mild to moderate hypertension in pregnancy (Magee and Duley 2003). Large randomized control trials are needed to compare antihypertensive therapies in general with placebo. This trial is currently underway (Magee, von Dadelszen *et al.* 2007). In the light of the findings of Human Study 2 further investigation of catecholamines and the sympathetic nervous system response to this disease is required.

Within the heart failure literature there are mechanisms such as a pathological injury response model which leads to inappropriate and ongoing activation of the sympathetic nervous system in low cardiac output states (Kudo, Mikuniya *et al.* 1998; Sackner-Bernstein and Mancini 1995). The response to injury by the body usually only lasts minutes to hours such as that which may occur during a myocardial ischaemia (Esler and Kaye 2000; Floras 2009; Grassi, Seravalle *et al.* 2009; Nisell and Lunell 1984). The injury to the body that occurs in preeclampsia is ongoing, commencing well before the clinical symptoms and signs develop at 20 weeks gestation. The chronic unremitting nature of the injury to the body and the possible chronic sympathetic nervous system response may lead to maladaptive changes. The temporal nature of improvement in blood pressure measurements that occurs after the birth of the baby and delivery of the placenta supports the concept that sympathetic nervous system activity may account for some of the secondary injury that occurs in preeclampsia. Further investigation of this pathological response to injury model concept is required.

A group of substances with inotropic properties that are of current interest in the pharmacological and laboratory research setting are the bufodienolides. These are cardiotonic steroid molecules that have been synthesized and are associated with vasoconstriction and increased inotropy (Averina, Tapilskaya *et al.* 2006; Bagrov, Shapiro *et al.* 2009; Dmitrieva and Doris 2002). There is preliminary work investigating these substances and their role in preeclampsia and the results of Human Study 2 here would support further investigation of these substances and their role in preeclampsia.

5.4.3. Height and weight

As was previously discussed in Chapter 4 there is controversy regarding the indexing of cardiac measurements in pregnancy and lack of agreement about what equation, if any, to use to calculate body surface area in pregnancy. Nonetheless it is important to examine the changes in cardiac output relating to weight given that the women with preeclampsia were of higher body mass index than healthy pregnant women. Weight gain during pregnancy, prepregnancy weight and absolute weight, have all been associated with the development of preeclampsia.

The importance of the finding of increased cardiac output in the group of women with untreated preeclampsia lies not in the fact that it is necessarily larger, as one might expect given the larger body mass index in this group, but rather in the increase in inotropy that was demonstrated in this group. The usual situation regarding cardiac output is that if one person is much larger than another person, then their cardiac output is usually larger. The reason for the larger cardiac output is that their heart is larger, their end diastolic volumes are larger, their stroke volumes are subsequently larger however their fractional shortening, fractional area change and ejection fraction remain similar to the smaller person.

Human Study 2 has demonstrated that the reason for the increased cardiac output in women with untreated preeclampsia is an increase in inotropy not an increase in left ventricular end diastolic volume. The increase in cardiac output is due to an increase in stroke volume which occurs from a similar end diastolic volume to healthy pregnant women. The fractional area change and fractional shortening are both increased in women with untreated preeclampsia which is caused by increased inotropy. Large women who are healthy and non-pregnant do not exhibit increased inotropy compared to smaller women.

5.4.4. Diastolic changes

Human Study 2 showed that there are alterations in the diastolic cardiac flows, diastolic time intervals and diastolic dimensions in women with untreated preeclampsia.

Compared with healthy pregnant women, women with untreated preeclampsia demonstrate changes of:

1. Significantly reduced mitral valve E/A wave ratio
2. Significantly reduced septal e' wave velocity
3. Significantly reduced septal a' wave velocity
4. Significantly increased isovolumetric relaxation times and mitral valve deceleration times
5. Increased left ventricular mass
6. Significantly more pericardial effusions

These data demonstrate that women with untreated preeclampsia have significant changes in left ventricular diastolic function. These data are consistent with recent work by other authors (Rafik Hamad, Larsson *et al.* 2009; Zentner, du Plessis *et al.* 2009).

Diastolic heart failure is more common in elderly women than elderly men. It is accepted that there are aetiological and myocardial structural and functional differences between systolic and diastolic heart failure (Leite-Moreira 2006; Pirracchio, Cholley *et al.* 2007; Zile and Brutsaert 2002). Given that there are major diastolic changes in women with untreated preeclampsia it needs to be considered that the aetiology of the diastolic failure that occurs in some elderly women has its origins during pregnancy complicated by preeclampsia (Mosca, Banka *et al.* 2007; Newstead, von Dadelszen *et al.* 2007). In addition, the increased long term cardiac morbidity among women who have had preeclampsia may be explained by these abnormalities in diastolic function (Hermes, Franx *et al.* 2010; Irgens, Reisaeter *et al.* 2001).

5.4.5. Structural changes

Increased left ventricular mass

Studies have demonstrated that there exists increased left ventricular mass during pregnancy and more so with hypertensive diseases of pregnancy. It is unclear from the literature whether this increase in left ventricular mass calculated by various equations is myocardial cell mass, myocarditis or myocardial oedema. The resolution of hypertrophy has not been clearly demonstrated as few women are followed longitudinally, however it may be that the increase in observed left ventricular mass is myocardial oedema and not increased muscle mass caused by increased afterload (Bijnens and Sutherland 2008; Dent, Scott *et al.* 2000; Fatema, Hirono *et al.* 2002; Hauser, Gordon *et al.* 1983; Hosenpud, Norman *et al.* 1987; Maeder, Wolber *et al.* 2006; Spotnitz and Hsu 1994).

In normal pregnancy there is increased left ventricular wall thickness which may contribute to some impairment of diastolic function. In women with preeclampsia there is additional left ventricular wall increases leading to increased left ventricular mass calculations.

Pericardial fluid

The amount of pericardial fluid and the number of women demonstrating pericardial fluid was large in the women with untreated preeclampsia compared to healthy pregnant women. Pericardial effusions may contribute to the development of pericardial constraint and also the presence of pericardial fluid may lead to diastolic ventricular interaction. In preeclampsia myocardial oedema may further contribute to diastolic dysfunction. The size of the pericardial effusion however did not appear to influence cardiac output.

5.4.6. Threshold values for abnormal cardiac function

Observations in the healthy pregnant women in Human Study 2 showed that despite being healthy they have objective measures of reduced diastolic and systolic function with reductions in mitral valve E/A ratios, septal e' velocities, increased mitral valve E/septal e' ratios and reductions in systolic function. These results are consistent with the findings in Human Study 1, which showed that some women in the healthy term pregnant group had reduced systolic function, evidenced by very low cardiac outputs, and became symptomatic in the head-down position with a reduction in stroke volume in that position. The diastolic changes demonstrated in Human Study 2 may account for some of the changes observed in Human Study 1.

Quantifiable abnormalities in diastolic function are more widespread in the group of women with untreated preeclampsia. The two most striking findings were:

1. 85% of women with untreated preeclampsia had mitral valve E/septal e' ratios of greater than 8. Increases in left atrial pressure and left ventricular end diastolic pressure are associated with values > 8 in the non-pregnant population. This result may indicate raised left atrial pressure and left ventricular end diastolic pressure in this group of women as well.
2. 50% of women with untreated preeclampsia had septal a' velocities $>$ septal e' velocities. None of the healthy women had these changes.

5.4.7. The septal tissue Doppler waveform

This waveform warrants particular emphasis. In the 100 women examined in this study, this waveform was easy to obtain and gave reliable velocities. Doppler alignment was excellent when the sample volume was placed at the septal region of the mitral valve annulus. The information that can be obtained from this waveform is extensive as shown in Figure 59 with systolic and diastolic measurements being shown on one waveform. Furthermore significant changes occurred in this waveform in women with preeclampsia (reduced septal e' velocity, septal a' velocity > septal e' velocity, reduced septal s' time, increased isovolumetric relaxation time) which can be easily compared to healthy women, in whom few of these changes occurred.

The absence of changes in healthy women is consistent with data in the non-pregnant literature involving athletes. Pregnancy has often been referred to as a physiological stress test similar to exercise. Tissue Doppler findings in athletes show that the tissue Doppler indices are normal in physiological hypertrophy such as that which occurs in the exercise situation (Butz, van Buuren *et al.* 2010). In addition, the measurement of tissue Doppler indices can be used to detect abnormalities in hypertensive individuals prior to the development of left ventricular hypertrophy and so may have an application in serially following women throughout pregnancy to monitor the development of left ventricular hypertrophy. Apart from a research tool it has not been reported to be used in the clinical setting in pregnancy although in other areas of medicine it is becoming more widely utilized (Combes, Arnoult *et al.* 2004; Germing, Ulrich *et al.* 2008; Salem, Vallee *et al.* 2008; Skubas 2009; Sturgess, Marwick *et al.* 2007; Tigen, Karaahmet *et al.* 2009; Yu, Sanderson *et al.* 2007)

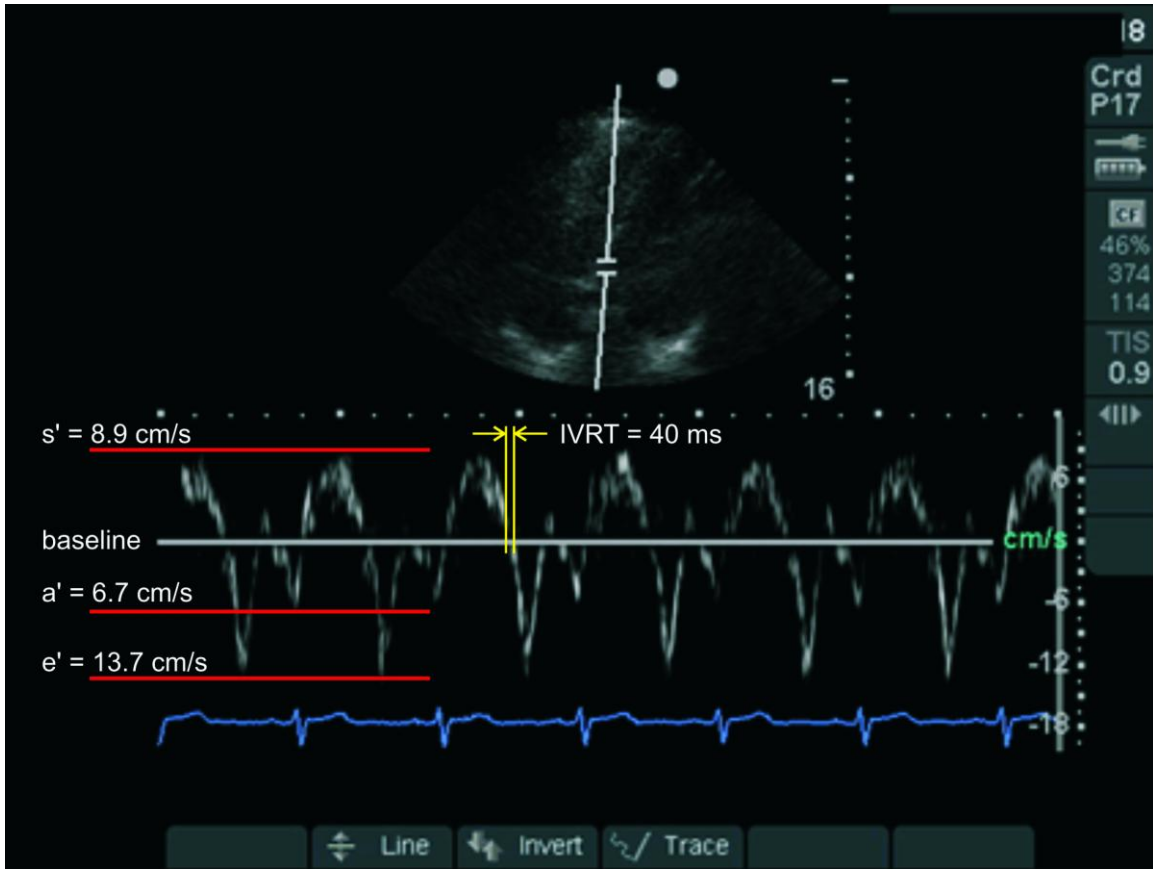


Figure 59 Septal tissue Doppler waveform in a healthy pregnant woman

This is the appearance of the septal tissue Doppler waveform in a healthy pregnant woman. The waveform consists of three main deflections. The first deflection above the baseline (0 cm/s line) that occurs during systole is the s' wave. The peak velocity is recorded as the s' velocity. In this case the s' velocity is 8.9 cm/s. The first deflection below the baseline in diastole is the e' wave. The peak velocity of this wave is recorded as the e' velocity. In this example it is 13.7 cm/s. The time period between the end of the s' wave and the beginning of the e' wave is known as the isovolumetric relaxation time (IVRT). In this example it is 40 ms. The second downward deflection during diastole is the a' wave. The peak deflection is recorded as the a' velocity and in this case it is 6.7 cm/s. This waveform obtained from a different healthy pregnant woman is also described in Chapter 3 Human Experimental Methods Figure 17 (page 79). The use of the Cardiac Function waveform diagram Figure 6 (page 65) assists with understanding how this waveform pattern is related to the other velocities, pressures and volumes in systole and diastole.

5.4.8. The Pressure Volume loop construct

The Frank Starling mechanism describes the relationship between the resting fibre length of the myocardial tissue and the tension or force developed by the ventricle during myocardial contraction. Resting fibre length is equated to end diastolic volume. This relationship was first shown in cardiac experiments in dogs. From this relationship the classic pressure volume loop of the left ventricle is created (Katz 2002; Markwalder and Starling 1914; Patterson, Piper *et al.* 1914; Patterson and Starling 1914; Starling 1914).

The concept of the left ventricular pressure volume loop allows visualization of the data from the three different groups in this study. For the purposes of the discussion and accepted description of the graph, the Pressure Volume loop will be referred to as such with the Y axis dependent variable (pressure) being described first followed by the X axis independent variable (volume) being stated second. This is shown in Figure 60, Figure 61 and Figure 62.

Figure 60 represents the situation in healthy non-pregnant women, with a reference diastolic and systolic curve and the cardiac cycle. The closure of the aortic valve is the commencement of diastole with the isovolumetric relaxation time. At the end of this time period the ventricle contains the end systolic volume. The left ventricle then fills during diastole to an end diastolic volume. At this point the interventricular pressure rises during the first phase of systole, the isovolumetric contraction time. The aortic valve then opens and ejection of blood occurs into the aorta. When pressure in the left ventricle decreases below that of the aorta the aortic valve closes and diastole once again commences. It is useful to think of the cardiac cycle beginning at the commencement of diastole as the left ventricle fills and then expels that volume of blood. The pressure, volume and time conditions that exist during diastole facilitate the effective stroke volume of that specific heart beat.

Figure 61 superimposes the situation in the case of healthy pregnant women. The resting tension curve of the left ventricle is shifted upward slightly due to the findings of reduced diastolic function compared with non-pregnant women. The aortic valve opens at a lower

pressure due to lower mean arterial pressure in healthy pregnant women compared to healthy non-pregnant women. There were no changes in systolic function between healthy non-pregnant women and healthy pregnant women therefore the systolic function curve remains unchanged and the healthy pregnant cardiac cycle can be generated.

Figure 62 demonstrates the changes that have been shown in Human Study 2 in women with untreated preeclampsia compared with healthy pregnant women and healthy non-pregnant women. The greater reduction in diastolic function compared with healthy pregnant women means that the resting tension curve is shifted upwards beyond the healthy pregnant women's curve. Left ventricular end diastolic areas, translated here to left ventricular end diastolic volume were the same in healthy pregnant women and in women with untreated preeclampsia. The aortic valve opens at a higher pressure due to the presence of hypertension. The increase in systolic function with increased cardiac output due to increased stroke volume means that the systolic curve is shifted to the left indicating increased inotropy.

The area of the cardiac cycle shape for Figure 60, Figure 61 and Figure 62 represents cardiac work. Cardiac work is significantly greater in women with untreated preeclampsia compared to the other two groups.

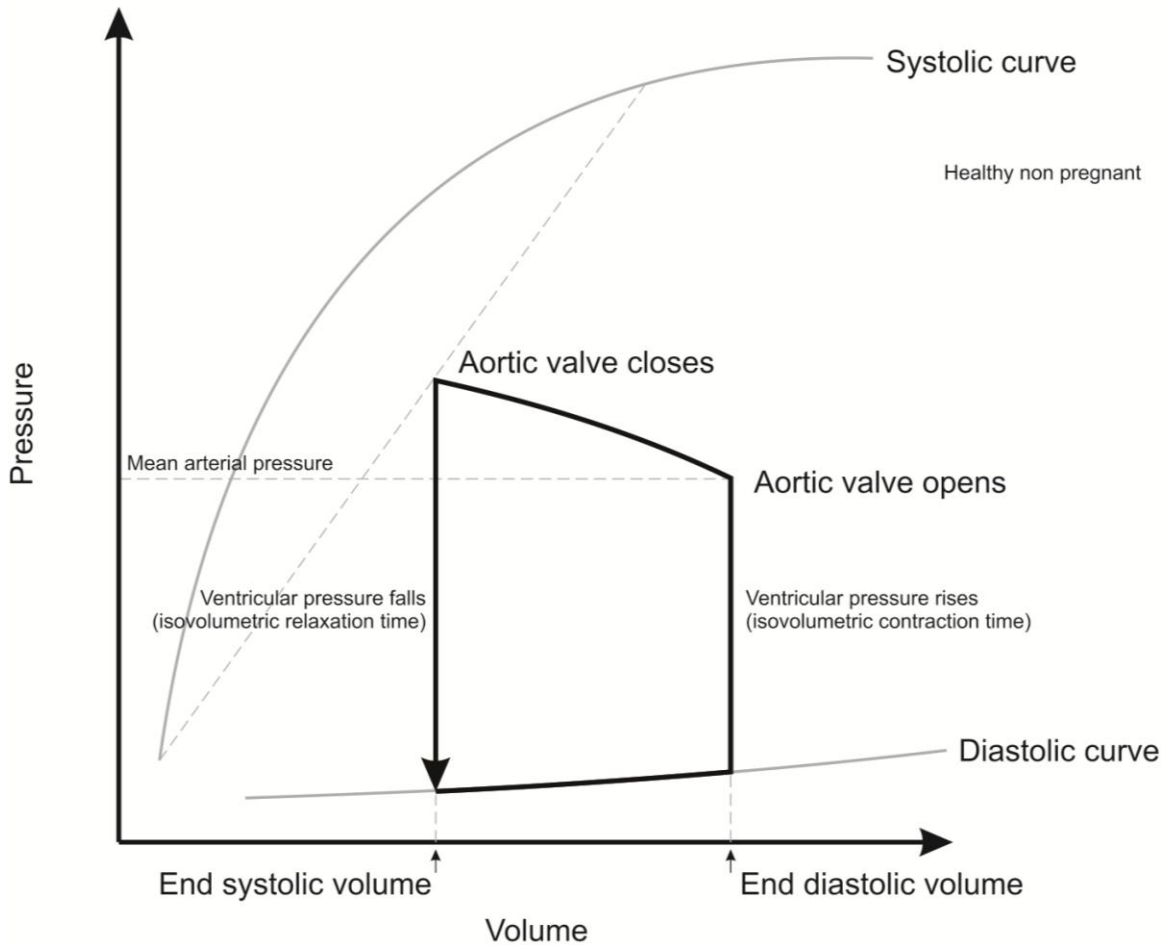


Figure 60 Pressure Volume diagram - Healthy non-pregnant women

This Pressure Volume diagram shows a single cardiac cycle in a healthy non-pregnant woman. The heart fills from an end systolic volume to an end diastolic volume along the diastolic curve or resting tension curve. This occurs during diastole. The mitral valve then closes and the ventricular pressure rises. This time period is known as the isovolumetric contraction time and is the first phase of left ventricular systole. The aortic valve opens when the pressure in the left ventricle exceeds the pressure in the aorta. The stroke volume is ejected. Contraction of the left ventricle is governed in part by factors intrinsic to the myocardium and is related to the systolic curve of the left ventricle. The aortic valve closes when the pressure in the left ventricle falls below the pressure in the aorta. This completes left ventricular systole. The first phase of diastole then occurs with active relaxation of the left ventricle during the time known as the isovolumetric relaxation period.

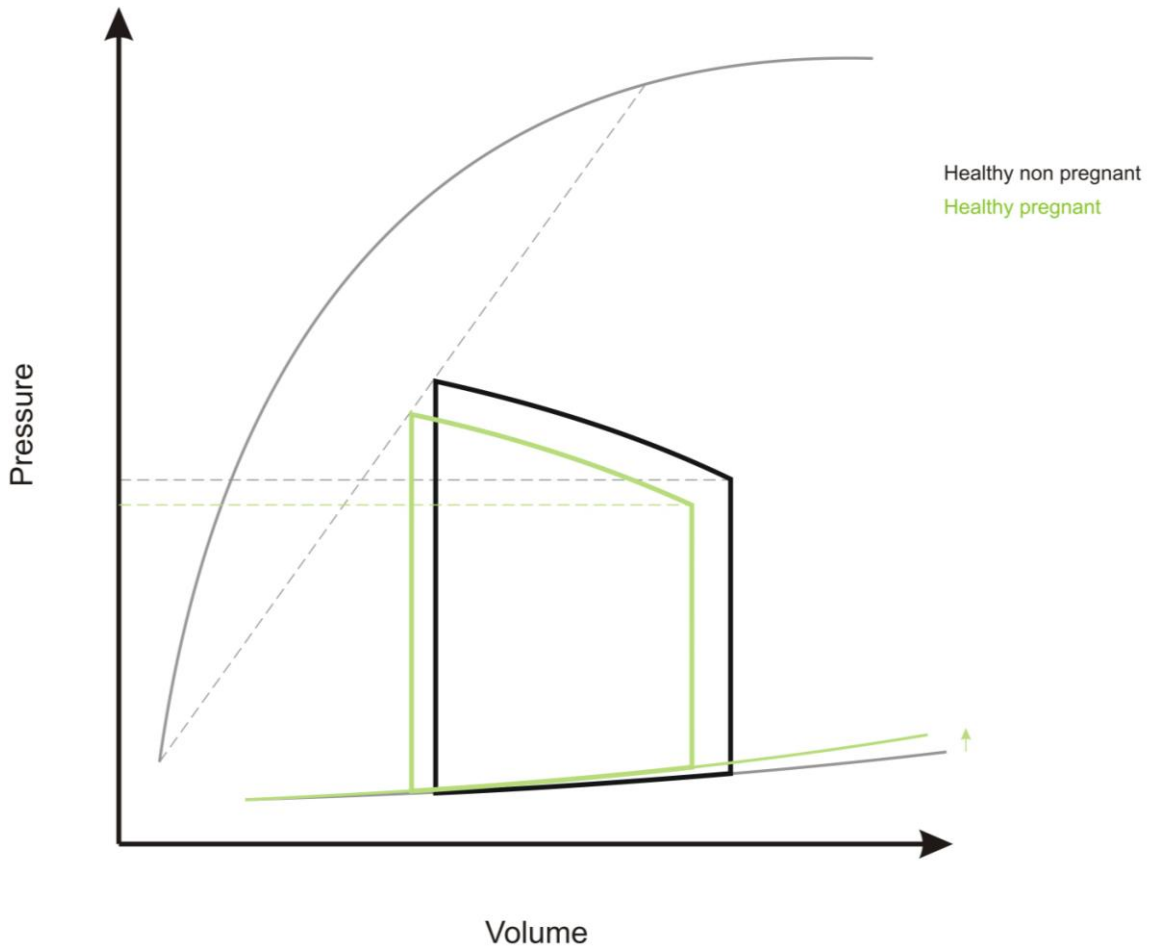


Figure 61 Pressure Volume diagram - Healthy non-pregnant women compared with healthy pregnant women

This diagram shows the superimposed healthy pregnant woman's cardiac cycle (green). The resting tension or diastolic curve is shifted upwards due to the impairment in diastolic function that occurs during healthy pregnancy (data from Table 24 page 127) when compared to non-pregnant women. The mean arterial pressure is reduced allowing the aortic valve to open at a reduced intraventricular pressure (green dotted line) (data from Table 23 page 124). The left ventricular end systolic area and the left ventricular end diastolic areas are slightly reduced compared with a non-pregnant woman however they did not reach statistical significance. The stroke volume is similar in healthy pregnant and non-pregnant women (data from Table 23 page 124) therefore the systolic curve is unchanged.

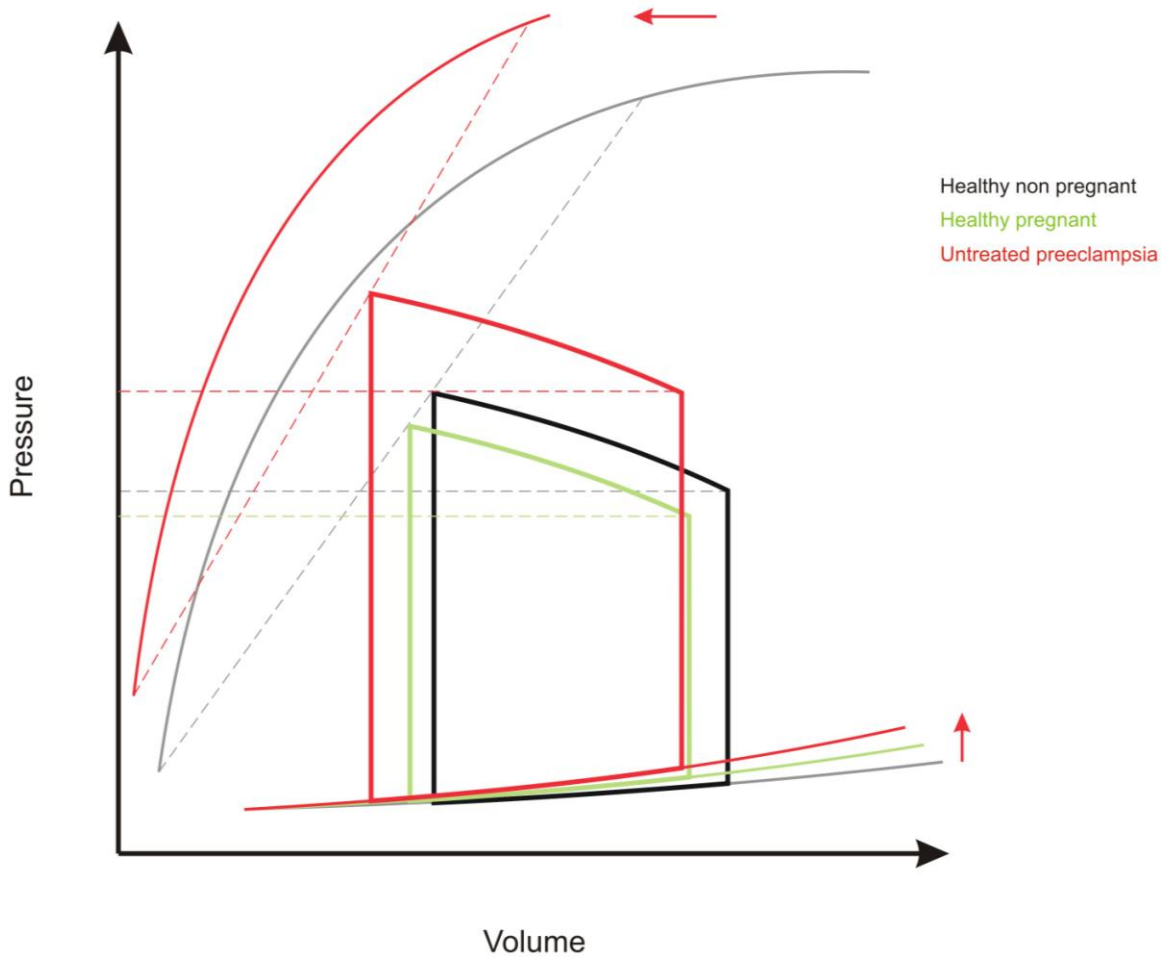


Figure 62 Pressure Volume diagram - Healthy non-pregnant women and healthy pregnant women compared with women with untreated preeclampsia

This diagram illustrates the addition of the red curve which is the situation that occurs in women with untreated preeclampsia. The left ventricular end diastolic volume is similar to the healthy pregnant woman however due to the pronounced diastolic changes, the diastolic curve is shifted upwards compared to healthy pregnant women (red arrow) (data from Table 24 page 127). This means that the end diastolic pressure is greater in women with untreated preeclampsia compared with healthy pregnant woman. The mean arterial pressure is significantly greater in women with untreated preeclampsia leading to the aortic valve opening at a higher intraventricular pressure (red dotted line). The end systolic volume is reduced compared to healthy pregnant women which leads to an increased fractional area change (data from Table 23 page 124). In the presence of increased mean arterial pressure this is caused by an increase in inotropy of the heart as indicated by the leftward shift of the systolic curve.

5.4.9. Acute pulmonary oedema in preeclampsia

Acute pulmonary oedema is a cause of morbidity and mortality in women with preeclampsia. Intravenous fluid therapy may lead to acute pulmonary oedema. Intravenous fluid therapy may also exacerbate acute respiratory distress syndrome (ARDS), leading to hypoxaemia, high airway pressures and difficulty with ventilation. Fluid therapy however may be indicated if there are concerns about placental perfusion so the ability to tailor intravenous fluid therapy to the women that need it most is important.

Acute pulmonary oedema occurs in women with preeclampsia in association with severe hypertension. This is different to acute pulmonary oedema that occurs with left ventricular systolic failure. It can be seen from the results in Human Study 2 that women with untreated preeclampsia have impaired diastolic function in the presence of severe hypertension. Thus the acute pulmonary oedema that occurs in women with preeclampsia is one of hypertensive cardiac failure or diastolic heart failure. This is most likely to occur in the presence of a preserved left ventricular ejection fraction and thus adequate stroke volume and flow to the end organs. There is however reduced oxygen delivery as the presence of pulmonary oedema limits respiratory gas exchange and leads to hypoxaemia. This is a situation where the thinking regarding adequate cardiac function needs to be broadened. Adequate cardiac function needs to include the effective and efficient flow of blood from the lungs to the left side of the heart. Thus the term cardiac output with adequate pulmonary gas exchange describes a situation where the heart is functioning appropriately by incorporating effective cardiac output from the lungs and effective cardiac output to the systemic circulation.

In this setting, heart failure with pulmonary venous congestion resembles diastolic heart failure (heart failure with normal ejection fraction), rather than dilated cardiomyopathy. This condition is common in elderly people, accounting for approximately 40% of patients with symptoms and signs of cardiac failure. In conceptual terms, they have a small stiff heart rather than a dilated floppy heart. At the end diastolic volume, the intraventricular pressure is elevated, and small increases in volume lead to dramatic rises in pressure, as their end diastolic Pressure Volume curve is steep. In the presence of

hypertension, the heart is less able to eject blood, leading to an increase in the end diastolic volume, which translates into dramatically increased end diastolic pressure, with enhanced likelihood of pulmonary venous congestion.

Based on data from Human Study 2 and using the pressure volume loop construct the curve can be applied to the situation where a hypothetical intravenous fluid bolus is given to a healthy pregnant woman and also to a woman with preeclampsia. The use of this diagram in combination with the data obtained from Human Study 2 helps improve the understanding of why women with severe hypertension in pregnancy have an increased propensity to develop acute pulmonary oedema especially with fluid boluses.

Five determinants of cardiac output are frequently taught in the textbooks and current literature; preload, contractility, afterload, rate and rhythm. It is important to consider the sixth; cardiac relaxation (lusitropy) as creating the diastolic conditions at the end of which an adequate stroke volume is generated for appropriate cardiac output and also adequate lung emptying into the left atrium during both systole and diastole. It is different to preload in that it describes times and velocities during diastole and not purely pressure or volume as in preload. Therefore in Figure 63 the term lusitropy (relaxility) is incorporated into the pressure volume loop construct.

Figure 64 demonstrates the threshold left ventricular end diastolic pressure above which acute pulmonary oedema occurs. A fluid bolus is then given to a healthy pregnant woman Figure 65. The fluid bolus increases the left ventricular end diastolic volume however the threshold left ventricular end diastolic pressure is not reached for that particular person and acute pulmonary oedema does not occur.

Figure 66 shows the situation when the same fluid amount is given to a woman with untreated preeclampsia. The fluid bolus leads to an elevation in left ventricular end diastolic volume that corresponds to a left ventricular end diastolic pressure value above which acute pulmonary oedema (APO) occurs. Furthermore women with preeclampsia frequently have reduced colloid osmotic pressure with low total protein and albumin

levels and renal protein loss which further increases their propensity for developing acute pulmonary oedema.

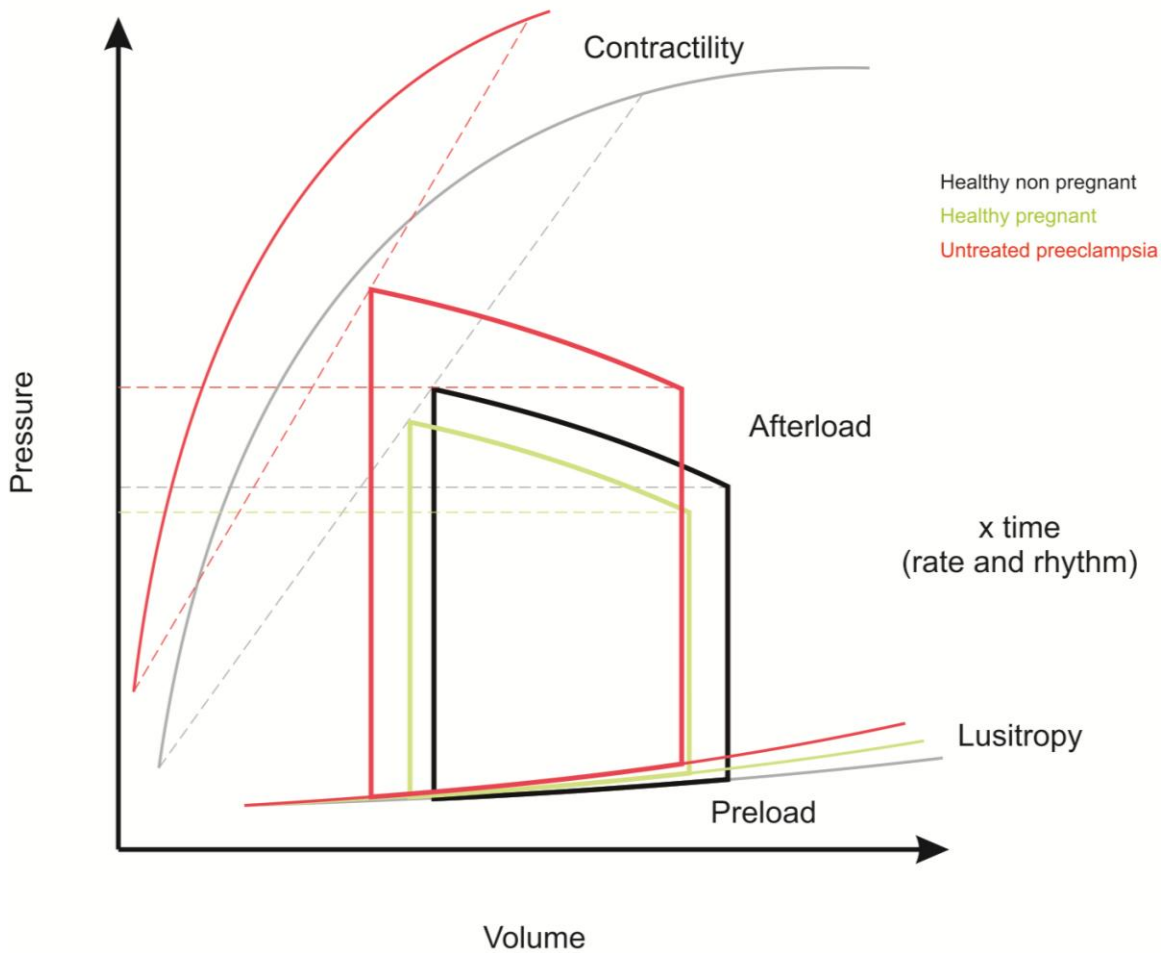


Figure 63 Pressure Volume diagram - Determinants of cardiac output

This diagram shows the determinants of cardiac function; left ventricular preload which corresponds on this diagram to a volume at a particular pressure; afterload which has both cardiac components (the aortic valve) and also the systemic vascular resistance reflected in the mean arterial pressure; contractility; relaxility or lusitropy, and cardiac rate and rhythm.

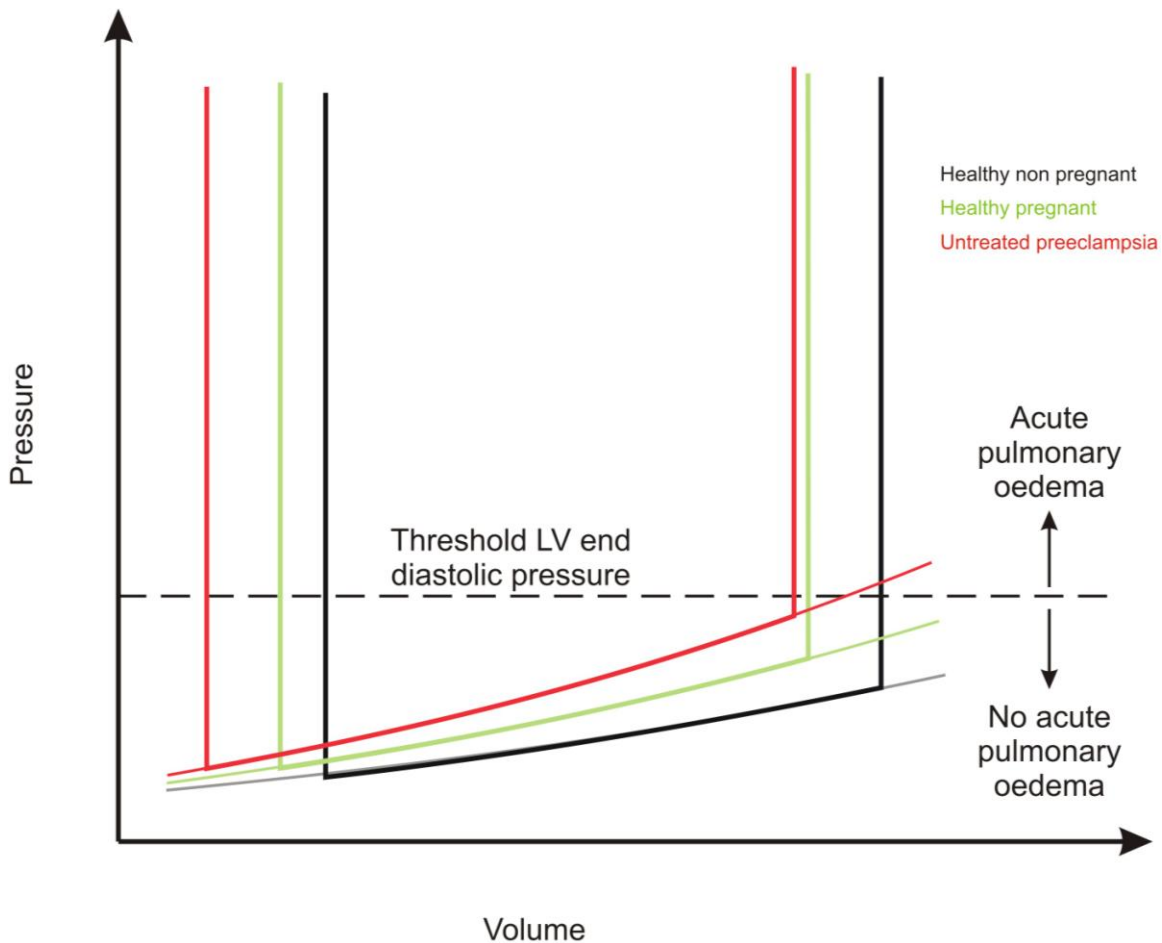


Figure 64 Pressure Volume diagram - Left ventricular end diastolic volume relationship to threshold left ventricular end diastolic pressure for acute pulmonary oedema

This diagram focuses on the filling of the heart and the diastolic curve. If left ventricular end diastolic volume and therefore its corresponding pressure, were to rise above a threshold value, filling of the left atrium from the lungs would be impaired. Back pressure and volume would accumulate leading to acute pulmonary oedema.

In the healthy non-pregnant woman, the threshold pressure and volume value is very high compared to their functioning state. Therefore it is very difficult for a healthy non-pregnant woman to experience an episode of acute pulmonary oedema even in the presence of large amounts of intravenous fluid administration. There is less reserve in healthy pregnant women due to changes in diastolic function (data from Table 24 page 127) represented in the diastolic curve. The functional reserve is further reduced in women with untreated preeclampsia due to the marked changes in diastolic function resulting in the alteration in the diastolic resting tension curve (data from Table 24 page 127).

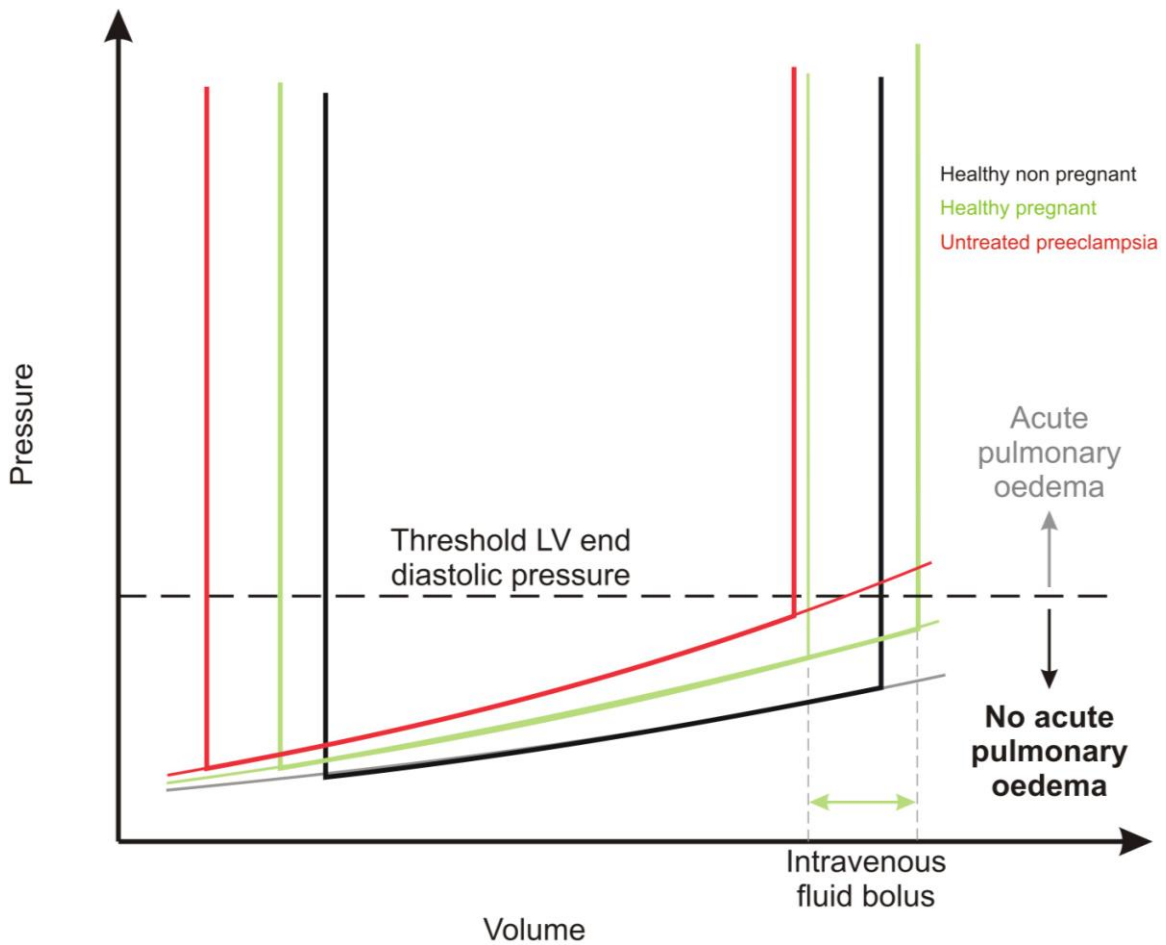


Figure 65 Pressure Volume diagram - Hypothetical intravenous fluid bolus in a healthy pregnant woman

A hypothetical fluid bolus is given to a healthy pregnant woman (green arrow). This shifts the left ventricular end diastolic volume to the right, increasing the left ventricular end diastolic pressure. The corresponding pressure however, is still below that which would cause acute pulmonary oedema.

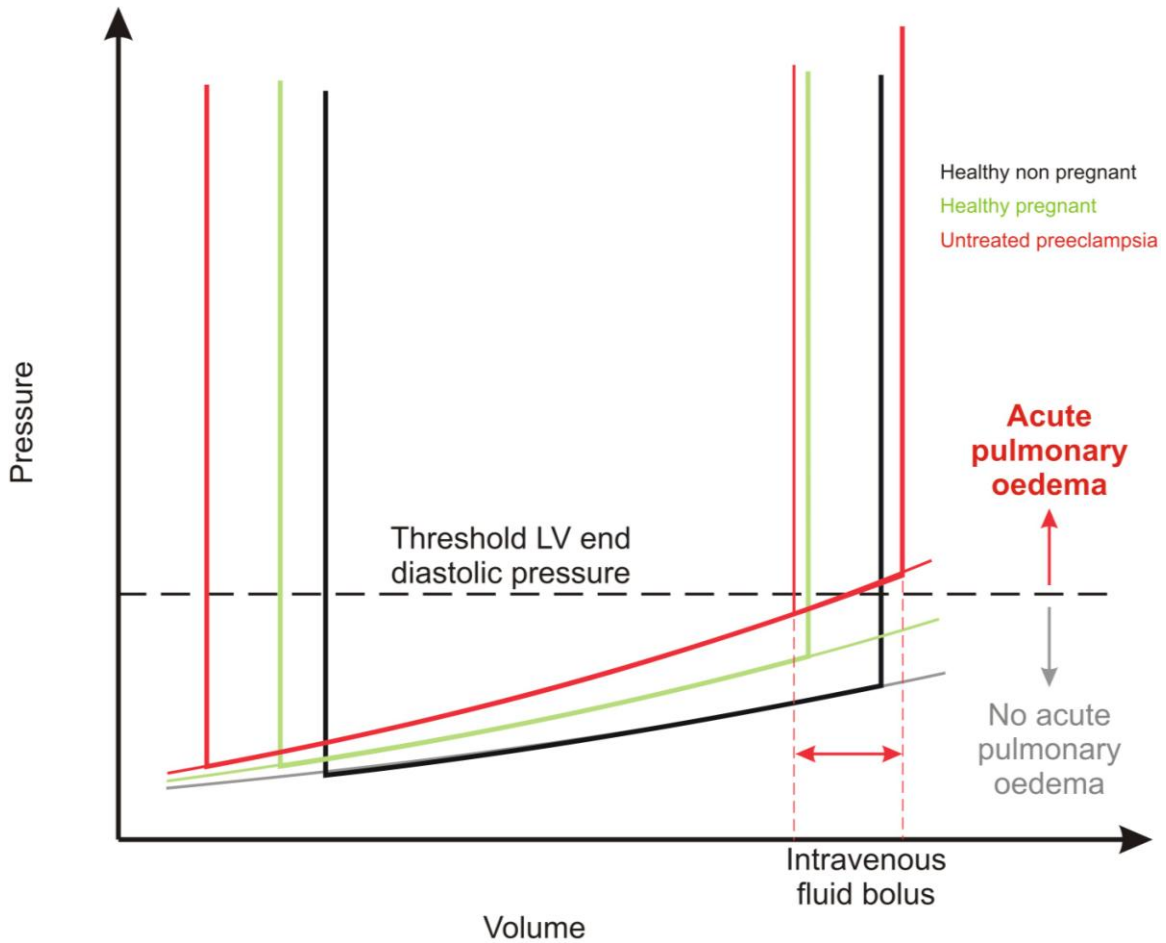


Figure 66 Pressure Volume diagram - Hypothetical equivalent fluid bolus in a woman with untreated preeclampsia

In this diagram, the same volume of intravenous fluid that was given to the healthy pregnant woman in Figure 65, is given to a woman with untreated preeclampsia. The equivalent fluid bolus in this situation increases the left ventricular end diastolic volume, and corresponding diastolic pressure, to a value that is above the threshold value for acute pulmonary oedema. This is because of the pronounced diastolic impairment that exists in the woman with untreated preeclampsia (data from Table 24 page 127) leading to increased left ventricular intraventricular pressure at end diastole. In addition women with untreated preeclampsia may have reduced colloid osmotic pressure due to significant protein loss in the urine. This may further reduce their capacity to effectively compensate for increases in left ventricular end diastolic volume that may occur with intravenous fluid.

5.5. Key findings

Compared with healthy pregnant women, women with untreated preeclampsia demonstrate

1. Increased systolic function
 - Increased cardiac output due to increased stroke volume
 - Increased inotropy

2. Reduced diastolic function
 - Altered mitral valve velocities and relaxation times
 - Altered interventricular wall velocities

3. Altered cardiac structure
 - Increased left ventricular wall dimensions
 - Increased left ventricular mass
 - Pericardial effusions

Chapter 6. Animal Study 1 Cardiac function in healthy pregnant and non-pregnant baboons (*Papio hamadryas*)

6.1. Introduction

The previous part of this thesis explored cardiac function in healthy pregnant women and then led on to the exploration of cardiac function in women with untreated preeclampsia. This part of the thesis explores the use of transthoracic echocardiography in pregnant and non-pregnant baboons, application as a serial monitor, and use as a device to measure changes in cardiovascular system variables during an intervention study in baboons.

Animal preparations of preeclampsia have been developed in the baboon however quantification of systolic and diastolic function by transthoracic echocardiography (TTE) has not previously been reported (Makris, Thornton *et al.* 2007). An understanding of these values prior to pregnancy and serially during induced preeclampsia is important in order to examine the effect of the disease on cardiac function and to examine changes that occur due to interventions.

The baboon surgical and pharmacological preparation of preeclampsia is well established and the need to serially monitor the cardiac function in these animals in a non-invasive low risk way would be very important as invasive monitoring devices have been associated with pregnancy loss in the baboon.

Non-invasive technology in this setting would enable minimal anaesthesia and restraint techniques, and facilitate more frequent assessment of the native disease state in these animals over time. The non-invasive nature of the measurement device also means that risks of injury are low to the animal which is very important in the setting of a non-human primate model. TTE represents a novel way of determining cardiac function in the baboon and has not been previously applied in this setting. Therefore the following two investigations were designed to apply this technology to examine the cardiac function in a group of healthy non-pregnant and healthy pregnant baboons (Animal Study 1) and

generate baseline cardiac systolic, diastolic and structural data for the baboon, and serially after an intervention (Animal Study 2), and thus potentially facilitating the translation of the results of animal work to the human situation if the same data set and non-invasive technology is used in both research settings.

The aim of this study was to

1. Determine
 - a. systolic variables of cardiac output (left ventricular tract diameter, left ventricular tract velocity time integral), fractional shortening, ejection fraction, fractional area change,
 - b. diastolic variables of mitral valve inflow, tissue Doppler indices, left atrial size, and
 - c. derived variables of mean arterial pressure, left ventricular mass, and systemic vascular resistance.
2. Compare the data obtained with historical data that determined cardiac output using thermodilution to provide a measure of device accuracy in this setting.
3. Determine the impact of this method of measurement on the animal by measuring behavioral characteristics.

6.2. Method

6.2.1. Study protocol

The study protocol was approved by the Sydney South West Area Health Service (SSWAH) Animal Welfare Committee (Appendix A) and was performed according to The Australian Code of practice for the care and use of animals for scientific purposes (NHMRC 2004).

6.2.2. Animal subjects

All the baboons used in the experiments were from the NHMRC National Baboon colony which was established in 1982. It contains a large number of baboons of varying ages and sexes. The colony has a very successful breeding program and is used for scientific purposes. The baboons in the colony have previously been involved in the development of the uteroplacental ischaemia (UPI) model for the generation of preeclampsia (Makris, Thornton *et al.* 2007). The animals used in this study were eight healthy non-pregnant lactating baboons and six healthy pregnant baboons. The studies were conducted at either the Royal Prince Alfred Hospital (RPAH) or at the NHMRC Australian National Baboon Colony in Wallacia NSW. The experimental method and timeline is outlined in Figure 67.

6.2.3. Animal handling and anaesthesia

There was minimal interference and handling of the animals. The experimental environment was quiet. The healthy non-pregnant baboons had echocardiography performed at the Royal Prince Alfred Animal Care facility having been transferred with their offspring earlier that morning from the colony (Figure 68 and Figure 69). The animals remained with their female companions up until the time of initial injection of ketamine when they were spatially but not visually separated from their companion in order to reduce anxiety. The pregnant animals were scanned in their home environment at the baboon colony (Figure 70, Figure 71 and Figure 72).

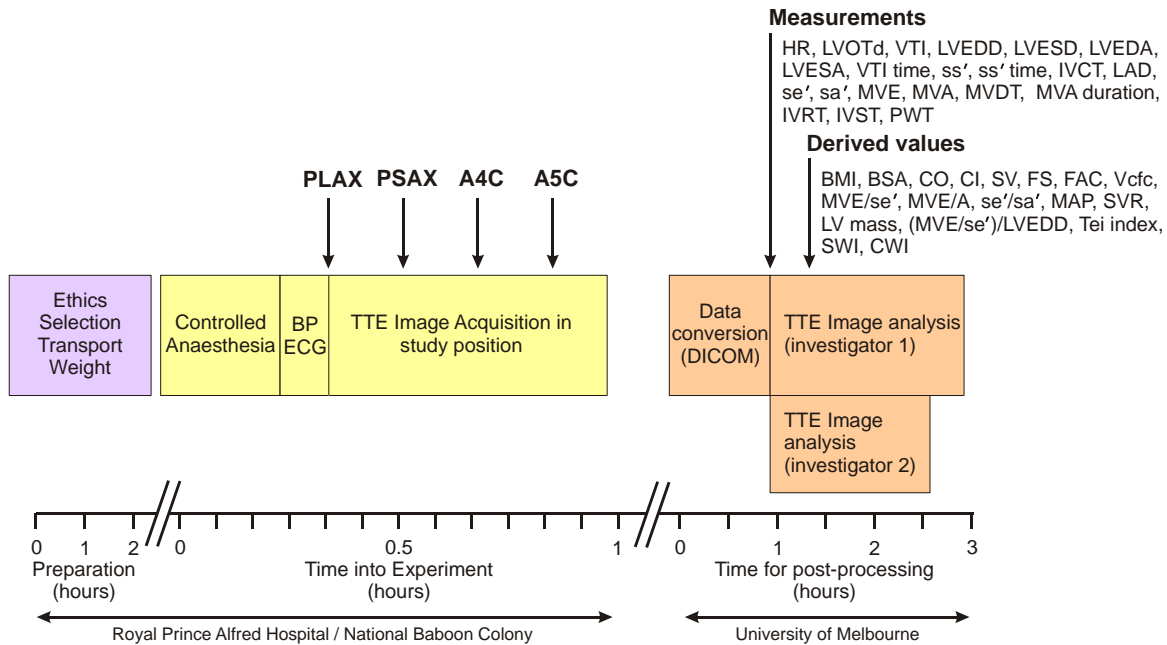


Figure 67 Animal Study 1 Experimental procedure and timeline

This figure is divided into three sections – the preparation time involving transport of the animals (purple section), the experimental procedure including the administration of standardized anaesthesia (yellow section) and the post-processing procedure where the data are converted and measured by two independent observers (orange section).

TTE = transthoracic echocardiography; BP = blood pressure; ECG = electrocardiograph; PLAX = parasternal long axis view; PSAX = parasternal short axis view; A4C = apical 4 chamber view; A5C = apical 5 chamber view; HR = heart rate; LVOTd = left ventricular outflow tract diameter; VTI = left ventricular outflow tract velocity time integral; LV = left ventricular; EDD= end diastolic diameter; ESD = end systolic diameter; EDA = end diastolic area; ESA = end systolic area; s = septal, IVCT = isovolumetric contraction time; LAD = left atrial diameter; MV = mitral valve; DT = deceleration time; IVRT = isovolumetric relaxation time; IVST = interventricular septum thickness; PWT = posterior wall thickness; BMI = body mass index; BSA = body surface area; CO = cardiac output; CI = cardiac index; SV = stroke volume; FS = fractional shortening; Vcfc = circumferential fibre length shortening; MAP = mean arterial pressure; SVR = systemic vascular resistance; SWI = stroke work index; CWI = cardiac work index

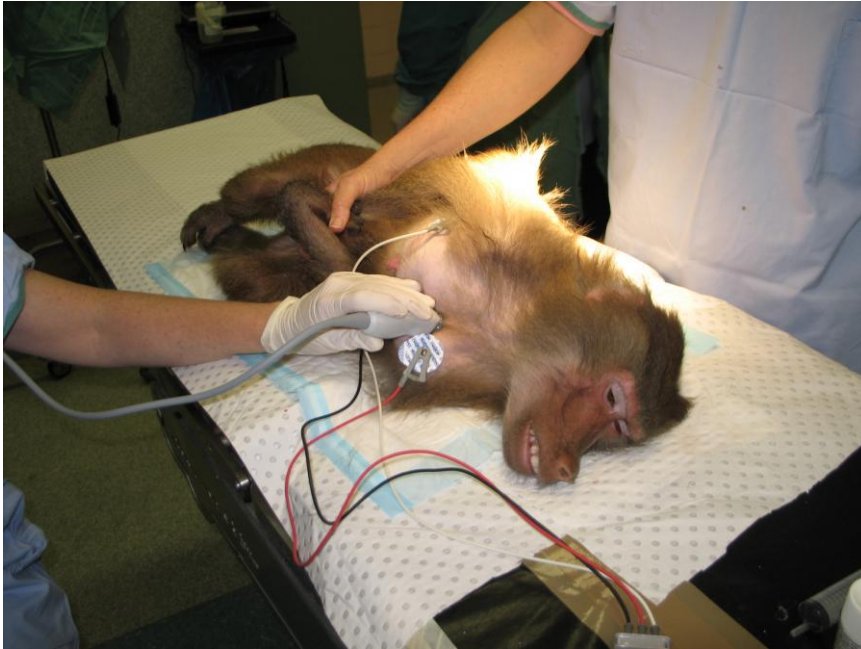


Figure 68 Animal Study 1 Experimental setup Royal Prince Alfred Hospital Sydney – Non-pregnant baboon Parasternal long axis view



Figure 69 Animal Study 1 Experimental setup Royal Prince Alfred Hospital Sydney – Non-pregnant baboon Apical four chamber view



Figure 70 Animal Study 1 Experimental setup National Baboon Colony – Healthy Pregnant baboon



Figure 71 Animal Study 1 Experimental setup National Baboon Colony – Equipment



Figure 72 Adult female baboon with infant

All animals were given a standardized anaesthetic consisting of a bolus intramuscular injection of 120 mg ketamine followed by a further 60 mg bolus 20 minutes later. No intravenous access was obtained and no atropine or additional medications were administered. The animals were not fasted and received a standard diet. After signs of sedation were seen such as laying on the ground of the cage and eye closure the animal was picked up, weighed on a standardized calibrated scale and placed on the scanning table. A mercury sphygmomanometer with a paediatric cuff was used to measure the blood pressure at the commencement of the procedure in the left upper limb of the animal according to the American Heart Association Guideline, blood pressure measurement in experimental animals (Kurtz, Griffin *et al.* 2005).

6.2.4. Haemodynamic method

The animals were placed in the left lateral position on an operating table or covered bench and attached to a three lead electrocardiograph (ECG) (Figure 68). A SonoSite MicroMaxx® transthoracic echocardiography machine with an 8 - 4 MHz phased array cardiac transducer with depth of view of 14 cm and a 10 mm footprint was used by a single operator (ATD) to obtain the images according to guidelines of the American Society of Echocardiography.

A parasternal long axis (PLAX), parasternal short axis (PSAX), apical 4- and 5- chamber (A4C, A5C) TTE examination including two-dimensional imaging and continuous, pulse wave, colour flow and tissue Doppler were performed according to guidelines of the American Society of Echocardiography (Gottdiener, Bednarz *et al.* 2004; Lang, Bierig *et al.* 2005; Nagueh, Appleton *et al.* 2009; Quinones, Otto *et al.* 2002).

In the left lateral position the left ventricular outflow tract (LVOT) image was obtained from the PLAX view. A zoomed two dimensional image in systole during quiet breathing was recorded. The LVOT velocity time integral (VTI) was recorded using the A5C view, with the Doppler integration angle $< 20^\circ$ to flow. Pulse wave Doppler was used with a 3 mm sample volume placed within the LVOT approximately 0.5 cm or less, proximal to the aortic valve. At least three consecutive beats were recorded.

Images were converted to Digital images and communications in medicine (DICOM) format and analysed off-line using ProSolv® software.

LVOT measurement

From the PLAX image, the zoomed LVOT image was frozen during systole. End systole was defined as the frame preceding early diastolic mitral valve opening. End diastole was defined as the onset of the Q wave of the QRS complex. LVOT was measured perpendicular to the aortic root with the anterior caliper positioned at the junction of the anterior coronary cusp and the interventricular septum and the posterior caliper was

positioned at the junction of the posterior non-coronary aortic cusp and the anterior mitral valve leaflet (Figure 73). Three consecutive measurements were averaged.

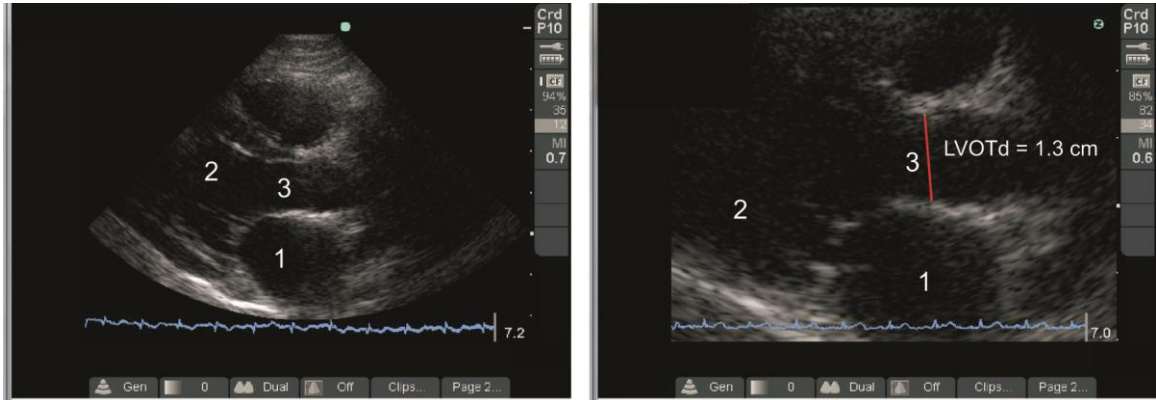


Figure 73 Parasternal long axis image in a healthy pregnant baboon – Transthoracic echocardiography images

The view on the left is obtained by freezing the two dimensional video image. The image on the right is the frozen zoomed image of the left ventricular outflow tract during systole with the red line showing the left ventricular outflow tract measurement. The left ventricular outflow tract diameter is 1.3 cm. 1 = left atrium, 2 = left ventricle, 3 = left ventricular outflow tract. Note the similarity to Figure 8 (page 67).

VTI and heart rate (HR) measurement

The VTI was determined by tracing the leading edge of the velocity spectrum of three consecutive beats. The VTI was the average of these three consecutive beats. Heart rate was measured from the Doppler spectral display from the R-R interval of the ECG (Figure 74).

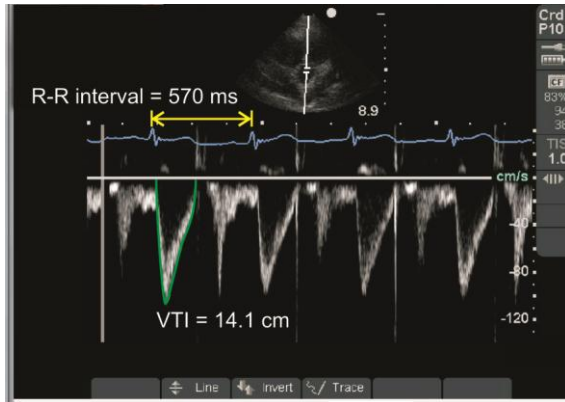


Figure 74 Apical 5 chamber image in a healthy pregnant baboon – Velocity time integral and R-R interval

The image shows the measurement of the velocity time integral (green line, 14.1 cm) and the R-R interval (yellow line, 570 ms). The cardiac output is 1979.0 ml/min (Figure 73 and Figure 74). Note the similarity between this figure and Figure 10 (page 69).

Calculation of cardiac output

Cardiac output (CO) was calculated using the formula:

$$\text{CO (ml/min)} = \text{HR (beats/min)} \times [(\text{LVOT diameter}^2) \times 0.785] (\text{cm}^2) \times \text{VTI (cm)}.$$

[Where $(\text{LVOT diameter}^2) \times 0.785 = \pi \times \text{LVOT radius}^2 =$ cross sectional area of the LVOT.]

Mitral valve Doppler inflow velocities

From the Apical 4 chamber view (Figure 75) pulse wave Doppler with a sample volume of 2 mm was positioned centrally at the tips of the open mitral valve in the left ventricle. The first peak velocity during diastole was recorded as the E wave. The second peak velocity during diastole was recorded as the A wave (Figure 76). The mitral valve deceleration time is shown in Figure 76.

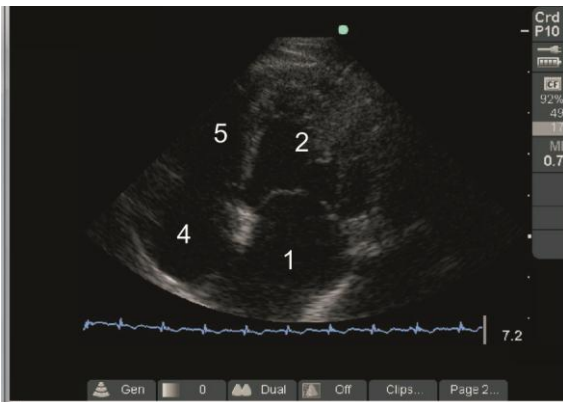


Figure 75 Apical 4 chamber image in a healthy pregnant baboon – Transthoracic echocardiography image

This still image during diastole is taken from the two dimensional video image in the apical 4 chamber view. 1 = left atrium, 2 = left ventricle, 4 = right atrium, 5 = right ventricle. Note the similarity between this image and Figure 15 (page 74).

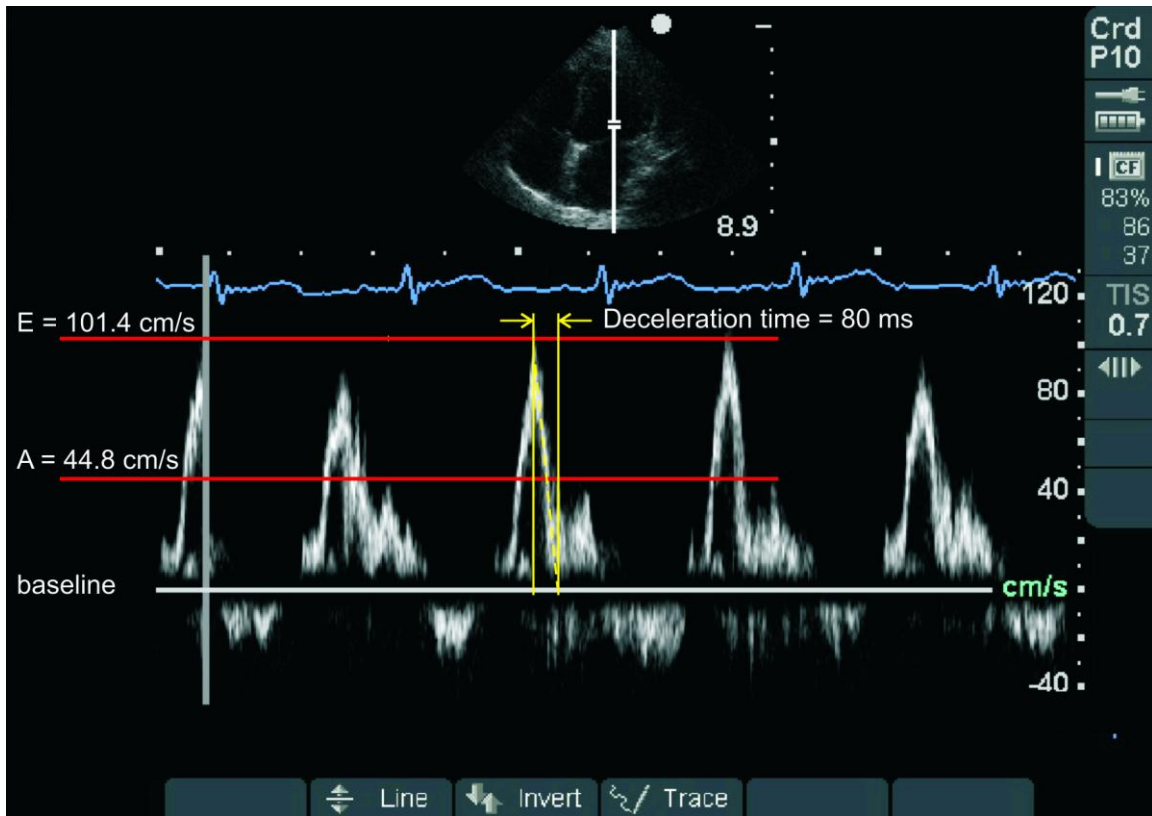


Figure 76 Apical 4 chamber view in a healthy pregnant baboon – Mitral valve inflow Doppler waveform

This image shows the mitral valve E wave velocity (101.4 cm/s) and the mitral valve A wave velocity (44.8 cm/s). The mitral valve deceleration time is 80 ms (yellow line). Note the similarity between this image and Figure 16 (page 77).

Tissue Doppler velocities

From the Apical 4 chamber view, tissue Doppler was used with a sample volume of 5 mm placed at the septal junction of the left ventricular wall with the fibrous mitral annulus. The peak systolic velocity was recorded as the septal s' velocity (Figure 77), the first peak velocity during diastole was recorded as the septal e' velocity and the second peak velocity during diastole were recorded as the septal a' velocity.

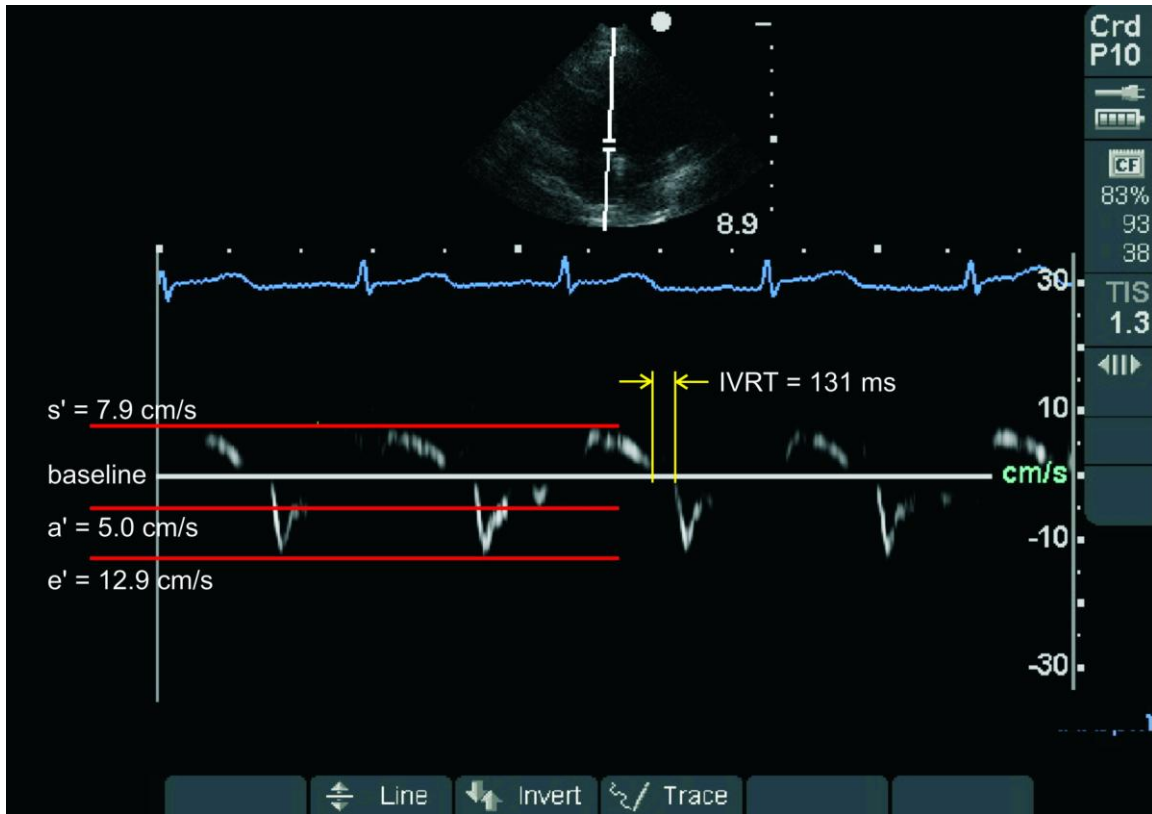


Figure 77 Apical 4 chamber view in a healthy pregnant baboon – Septal tissue Doppler waveform

The septal s' wave velocity is 7.9 cm/s. Figure 77 in the baboon is very similar to Figure 15 (page 74) in the human. The septal e' wave velocity is 12.9 cm/s. The septal a' wave velocity is 5.0 cm/s. The isovolumetric relaxation time (IVRT) is 131 ms.

A second observer (JC) who was unaware of the haemodynamic or pregnancy status of animals measured the LVOT diameter and the LVOT VTI in random order. An average of three consecutive beats was used for all measurements.

Comparison between the averages of JC and ATD measurements for LVOT diameters and LVOT VTI were used for interobserver reliability assessment. Comparisons between three consecutive or repeated measurements from observer 1 (ATD) were used to assess intraobserver reliability.

6.2.5. Statistical methods

Statistical analysis used the General Linear Model and unpaired t-test comparisons. Bland-Altman methodology was used to assess inter- and intraobserver differences.

6.3. Results

The characteristics of the baboons are shown in Table 33. The cardiac function data is shown in Table 34. Images were able to be obtained in all animals and haemodynamic data was able to be obtained in the two acoustic windows. Animals were assessed with respect to their recovery from anaesthesia. No animal vomited and there was resumption of routine daily activities after anaesthesia (Table 35).

Table 33 Animal Study 1 Characteristics of the baboons

Characteristic	Non-Pregnant baboons n = 8	Pregnant baboons n = 6	<i>P</i> value
Age (years)	8.4 ± 2.6	7.2 ± 1.4	0.352
Weight (kg)	11.4 ± 1.7	13.2 ± 1.1	0.039
Gestation (days)	N/A	132 ± 37	N/A

Mean ± standard deviation (SD).

Table 34 Animal Study 1 Haemodynamic, systolic, diastolic and structural measurements in healthy non-pregnant and pregnant baboons

Variable	Healthy non-pregnant baboons n = 8	Healthy pregnant baboons n = 6	P value
MAP (mmHg)	89 ± 3.4	85 ± 5.1	0.104
Cardiac output (ml/min)	1317 ± 134	1615 ± 121	0.001
Heart rate (BPM)	105 ± 6	120 ± 11	0.018
Stroke volume (ml)	13 ± 2	14 ± 2	0.316
SVR (dyne.cm/sec ⁵)	5476 ± 579	4201 ± 211	< 0.001
Septal s' (cm/s)	8.1 ± 1.2	7.9 ± 0.7	0.745
Mitral valve E/A	1.8 ± 0.3	1.9 ± 0.6	0.577
Septal e' (cm/s)	12.1 ± 1.9	11.2 ± 1.5	0.346
Septal a' (cm/s)	6.7 ± 1.1	6.5 ± 0.9	0.689
Mitral valve E/septal e'	7.5 ± 1.4	8.0 ± 1.3	0.101
Pericardial effusion	0	3 (50)	0.182

MAP = mean arterial pressure; number (%); BPM = beats per minute; SVR = systemic vascular resistance
Data are mean ± standard deviation (SD); number (%) as appropriate.

Table 35 Animal Study 1 Behavioral characteristics of the baboons after sedation and transthoracic echocardiography

Variable	Non-pregnant baboons n = 8	Pregnant baboons n = 6
Vomiting	0	0
Eating/drinking at next scheduled feeding time	8 (100)	8 (100)
Breastfeeding	8 (100)	0

Data are expressed as number of baboons and (%)

Interobserver and intraobserver reliability

The interobserver reliability (mean difference \pm SD) for the left ventricular outflow tract diameter was 0.05 ± 0.07 cm. The interobserver reliability (mean difference \pm SD) for the left ventricular outflow tract velocity time integral was 0.47 ± 0.97 cm/s. The intraobserver reliability (mean difference \pm SD) for the left ventricular outflow tract diameter was 0.01 ± 0.03 cm. The intraobserver reliability (mean difference \pm SD) for the left ventricular outflow tract velocity time integral was 0.01 ± 0.11 cm/s. These results indicate close agreement between the two observers' measurements and the single observer's repeated measurements.

6.3.1. Comparison measurements with historical controls

Data from historical control baboons was available from previous work done with animals from the colony. A comparison of cardiac output derived by current non-invasive techniques was undertaken to examine the determinants of cardiac output using the two different techniques (Table 36).

Table 36 Animal Study 1 Individual values of cardiac output measured by thermodilution in historical controls versus transthoracic echocardiography in current baboons

Variable	Cardiac output (ml/min) (TTE)	Stroke volume (ml) (TTE)	Heart rate (BPM) (TTE)	Variable	Cardiac output (ml/min) (TD)	Stroke volume (ml) (TD)	Heart rate (BPM) (TD)
Animal 1	1170	10.7	109	Animal 1H	1730	14.4	120
Animal 2	1363	13.9	98	Animal 2H	1630	12.9	126
Animal 3	1147	11.1	103	Animal 3H	1550	11.1	140
Animal 4	1198	10.6	113	Animal 4H	1910	14.7	130
Animal 5	1585	14.8	107	Animal 5H	1760	13.5	130
Animal 6	1539	14.2	108	Animal 6H	1460	13.5	108
Animal 7	1301	12.2	107	Animal 7H	1630	14.1	116
Animal 8	1439	13.2	109	Animal 8H	1730	14.4	120

TTE = transthoracic echocardiography; TD = thermodilution; H=historical controls

(Phippard, Horvath *et al.* 1986).

The cardiac output comparison with historical controls showed that stroke volume is very similar between the two different groups and two different techniques. The differences in cardiac output between the two groups were due to differences in heart rate between the groups.

6.4. Discussion

Animal Study 1 demonstrated the utility of transthoracic echocardiography in the setting of animal research with the baboon and is an accurate and reliable form of non-invasive monitoring. The animals were able to be examined and have their systolic and diastolic variables measured with sedative, short duration anaesthesia with minimal impact to their daily activities. There were no complications and minimal interruption to their daily schedule. This technology enabled evaluation of their haemodynamics with little stimulation and would thus reflect better than invasive devices, their baseline haemodynamics.

Animal Study 1 represents the first time that diastolic variables have been measured in the baboon and forms the baseline from which measurements can be compared when disease states such as the uteroplacental ischaemia model of preeclampsia are induced. The examination technique in these animals was identical to that employed in pregnant humans and the waveform patterns and velocities obtained were very similar to humans (Table 37 and Figure 78). The use of TTE in these animals and its ability to non-invasively monitor changes in systolic and diastolic measurements means that results from future studies can be more easily translated into the human clinical situation as this technology has an emerging application in obstetric anaesthesia and critical care. The similarity in technique and data obtained means that translation to the human disease is possible.

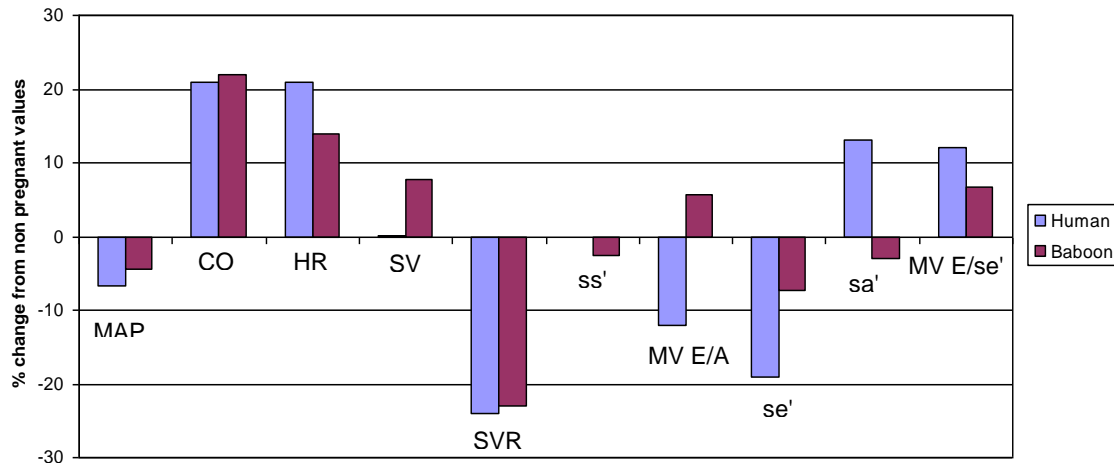
Historical control animals were ex-circus animals that were more intrinsically stressed which may explain their higher heart rates under anaesthesia. In addition the invasive

nature of the measurement procedure may have caused an elevation in their heart rate. The differences in cardiac output were due to differences in heart rate not stroke volume.

Table 37 Comparison between human and baboon variables

Variable	Healthy non-pregnant women n = 40 mean ± SD	Healthy pregnant women n = 40 mean ± SD	Difference between NP and HP women (% change)	Healthy non-pregnant baboons n = 8 mean ± SD	Healthy pregnant baboons n = 6 mean ± SD	Difference between NP and HP baboons (% change)
MAP (mmHg)	86.5 ± 8.6	80.8 ± 8.3	- 5.7 (6.6%)	89 ± 3.4	85 ± 5.1	- 4 (4.5%)
Cardiac output (ml/min)	3400 ± 752	4109 ± 595	+ 709 (21%)	1317 ± 134	1615 ± 121	+ 298 (22%)
Heart rate (BPM)	64.2 ± 18.4	77.8 ± 9.6	+ 13.6 (21%)	105 ± 6	120 ± 11	+ 15 (14%)
Stroke volume (ml)	53.1 ± 9.5	53.2 ± 7.9	+ 0.1 (0.2%)	13 ± 2	14 ± 2	+ 1 (7.7%)
SVR (dyne.cm/sec ⁵)	2116 ± 457	1612 ± 315	- 504 (24%)	5476 ± 579	4201 ± 211	- 1275 (23%)
Septal s' velocity (cm/s)	8.9 ± 0.7	8.9 ± 1.2	0 (0%)	8.1 ± 1.2	7.9 ± 0.7	- 0.2 (2.5%)
Mitral valve E/A	1.7 ± 0.4	1.5 ± 0.2	- 0.2 (12%)	1.8 ± 0.3	1.9 ± 0.6	+ 0.1 (5.6%)
Septal e' velocity (cm/s)	14.2 ± 1.5	11.5 ± 2.3	- 2.7 (19%)	12.1 ± 1.9	11.2 ± 1.5	- 0.9 (7.4%)
Septal a' velocity (cm/s)	6.4 ± 1.4	7.2 ± 1.2	+ 0.8 (13%)	6.7 ± 1.1	6.5 ± 0.9	- 0.2 (3.0%)
Mitral valve E/septal e'	6.0 ± 1.1	6.7 ± 1.3	+ 0.7 (12%)	7.5 ± 1.4	8.0 ± 1.3	+ 0.5 (6.7%)

MAP = mean arterial pressure; BPM = beats per minute; SVR = systemic vascular resistance;



MAP = mean arterial pressure; CO = cardiac output; HR = heart rate; SV = stroke volume; SVR = systemic vascular resistance; ss' = septal s' velocity; MV E/A = mitral valve E/A ratio; se' = septal e' velocity; sa' = septal a' velocity; MV E/se' = mitral valve E wave velocity/septal e' wave velocity

Figure 78 Changes from non-pregnant values – Human and baboon

This figure shows the relative changes from the non-pregnant state for both pregnant humans and pregnant baboons. The direction of the changes is similar for both humans and baboons except for mitral valve E/A ratios and septal a' velocities. Importantly changes in heart rate, stroke volume, systemic vascular resistance, blood pressure and cardiac output were all in the same direction. The changes in septal e' velocity and mitral valve E/septal e' ratio were also altered in a similar way in the human and the baboon. The differences in the directions of change for the mitral E/A ratio and septal a' velocity for the human and baboon may partly be explained by ergonomic mechanisms. Pregnant baboons appear to move in a way that seems unaffected by the pregnancy. There is less impact of the gravid uterus on the thorax in the baboon compared with the humans. Humans frequently display characteristic stances, positions and walking postures related to being near term pregnancy. The implications of these differences may be that diastolic cardiac function evidenced by the mitral valve E/A ratio and the septal a' velocity, may be differently affected by pregnancy in the human and the baboon.

6.5. Key findings

1. Cardiac function can be easily measured using transthoracic echocardiography in the baboon.
2. The experimental setup is very similar to the human using similar equipment and positioning.
3. Prepregnancy systolic, diastolic and structural variables have been quantified providing baseline values for subsequent use in pregnancy and induced preeclampsia studies in these animals.

Chapter 7. Animal Study 2 Cardiovascular and renal effects of relaxin in baboons (Papio hamadryas)

7.1. Introduction

Relaxin is a circulating hormone produced predominantly by the ovary. It belongs to the insulin-like growth factor family. Relaxin has a relative molecular mass of approximately 5000-6000 and it acts via the relaxin receptors. These receptors are a family (relaxin family peptide (RXFP)) of seven transmembrane spanning receptors consisting of four main types – RXFP 1/2 and RXFP 3/4, all acting via G protein activation (Alexander, Mathie *et al.* 2008). The actions of relaxin are to facilitate remodeling of maternal joints and cervix and alteration of breast tissue. It also has an effect on pituitary hormone secretion and causes renal vasodilatation and effects smooth muscle in the heart and gastrointestinal tract (Sherwood 2004). Relaxin may be important in the pathophysiology of preeclampsia based on observations that women who have undergone assisted reproductive technologies (ART) to become pregnant have an increased rate of preeclampsia. Twenty five percent of women who receive donor eggs develop preeclampsia and other forms of ART have rates as high as 20%. It has been hypothesized that women undergoing ART have reduced or absent functioning ovaries and thus have reduced relaxin levels (Danielson, Sherwood *et al.* 1999).

If a reduction in relaxin levels, and loss of vasodilation in the kidney, which may also reflect a loss of vasodilation in the uterine circulation in pregnancy, is one of the reasons why women undergoing ART are susceptible to preeclampsia, then replacement of the hormone may decrease the rate of this complication and improve pregnancy outcomes.

As previously discussed, the baboon and human share many similarities. In particular regarding this experiment, the increased renal blood flow that occurs during pregnancy in humans also occurs in the baboon and therefore they are suitable to study in the model which measures renal blood flow changes to relaxin administration as well as cardiac changes.

Aims of this study were to perform a pilot study in two non-pregnant baboons to

1. serially investigate the systolic and diastolic effects of a relaxin infusion,
2. investigate the renal effects of a relaxin infusion over the same time course and
3. assess the applicability and accuracy of TTE in measurement serial changes during an intervention study.

7.2. Methods

The study protocol was approved by the Sydney South West Area Health Service (SSWAH) Animal Welfare Committee (Appendix A) and was performed according to The Australian Code of practice for the care and use of animals for scientific purposes (NHMRC 2004).

Transthoracic Doppler echocardiography was used to determine cardiac output, mitral valve Doppler inflow velocities and septal systolic and diastolic velocities in the two baboons at five discrete time points under standardized anaesthesia during the relaxin infusion experiment (Day 0 – pre-relaxin, Day 2 of infusion, Day 4 of infusion, Day 6 of infusion, Day 10 (equivalent to Day 4 post infusion cessation). The relaxin infusion was administered via osmotic pumps placed subcutaneously between the scapulae on the animals' back. The relaxin was infused over a 6 day period (Day 0 to Day 6).

7.2.1. Anaesthesia

The two animals were fasted prior to anaesthesia and all readings were obtained between 0700 – 1000 hours. The animals were given a loading dose of approximately 9 mg/kg intramuscular ketamine. Intravenous access was obtained, blood tests taken and a ketamine infusion was commenced 0.2 mg/kg/min. 600 ug atropine, 2.5 mg metoclopramide and 0.1 mg clonazepam were given. Blood pressure was recorded with a manual mercury sphygmomanometer with a paediatric cuff on the animal' upper left limb.

7.2.2. Haemodynamic method

The haemodynamic methodology was identical to that outlined in Chapter 6.2.4.

Day 2 Measurements – on Day 2 the planned venous blood sample and the planned TTE examination occurred under different anaesthetic conditions. The animals were only given a bolus and single follow-up dose of ketamine without atropine, clonazepam or metoclopramide as procedures on this day were less invasive.

7.3. Results

The animals remained still during all the procedures. No complications occurred and the animals tolerated the placement of the pump, the presence of the pump and removal of the pump. Baseline baboon characteristics are presented in Table 38.

Regarding TTE, good image quality and qualitative data were able to be obtained in the PLAX, PSAX and apical 4 and 5 chamber views. Urine was collected on Days 0, 4 and 10 via specially designed trays at the bottom of the animals' cages (Table 39).

Mitral valve inflow beat fusion occurred at all the measured heart rates on Day 0, Day 4, Day 6 and Day 10, however this did not occur at a lower heart rate on Day 2 when a different anaesthetic technique was employed. Thus for Day 0, Day 4, Day 6, and Day 10 the A wave velocity, DT or A wave duration were not able to be recorded. The influence of different experimental methodology and anaesthetic agents on cardiac function is shown in Figure 79 and Figure 80. Day 2 animals only received ketamine anaesthesia because the procedures they needed to undergo were simple blood tests and TTE.

Changes were observed in cardiac output and creatinine clearance however they did not reach statistical significance in this two animal pilot study. As these are pilot data interobserver reliability was not measured with a second observer.

Table 38 Animal Study 2 Baseline baboon characteristics

Variable	Baboon 1 - Tabitha	Baboon 2 - Scila
Age (years)	4.6	4.2
Weight (kg)	9.9	9.0

Table 39 Animal Study 2 Left ventricular systolic and diastolic values under standardized anaesthesia

Variable	Day 0	Day 4	Day 6	Day 10
	Pre Relaxin			
Cardiac output (ml/min)	1222	1402	1385	1214
Heart rate (BPM)	144	163	163	148
Stroke volume (ml)	9	9	9	8
Septal s' velocity (cm/sec)	8.7	9.7	10	10.7
Septal e' velocity (cm/sec)	11.4	11.6	10.5	13.8
Mitral valve E (cm/sec)	82.5	93	97.5	115
Mitral valve E/septal e'	7.2	8.0	9.3	8.3
Creatinine Clearance (ml/min)	26.1	34.3		29.6

BPM = beats per minute; mean values (n=2)

Serial CO & HR changes

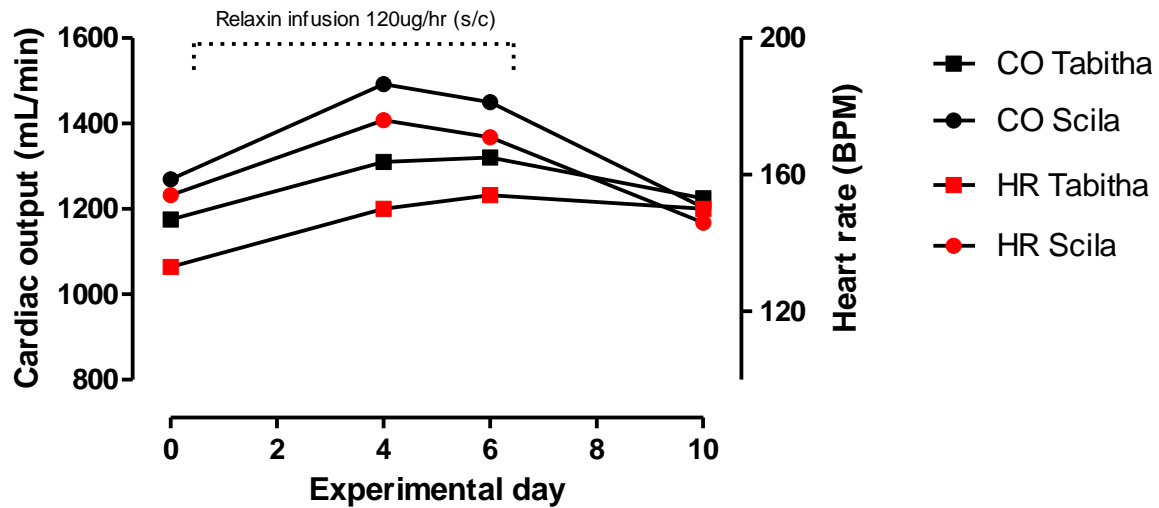


Figure 79 Animal Study 2 Individual values for cardiac output and heart rate excluding Day 2 values

This figure shows the changes in cardiac output over the 10 days of the experiment. Day 0, 4, 6 and 10 involved the administration of the same anaesthetic agents. These included atropine, an anticholinergic agent which increases heart rate. CO = cardiac output; HR = heart rate; s/c = subcutaneous; BPM = beats per minute.

Serial CO & HR changes

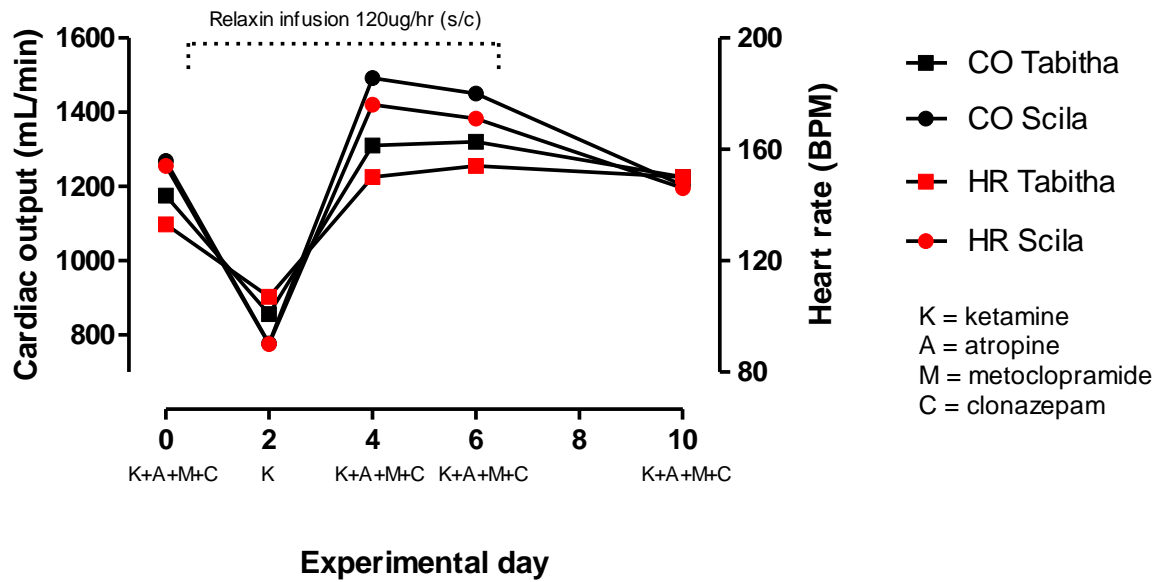


Figure 80 Animal Study 2 Individual values for cardiac output including Day 2 values

This figure includes the Day 2 values which were obtained under different anaesthetic conditions. It demonstrates the influence of the anaesthetic technique on experimental outcome. The administration of atropine on Day 0, 4, 6 and 10 greatly influenced the cardiac output. This can lead to erroneous conclusions about the effects of the experimental agents if this is not taken into account. CO = cardiac output; HR = heart rate; s/c = subcutaneous; BPM = beats per minute.

7.4. Discussion

Animal Study 2 showed that relaxin can be administered by a continuous infusion in the baboon and is well tolerated.

The non-invasive technique of transthoracic echocardiography is applicable for the serial assessment of systolic and diastolic function in this setting in baboons. The influence of anaesthetic agents on cardiac output can be seen and highlights the importance of using minimal anaesthesia wherever possible with minimal disruption to the animals' daily routine, or alternatively a rigid anaesthetic protocol to limit these influences. This can enable serial cardiac function measurements under stable haemodynamic conditions to be made.

Key findings

1. Relaxin appears safe and easy to administer in this limited non-pregnant baboon study
2. Cardiac and renal function changes that occurred did not reach statistical or clinical significance in this pilot study
3. Cardiac function can be easily measured serially after interventions using transthoracic echocardiography in the baboon

Chapter 8. Summary of studies

8.1. Key implications

1. Human Study 1

- a. Healthy term pregnant women have reduced cardiac output due to reduced stroke volume in the head-down position. Care needs to be taken when positioning term pregnant women. Assessment of cardiac function using TTE should occur in most pregnant women in order to assess their cardiac function.
- b. There is a very large range of cardiac output despite a very similar cohort of women.

2. Human Study 2

- a. Cardiac output and inotropy are increased in women with untreated preeclampsia.
- b. Diastolic function is reduced.
- c. Cardiac function can be assessed in women with preeclampsia using transthoracic echocardiography and may help to guide therapies especially fluid therapy in women with this disease.
- d. The septal tissue Doppler waveform is an important source of systolic and diastolic information in women with preeclampsia.
- e. Acute pulmonary oedema occurring in women with preeclampsia is due to diastolic heart failure also known as hypertensive cardiomyopathy.
- f. The presence of an inovasconstrictor needs to be searched for to explain the findings.

3. Animal study 1

- a. Transthoracic echocardiography is an applicable technology to measure cardiac function in pregnant and non-pregnant baboons under sedative anaesthesia.
- b. Cardiac changes during the baboon pregnancy are similar to changes in the human pregnancy.

4. Animal study 2

- a. Transthoracic echocardiography is applicable as a serial monitor of cardiac function in intervention studies in the baboon.
- b. Relaxin has no significant side effects when administered subcutaneously to a non-pregnant baboon allowing consideration of its use more broadly in humans.

8.2. Limitations

This work focused on left ventricular systolic and diastolic function and left ventricular structure and as such did not specifically examine right heart function. The measurement of inferior vena caval size is difficult in pregnancy due to the gravid uterus and the inability of the women to lie supine due to aortocaval compression. Therefore inferences about the overall intravascular volume status of women with untreated preeclampsia, especially in the presence of the significant diastolic changes demonstrated, cannot be reliably made (Jansen, Maas *et al.* 2010; Magder 2006; Monnet and Teboul 2006; Parkin and Leaning 2008).

Women with both mild and severe disease were recruited in the preeclampsia cohort in Study 2. The study methodology was based on 40 women with any form of preeclampsia, compared to healthy pregnant women. Therefore, whilst statistical and clinical differences were found between these groups, future studies should propose an hypothesis to test and then design a methodology specifically to examine this question in groups of women with mild disease compared with women with severe disease. The inclusion of women with twin pregnancies was a methodological limitation. Whilst it did not affect the overall results as the data were examined with and without the inclusion of these women, in designing future studies women with multiple gestations should not be included as their addition adds heterogeneity to the study group.

Apart from the baboon intervention study, the other three studies reported in this thesis were single time point studies and did not involve serial transthoracic echocardiography examinations. Therefore inferences cannot be made regarding the time course of development of the changes demonstrated or the resolution of such changes.

Chapter 9. Recommendations for future work

Future work involves

1. Applying the developed transthoracic echocardiographic methodology to large numbers of women before, during and after pregnancy and the serial follow up of women, after their pregnancy, who develop severe preeclampsia to observe the time course of changes over many years.
2. Observing cardiac function in women with preeclampsia after treatment interventions.
3. Observing cardiac function in women with gestational hypertension compared to pregnant women with hypertension and an additional organ system involvement (preeclampsia).
4. Assessment of intravascular volume and examination of right heart function.
5. Recording transthoracic echocardiographic changes in response to fluid boluses to examine fluid responsiveness and measure objective outcomes of morbidity.
6. Examination of the cause of the left ventricular mass increase through the use of cardiac magnetic resonance imaging (MRI) in the human and myocardial biopsy in the baboon preparation of preeclampsia.
7. Using the Pressure Volume loop construct to model changes after treatment interventions in women with preeclampsia.
8. Searching for a circulating ino vasoconstrictor as a mediator in the disease.
9. Applying the pathological response to injury model to preeclampsia to assist with investigations into aetiology.

10. Exploring of the relationship between the presence of diastolic heart failure in elderly women and its relationship to preeclampsia during their pregnancies.

11. Developing a registry of women who have experienced severe preeclampsia starting first with those that developed hypertensive cardiac failure (acute pulmonary oedema) and eclampsia to monitor their cardiovascular and neurological system over time.

Chapter 10. Conclusions

This project has developed a reproducible methodology of transthoracic echocardiographic examination in healthy pregnant women and in women with preeclampsia. The use of clinician performed transthoracic echocardiography in pregnant women and its growing acceptance by the obstetric anaesthetic community has been achieved through work performed during this thesis. Transthoracic echocardiography is now seen as an emerging application in this area.

The work has developed a new method for serial monitoring of cardiac function in healthy pregnant and non-pregnant baboons and serially in baboons after an intervention. It has recorded systolic, diastolic and structural variables and the reference ranges for cardiac function in healthy pregnant and non-pregnant baboons.

The reference range for cardiac output in healthy pregnant women at term has been defined, thereby improving the understanding of cardiac output in healthy women at term.

This work has defined the native disease state in women with untreated preeclampsia and improved the understanding of cardiac function in women with untreated preeclampsia. Women with untreated preeclampsia have increased systolic function with increased cardiac output and increased inotropy, and reduced diastolic function in the presence of systemic vascular vasoconstriction.

Appendix A Study listing

Human Research Ethics Approval

Human Study 1 – Mercy Hospital for Women

1. R07_32 Patient Information and Consent Form (PICF) Versions 1 – 8. Current PICF 10_3_2010.
2. R07_32 Protocol Version 1-3. Current protocol 09_06_2010.

Human Study 2 – Mercy Hospital for Women

1. R09_02 Patient information and Consent Form (PICF) Versions 1-4. Current PICF 08_06_2010.
2. R09_02 Protocol Version 2. Current protocol 21_7_2010.

Animal Research Ethics approval

Animal Study 1 and 2 – Sydney South West Area Health Service (SSWAHS) Animal Welfare Committee – 2007_010C, 2009_016A.

Appendix B Recruitment posters

Recruitment posters as part of the hospital wide awareness for Human Study 2.



Recruitment poster July 2009

Are you seeing women with untreated Preeclampsia or Gestational Hypertension?

We are currently recruiting women for a PhD research project.

Dr Alicia Dennis is performing:

- non invasive transthoracic echocardiography on women affected with preeclampsia or gestational hypertension
- and**
- who have not commenced antihypertensives or anticonvulsants.

Please call **Caroline Carr** – on phone or page 4088 (Monday to Friday 0800-1630hrs) or **Dr Alicia Dennis** on 0407 685 054 (out of hours) to discuss this important study with women and gain consent.



Are you a healthy woman who would like to take part in a research study?

We are currently looking for volunteers to participate in a PhD research study conducted by Dr Alicia Dennis. A healthy control group of 20 participants is needed to undergo Transthoracic Echocardiography (TTE).

This scan will take approximately 15 minutes and can be arranged at a time which suits you. It is a portable scan which will be performed by Alicia.

Inclusion criteria: able to give consent, age 18-39, body mass index (BMI) less than 33, American Society Anesthesiologists (ASA) classification 1 (healthy).

Exclusion criteria: pregnant (any gestation), diseases associated with peripheral vascular insufficiency, smoker, antihypertensive medication, thyroxine, salbutamol.

If you are interested in volunteering to be part of this study, or would like further information regarding the study, please contact Caroline Carr – Phone 8458 4088, or pager 4088.



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