Imperial College London

Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation and Fetal Outcomes

The HDiP – MCAFO Study

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Declaration of originality

I declare that this thesis is my original work. I have appropriately referenced where the work of others has been used in my thesis.

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List of abbreviations

4Q	4-quadrant plot
A5C	Apical 5-chamber
AC	Abdominal circumference
ACHD	Adult congenital heart disease
ACOG	American College of Obstetricians and Gynecologists
AEDF	Absent end-diastolic flow
ASD	Atrial septal defect
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
BPD	Biparietal diameter
BSA	Body surface area
BW	Birthweight
CAM	Cellular adhesion molecules
CHD	Congenital heart disease
C_i	Cardiac index
CI	Confidence intervals
CMR	Cardiac magnetic resonance imaging
CO	Cardiac output
CoA	Coarctation of the aorta
CPR	Cerebroplacental ratio
CRL	Crown-rump length
CSA	Cross-sectional area
CW	Chelsea and Westminster Hospital NHS Foundation Trust
DV	*Ductus venosus*
EBL	Estimated blood loss
ECG	Electrocardiogram
EDD	Estimated due date
EDF	End-diastolic flow
EDV	End-diastolic volume
EFW	Estimated fetal weight
EGF	Endothelial growth factor
ESV	End-systolic volume
FGR	Fetal growth restriction
FL	Femur length
GA	Gestational age
GRO	Growth-regulated oncogene
HC	Head circumference

HF	Heart failure
HR	Heart rate
HRA	Health Research Authority
HTN	Hypertension
ICAM	Intercellular adhesion molecule
ICC	Intraclass coefficient
ICF	Informed consent form
ICG	Impedance cardiography
IL	Interleukin
IFN	Interferon
IOL	Induction of labour
IQR	Interquartile range
IRAS	Integrated Research Application System
ISUOG	International Society of Ultrasound in Obstetrics and Gynaecology
IUGR	Intrauterine growth retardation
IWGMH	The International Working Group on Maternal Haemodynamics
LOA	Limits of agreement
LR	Low-risk
LV	Left ventricle
LVOT	Left ventricular outflow tract
MAP	Mean arterial pressure
MBRRACE	Mothers and Babies: Reducing Risk through Audit and Confidential Enquiries in the United Kingdom
MCA	Middle cerebral artery
MOD	Mode of delivery
NIHR CRN	National Institute for Health Research Clinical Research Network
NPV	Negative predictive value
NYHA	New York Heart Association
OFD	Occipitofrontal diameter
OR	Odds ratio
PE	Preeclampsia
PI	Pulsatility index
PIS	Participant information sheet
PLAX	Parasternal long-axis
PPH	Postpartum haemorrhage
RCOG	Royal College of Obstetricians and Gynaecologists
REC	Research Ethics Committee
RI	Resistance index
RR	Relative risk
RV	Right ventricle
SD	Standard deviation

SGA	Small-for-gestational age
SM-ICG™	Signal-morphology impedance cardiology
SpO_2	Peripheral oxygen saturations
SV	Stroke volume
Th	T-helper cell
TNF	Tumour necrosis factor
ToF	Tetralogy of Fallot
TTE	Transthoracic echocardiography
TVR	Total vascular resistance
UA	Umbilical artery
UAD	Umbilical artery Doppler
UK	United Kingdom
USA	United States of America
UtA	Uterine artery
UtAD	Uterine artery Doppler
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VSD	Ventricular septal defect
VTI	Velocity-time integral
Z	Impedance

Abstract

Introduction: The number of pregnant women with congenital heart disease (CHD) is increasing. Validation of non-invasive techniques to monitor cardiac output (CO) in this population is needed. Uteroplacental blood flow, impaired in women with CHD, is associated with their cardiac function. B-type natriuretic peptide (BNP), cytokines and cellular adhesion molecules (CAMs) could provide insight into the higher adverse pregnancy/cardiovascular events in women with CHD.

Methods: Low-risk pregnant women (LR-group) and pregnant women with CHD (CHD-group) were seen in each trimester. Visits included blood sampling for BNP, cytokine/CAM analyses and measurement of haemodynamic parameters at T1-rest, T2-immediately after exercise and T3-after recovery, simultaneously with transthoracic echocardiography (TTE) and impedance cardiography (PhysioFlow®). Fetal growth and Doppler studies performed in the third trimester.

Results: PhysioFlow® showed good trending ability (LR-group: concordance rate-CR=95.4%, angular bias=4.5^{o}; CHD-group: CR=100%, angular bias=4.1^{o}). Their blood pressure profile followed a J-shape pattern, heart rate increased, peripheral oxygen saturations decreased and CO remained stable with advancing gestation. No association found between uterine artery Doppler and CO/ΔCO or mean arterial pressure in either group. Umbilical artery Doppler was negatively associated with birthweight (BW-p=0.004, BW centile-p=0.006), as was the cerebroplacental ratio (BW-p=0.002, BW centile-p=0.003) in the LR-group. BNP decreased throughout pregnancy, without significant differences between groups. IL-6 was higher in the CHD-group (p=0.005) in the first trimester, IL-10 lower in the third trimester (p=0.018). High levels of IL-6 in the first trimester were associated with lower BW (p=0.002, BW centiles-p=0.020). Higher ICAM in the CHD-group in the first trimester was associated with lower BW (p=0.002, BW centiles-p=0.006).

Conclusion: PhysioFlow® can be used in low-risk pregnancy to study trends in CO. BNP decreased throughout pregnancy in both groups. The proinflammatory cytokine profile in women with CHD could explain the higher incidence of adverse outcomes, although more research is needed.

Chapter 1.
Introduction

Chapter 1. Introduction

1.1. Cardiac disease in pregnancy

Cardiovascular disease is the leading cause of mortality worldwide and there is an upward trend due to the pregnant population becoming increasingly unhealthy and older.[1] In pregnancy, cardiovascular disease complicates 1-4% of pregnancies.[2] This proportion is increasing which can be attributed to multiple factors such as a higher proportion of women with congenital heart disease (CHD) reaching childbearing age and choosing to become pregnant, women adopting sedentary lifestyles with greater rates of obesity and electing to delay pregnancy until older age with the consequent increase in age-associated risk factors such as hypertension (HTN) and diabetes.[2, 3] In the western world, cardiac disease in pregnancy is the main cause of maternal mortality.[3]

Cardiac disease in pregnancy has been the major cause of indirect and overall maternal deaths in the United Kingdom since the year 2000, at a rate of 2.10/100,000 maternities according to the latest MBRRACE (*Mothers and Babies: Reducing Risk through Audit and Confidential Enquiries in the United Kingdom*) report from 2019.[4-6] Over the last 15 years, deaths from cardiac disease in pregnancy have increased in the United Kingdom (UK) and are associated with an increasing maternal age at first pregnancy and increasing rates of obesity,[5] although there is a suggestion that the rate has stabilised since 2010.[4] The main cause of death was myocardial disease/cardiomyopathy, followed by ischaemia and sudden arrhythmia despite a normal heart (Figure 1.1) Most women (77%) died postnatally and CHD was responsible for a proportion of cases.[6]

Sub-classification	Number of deaths	Percentage of total (n=82)*
Ischaemic deaths	**20**	**24**
Atherosclerosis	14	
Coronary artery dissection	6	
Valvular heart disease	**6**	**7**
Valve disease	3	
Endocarditis	3	
Essential hypertension	**1**	**1**
Myocardial disease/ cardiomyopathy	**22**	**27**
Dilated cardiomyopathy	4	
Left ventricular hypertrophy (LVH) with or without fibrosis	2	
Left ventricular hypertrophy (LVH) in morbid obesity	2	
Myocarditis	2	
Peripartum cardiomyopathy	3	
Defined cardiomyopathy		
Hypertrophic cardiomyopathy	0	
Arrhythmogenic right ventricular cardiomyopathy	2	
Ventricular disease (not otherwise specified)	7	
Sudden arrhythmic cardiac deaths with a morphologically normal heart (SADS/MNH)	**15**	**18**
Aortic dissection	**9**	**11**
Others	**9**	**11**
Pulmonary arterial hypertension	4	
Undetermined cardiovascular disease	5	
TOTAL	**82**	
Women with Congenital Heart Disease (CHD) (included in figures above)		
Deaths from aortic dissection	1	
Deaths from valvular heart disease	1	
Deaths from arrhythmia	1	
Deaths from ischaemia	1	

Figure 1.1 *– Sub-classification of cardiac deaths for whom information was available for in-depth review, UK and Ireland 2015-2017. MBRRACE-UK – Saving Lives, Improving Mothers' Care 2019.[6] Image reproduced with permission of the rights holders, Healthcare Quality Improvement Partnership and National Perinatal Epidemiology Unit, University of Oxford © 2019.*

Medical advances have been responsible for a large increase in the number of adults with congenital heart disease (ACHD); the number of affected individuals was estimated to be 1.4 million in the United States of America (USA) in 2010 and this number was expected to grow by 5% annually.[7] Eighty-five percent of children with CHD survive into their adult years.[8] ACHD represents 80% of pregnancies in women with known cardiac disease in pregnancy in the western world.[3, 9, 10] The haemodynamic changes of pregnancy put women with ACHD at a greater risk of complications.[7] However, mortality and morbidity rates in pregnant women with CHD have remained stable with morbidity rates at approximately 11%[9, 10] mainly secondary to arrhythmias and heart failure.[9] Women with CHD experience higher incidence of pregnancy-related adverse outcomes such as postpartum haemorrhage (PPH), gestational hypertension and preeclampsia (PE).[11] Fetal outcomes are also impaired in the presence of maternal cardiovascular disease[3, 12, 13] with high rates of preterm labour/delivery, smallness-

for-gestational age (SGA) and fetal and perinatal mortality rates (perinatal mortality rates are four times higher than in the general population).[14]

Chelsea and Westminster Hospital NHS Foundation Trust (CW) offers, in conjunction with the Royal Brompton Hospital, a weekly high-risk Joint Cardiac Obstetric Medicine Clinic for women with cardiac disease in pregnancy, involving both an obstetrician and a cardiologist. We conduct an average of 600 antenatal consultations per annum. In the clinic we see, on average, 115 new cases per annum (of which 85% are women with ACHD). This is one of the largest obstetric cardiac services in the UK, and the largest in the southeast of England.[15] Once a month, we are joined by an obstetric anaesthetist to undertake delivery planning between 30 and 34 weeks of gestation. We also provide a monthly joint preconception counselling clinic at the Royal Brompton Hospital which is run by a cardiologist and an obstetrician. The 2016 MBRRACE report suggests that a lack of co-location of obstetric and cardiac services may affect the care of pregnant women with cardiac disease;[5] despite this, there have been no maternal deaths due to cardiac disease in women under the care of our service for the last 12 years.

1.2. Cardiovascular changes and adaptation in pregnancy

1.2.1. *Cardiovascular changes and adaptation in normal low-risk pregnancy*

Intravascular volume increases in pregnancy as early as six weeks of gestation.[2] It is considered that peripheral vasodilatation is the initial trigger (mediated by multiple vasoactive substances such as nitric oxide, calcitonin and cyclic guanosine 3',5'-monophosphate) which causes a reduction in the effective arterial blood volume.[16-18] This activates the renin-angiotensin-aldosterone system, which leads to sodium and water retention by the kidneys, resulting in a net 7-8L increase in total body volume.[16, 18-20] Additionally, there is an increase in the glomerular filtration rate which precedes the volume expansion.[20] The drop in vascular resistance also causes a compensatory increase in cardiac output (CO).[16, 21]

CO, defined as the amount of blood pumped into the aorta per minute, is a result of the product of stroke volume (SV, the amount of blood pumped into the aorta per cardiac cycle) and heart rate (HR).[22] This is known as aortic or systemic flow (Qs).[23] CO is known to increase by at least 30% in normal low-risk pregnancy in response to the increasing demands of the fetus and placenta.[22, 24] SV will depend on preload (the stretch of the cardiac muscle when the ventricles are filling), contractility and afterload (the resistance against which the heart pumps).[25] Robson *et al.* performed a longitudinal study in 1989[26] which showed that CO increased by 5 weeks of gestation and by 12 weeks of gestation the increase was 34-39% above non-pregnant values. This increased by up to 43-48% by 24 weeks of gestation and these levels were then maintained until term.

Discrepancies have been reported regarding the magnitude and pattern of increase of CO throughout pregnancy.[24] Such discrepancies are likely to be a result of different study designs and methodology in assessing CO.[26] Greater discrepancies have been reported regarding CO in the third trimester.[27] A recent meta-analysis (Figure 1.2) confirms that CO is increased compared to non-pregnant levels as early as the first trimester, reaching a peak by early third trimester, and that the increase is not linear.[24] The haemodynamic changes of normal low-risk pregnancy also include increases in the HR (15-25%) and SV (20-30%).[22, 28] SV is responsible for the increase in CO in the first half of pregnancy and the gradual increase in HR is responsible for the increase in CO thereafter.[29] Total vascular resistance (TVR) decreases until 20-28 weeks of gestation (30% decrease compared with non-pregnant state)[24] and this is followed by a small increase thereafter.[26] The fall in TVR and the increase in CO largely compensate for each other, hence there are only small differences in mean arterial pressure (MAP) during pregnancy.[22] Blood pressure (BP) is the result of the product of SV and TVR.[25] Some of these changes, a decrease in TVR and MAP and an increase in CO, occur prior to placentation.[17]

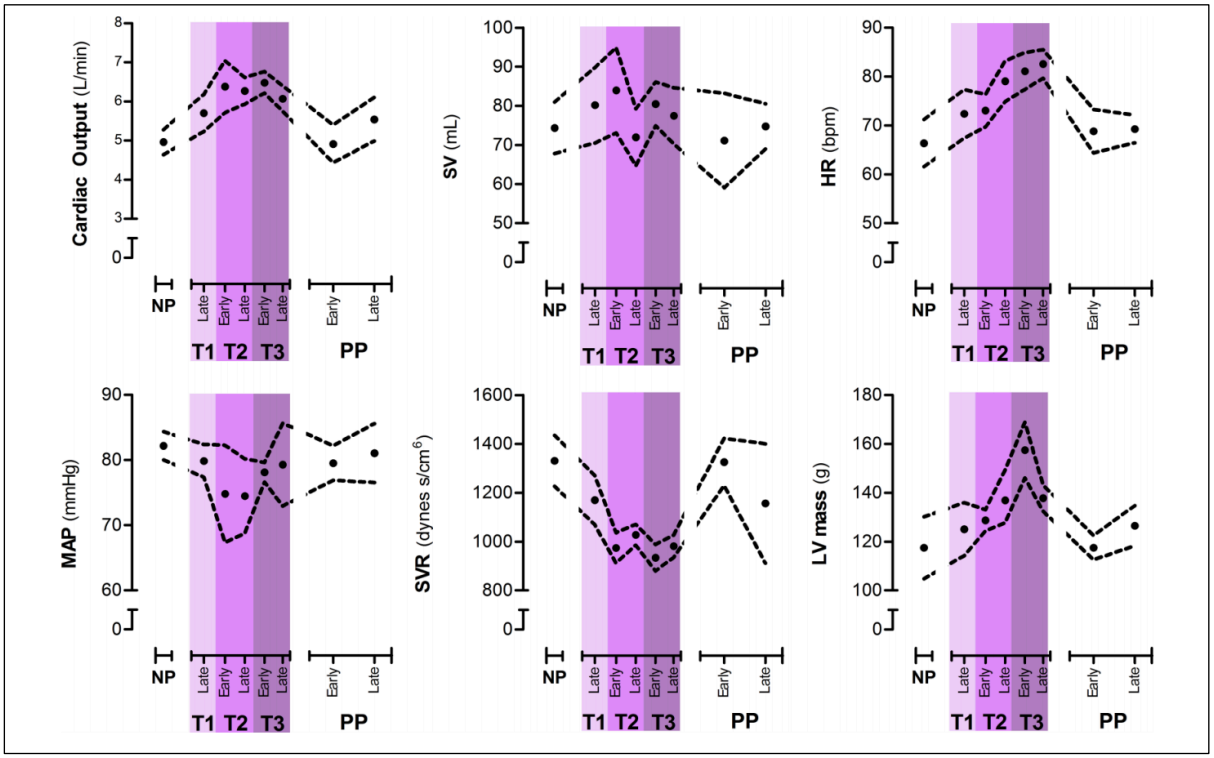


Figure 1.2 *– Compiled weighted mean and 95% confidence intervals derived from meta-analyses for cardiac output, heart rate (HR), stroke volume (SV), mean arterial pressure (MAP), systemic vascular resistance (SVR), and left ventricular (LV) mass at each gestational age. Coloured bars represent the first, second and third trimester of gestation. NP – non-pregnant, T1 – first trimester, T2 – second trimester, T3 – third trimester, PP – postpartum.*[24] *Image reproduced with permission of the rights holders, BMJ Publishing Group Ltd and the British Cardiovascular Society © 2016 BMJ Publishing Group Ltd and the British Cardiovascular Society.*

Cardiac hypertrophy in association with the increased volume has been demonstrated as an increase both in the wall thickness and the overall mass of the left ventricle (LV).[24, 26] Both these parameters are increased by 12 weeks of gestation and by 38 weeks of gestation LV wall thickness increases by approximately 28% and LV mass by 52% when compared to pre-pregnancy values.[26] These changes are similar to those induced in the heart by exercise[28] and are reversible as CO returns to non-pregnant values soon after delivery[28], with TVR also increasing to normal pre-pregnancy values by 12 weeks postpartum.[22, 24]

Robson *et al.*[30] and Hunter *et al.*[28] studied the haemodynamic changes (HR, SV and CO) at 38 weeks of gestation and at 2, 6, 12 and 24 weeks postpartum and showed that the haemodynamic changes after delivery are mostly complete by two weeks postpartum. The only parameters that had not completely regressed were LV wall thickness and mass, suggesting residual hypertrophy by 24 weeks postpartum but with unknown clinical significance.[28, 30]

1.2.2. *Cardiovascular changes and adaptation in pregnancy complicated by congenital heart disease*

Cardiovascular adaptation to pregnancy in women with CHD has not been extensively studied and most studies do not report on longitudinal data.[31] Although obstetric and offspring complications seem to be related to the cardiovascular function in women with CHD, the pathophysiology is not yet understood.[32] Research in this field is imperative considering there are growing numbers of women with ACHD becoming or wishing to become pregnant.

In women with structural heart disease, the cardiovascular adaptation to pregnancy is attenuated.[31, 33] There is an increase in CO and SV as observed in normal low-risk pregnancies, however, the magnitude of this increase is less and after a steep increase in the first half of pregnancy, it plateaus for the second half of pregnancy.[33] The largest longitudinal study on cardiovascular adaptation to pregnancy in women with CHD[31] showed that women with CHD when compared to controls had worse LV systolic function and the slope of LV ejection fraction was significantly different to controls. Nonetheless, pregnancy did not generally lead to worsening cardiac function in women with CHD, although the absolute values on LV function and dimensions were different to controls. Another study from 2010[34] reports no impairment in systolic function and no persistent dilatation of the left ventricle in women with pre-existing heart disease when compared to controls except for women with Tetralogy of Fallot (ToF) where a persistent dilatation of the subpulmonary ventricle was found.

The pattern of cardiovascular adaptation in patients with heart disease seems to be similar to those pregnancies complicated by fetal growth restriction (FGR) and pregnancies at high-altitude.[33] In FGR, the CO increase is less and TVR remains high compared to uncomplicated pregnancies.[35]At high altitude (defined as > 2500m), CO and SV increase to a maximum around 25 weeks of gestation and the magnitude of increase is lower when compared to women at sea level.[36]

1.3. Cardiac output and fetal growth

The physiological cardiovascular adaptations to pregnancy described previously are thought to be essential for adequate uteroplacental circulation and fetal growth.[12, 37] Placentation directly and/or indirectly influences these changes and there is an association between impaired placentation and impaired cardiovascular adaptation to pregnancy.[38] Placentation, including the maternal vascular response to placentation, is compromised in FGR.[39] It has also been demonstrated that defective placentation can precede FGR.[40] However, haemodynamic changes similar to pregnancy are observed in women in the luteal phase of the menstrual cycle and are exaggerated in ovarian hyperstimulation, suggesting that these changes do not depend solely on the placenta. It remains unclear whether the cardiovascular changes are essential for fetal growth or occur secondary to the endocrine changes in pregnancy.[17]

Adequate development of the human placenta is essential for a successful pregnancy outcome.[41] Following implantation, trophectodermal cells arising from the blastocyst epithelium form the primitive syncytium which invades the maternal endometrium.[41] After the formation of the lacunae system, the cytotrophoblasts give rise to primary villi which then differentiate into secondary and tertiary villi which are bathed in blood from the converted spiral arteries.[41] Spiral artery conversion is achieved by the extravillous trophoblast working in tandem with maternal immune cells.[41] The extravillous trophoblast target the musculature of the spiral arteries, converting these vessels to the large conduit vessels which are essential for adequate perfusion of the mature placenta.[41] Uteroplacental malperfusion which occurs secondary to incomplete spiral artery conversion can lead to FGR.[40] Intervillous flow starts around 7-8 weeks of gestation (and established at 12 weeks of gestation) but the presence of trophoblast plugging prevents the exposure of the fetus to high concentrations of oxygen and therefore higher oxidative stress.[40, 42] Premature loss of plugging can lead to miscarriage, PE, and/or FGR.[40, 42] After 12 weeks of gestation, the trophoblast plug breaks down and perfusion of the intervillous space begins, marking the start of true placental function after uterine artery remodelling transforms the spiral arteries into large diameter vessels with reduced contractility allowing oxygen delivery at low pressure.[41]

Birthweight (BW) is linked to neonatal, infant and childhood morbidity and mortality.[43] FGR, previously known as intrauterine fetal growth retardation (IUGR), is usually defined as a pathological condition in which the fetus fails to achieve its full biological growth potential.[44] However, there are inconsistencies in the literature and there is no universally agreed definition.[44-46] The commonest inconsistency is defining FGR as being SGA. The Royal College of Obstetricians and Gynaecologists (RCOG) define SGA as a fetus with an estimated fetal weight (EFW) or an abdominal circumference (AC) below the 10th percentile and severe SGA as a fetus with EFW or AC below the 3rd percentile.[46] However, many babies with BW < 10th percentile are constitutionally small normal babies and many babies who are FGR weigh > 10th percentile at birth.[47, 48] The RCOG do not provide a definition of FGR *per se* but state that FGR "*implies a pathological restriction of the genetic growth potential*" and that therefore these fetuses can show signs of fetal compromise such as abnormal Doppler studies or reduced liquor volume.[46] The American College of Obstetricians and Gynecologists (ACOG), uses FGR for fetuses whose EFW is below the 10th percentile and SGA for newborns whose BW is below the 10th percentile.[45] There is a common view that, to make a diagnosis of FGR, one needs to usually perform more than one measurement and show a growth rate below expected.[44, 46] A consensus from 2018 proposes the following definition (Figure 1.3):[44]

Consensus-based definitions for early and late FGR.

Early FGR: gestational age <32 weeks, in the absence of congenital anomalies
AC/EFW <p3 or AEDF in the umbilical artery
Or
1. AC/EFW <p10 **combined with**
2. PI in the uterine artery >p95 **and/or**
3. PI in the umbilical artery >p95

Late FGR: gestational age ≥32 weeks, in the absence of congenital anomalies
AC/EFW <p3
Or
At least 2 out of 3 of the following
1. AC/EFW <p10
2. Crossing centiles of more than 2 quartiles on growth centiles[a]
3. CPR <p5

[a] Growth centiles are noncustomized centiles.

Figure 1.3 *– Consensus-based definitions for early and late fetal growth restriction (FGR). pX – percentile; AEDF – absent end-diastolic flow; PI – pulsatility index; CPR – cerebroplacental ratio. Image reproduced with permission of the rights holders, Elsevier Ltd. © 2018 Elsevier Ltd.*

FGR is associated with a threefold increase in mortality and an increased incidence of necrotising enterocolitis and respiratory distress syndrome.[49]

Fetal growth is strongly correlated with maternal cardiovascular function.[50] There is a positive correlation between CO and BW[51] and an increasing recognition that FGR is associated with cardiovascular disorders and death from ischaemic heart disease later in life.[52] In the general population, FGR and PE are associated with lower CO and high TVR and in women with CHD it is postulated that their impaired haemodynamic adaptation can lead to inadequate placentation (rather than the reverse) and therefore a higher incidence of FGR and PE.[11]

FGR has been reported in up to 38% of pregnancies of women with CHD, depending on the type of cardiovascular abnormality.[53-55] TVR is inversely correlated with centile BW (in normotensive pregnancies) and TVR can be used as a surrogate marker for cardiovascular function during pregnancy.[50] In a study[56] comparing pregnant women (normotensive, non-smoker and not on any medications) who had IUGR babies to women who had non-IUGR babies, CO and SV were significantly lower in the IUGR group by 23% and 19%, respectively and TVR was higher by 33%. There was also a significant correlation between fetal weight and maternal cardiac output.[56] Another study reporting on 20 FGR pregnancies showed lower CO and higher TVR in this group compared to controls.[35]

Gelson *et al*.[55] reported perinatal complications of 34% in a cohort of 331 women with heart disease (as opposed to 15% in the control group) and the most common complication was SGA, accounting for 61% of cases. Median BW percentile was 31 in the heart disease group compared with 48 in the control group and women in the cyanotic or low CO group had the smallest babies.[55] A more recent review of 1321 pregnancies in women with heart disease reported that their babies were smaller compared to the control group and that the difference was more pronounced in those with Fontan circulation, pulmonary hypertension, prosthetic heart valves, systemic right ventricle, Marfan syndrome, repaired ToF and cardiomyopathy.[57]

Correlation of haemodynamic parameters assessed longitudinally throughout pregnancy but particularly during the first trimester, and its association with BW percentile may provide an insight into how haemodynamic (mal)adaptation influences fetal growth.

1.4. Uteroplacental circulation and its assessment

The use of ultrasound Doppler studies have for many years been used to study the fetal and placental circulations with a view to diagnosing and monitoring conditions such as FGR.[58] Commonly targeted vessels in clinical practice include the uterine artery (UtA), umbilical artery (UA), middle cerebral artery (MCA) and *ductus venosus* (DV).[58] Doppler ultrasound is a non-invasive and reliable way of assessing uteroplacental circulation.[59]

The main use of uterine artery Doppler (UtAD) has been to screen for PE, typically between 20-24 weeks of gestation, when a raised pulsatility index (PI), raised resistance index (RI) or presence of notching is considered to be abnormal.[58] It allows a distinction to be made between placental and other causes of FGR.[58] Cnossen *et al.*[60] performed a large systematic review and concluded that UtAD predicts PE better than FGR and that an increased PI with notching in the second trimester best predicted PE in both low-risk and high-risk women. An increased PI with or without notching best predicted severe FGR in a low-risk population and in high-risk subjects the best predictor was an increased RI.[60] Only women with risk factors should be referred for screening by means of UtAD as there is limited predictive value for low-risk women.[58] Screening can be started as early as the first trimester and the detection rate for early PE can be increased to nearly 90% when UtAD is used.[61] In women with a low-risk pregnancy affected by FGR or hypertensive disorders of pregnancy, placentation is often found to be impaired and this translates in abnormal Doppler studies of the uterine and umbilical arteries.[32] In women with an abnormal UtAD at 20-24 weeks of gestation (PI >95th centile and/or presence of notching) should have serial growth scans and umbilical artery Doppler (UAD) from 26-28 weeks of gestation as per RCOG guidance.[46]

Melchiorre *et al*.[62] studied the relationship between raised UtAD PI/notching and the presence of cardiac defects in the mother. The authors report ten cases of undiagnosed functionally significant structural cardiac anomalies (relative risk (RR) = 12.6, 95% confidence intervals (CI) [1.60-98.34]) in women with raised UtAD PI.[62] The authors conclude that high resistance UtAD PI may indicate suboptimal haemodynamic adaptation rather than defective placentation.[62] A systematic review of uteroplacental blood flow and pregnancy outcomes concluded that there is a place for routine UtAD in women with known cardiovascular dysfunction as this can reveal cardiovascular maladaptation and guide antenatal management.[63]

UAD is a surrogate marker of placental vascular resistance – as gestation progresses, there is reduced vascular resistance and indices like UAD PI decrease.[64] Abnormal UAD wave patterns (increased PI or absent/reversed end-diastolic flow (EDF)) precede decelerations on antenatal cardiotocography and therefore can be used as predictive of fetal compromise.[65]

The role of MCA Doppler in FGR is less clear than that of UAD.[58] The 'brain-sparing effect' was demonstrated by Wladimiroff *et al.*[66] in 1986 and was described as an "*increased peripheral vascular resistance in the fetal body and placenta and a compensatory reduction in peripheral vascular resistance in the fetal cerebrum*". MCA is not a useful predictor of adverse perinatal outcomes in preterm pregnancies and MCA Doppler should only be used to plan time of delivery if it is abnormal (even with a normal UAD) at term.[46] More recently, the cerebroplacental ratio (CPR), the ratio of the MCA PI divided by the UA PI was shown to correlate better than BW as a marker for placental insufficiency in the third trimester.[67]

Pieper *et al.*[32] performed UtAD and UAD studies at 20 and 32 weeks of gestation in over 200 women with CHD and compared the results to 70 low-risk women who acted as controls. UtAD and UAD PI and RI were higher throughout pregnancy in the CHD group compared to the low-risk group; PI/RI in both arteries decreased throughout pregnancy in both groups.[32] Doppler flow was associated with obstetric and offspring events in women with CHD – higher rates of hypertensive disorders/PE

(fourfold), preterm delivery and SGA (fourfold).[32] Another study by Goya *et al.*[68] including 127 women with CHD reported that UtAD PI at 20 weeks was statistically significantly higher in the heart disease group as was the UAD PI at 32 weeks of gestation. Lesion-specific studies such as the study by Kampman *et al.*[69] concluded that women with ToF have a higher incidence of abnormal UtAD and UAD which are indicative of abnormal placentation and that right ventricular (RV) function was correlated with the Doppler flow parameters. In women with repaired coarctation of the aorta (CoA), RV function was also impaired and correlated with Doppler flow parameters.[70]

1.5. Assessing cardiac output in pregnancy

Measurement of CO provides an indirect indication of tissue perfusion.[71] There is wide variability of CO between individuals and therefore to obtain a good picture of changes in pregnancy, CO should be studied longitudinally in the same subjects.[30] Many CO measurement techniques were introduced in the context of the critically-ill and then translated into obstetrics without validation.[25] When choosing a technique to measure CO in pregnancy, one should consider its availability, cost, operator dependence, ability to perform continuous *vs* intermittent measurements and accuracy and precision in providing absolute numbers or trends in CO change.[25] CO can be assessed using invasive, minimally invasive and non-invasive techniques and many techniques are used in clinical practice.[72]

1.5.1. *Invasive techniques*

Invasive studies, of the type which were performed before 1970, are associated with significant morbidity to both the mother and the fetus and are consequently not appropriate for serial investigation of CO in pregnancy.[27] Reported complications from pulmonary artery catheter insertion are rare and not associated with increased mortality, but include arterial injury, haemothorax, pneumothorax, arrhythmias, infection, thromboembolism and haematomata.[73-75]

1.5.1.1. The Fick Principle

Described by Adolph Fick in 1870, it uses the oxygen concentrations in arterial (pulmonary artery) and venous blood (pulmonary vein) to calculate flow (i.e. CO), which can be calculated using the following formula:[71, 76]

$$CO = \frac{VO2}{(CaO2 - CvO2) * 10}$$

Where:
VO2 – oxygen consumption (measured using a spirometer[25])
CaO2 – arterial oxygen content
CvO2 – mixed venous oxygen content.

This principle was initially tested in animals and only applied to humans from 1929.[76, 77] Although now rarely used in clinical practice, it is still regarded as the most accurate form of calculating CO.[25, 77, 78]

In the so called *direct Fick method*, oxygen consumption is measured as the difference in the content of inspired and expired oxygen in the respiratory cycle (spirometry) and the arterial-venous difference in oxygen is calculated from a sample of mixed venous blood obtained from the pulmonary artery (representing the combined/mixed venous saturation) and a simultaneous arterial sample (usually obtained from an arterial line).[25, 79] The accuracy of CO obtained via this technique depends on the individual being in a 'steady haemodynamic state' with stable oxygen consumption, respiratory and heart rates.[79] The variability of measurements with the direct Fick method is minimised by the fact that two out of the three components of the Fick equation are obtained directly: the arterial (CaO2) and venous blood oxygen content (CvO2).[80] Higher variations are seen in the oxygen consumption component of the equation due to occasional small and unavoidable leaks through the mouthpiece.[80] Despite this limitation, the direct Fick method is considered the true gold standard in CO measurement, although its use is limited to the catheter laboratory or in the context of research as it requires specialist personnel/equipment and is time consuming.[79] Like the direct Fick method, the indirect Fick method, uses a sample of mixed venous blood drawn from the pulmonary artery. However, rather than using direct measurement of total oxygen consumption and arterial blood oxygen, the indirect Fick uses pulse

oximetry devices to derive the arterial oxygen content.[79] The arterial venous difference is calculated as: [(1.36 * haemoglobin * oxygen saturations)-venous oxygen saturations].[79] Small errors resulting from inaccuracy of oximetry devices, errors in haemoglobin levels or in oxygen levels analysis in blood samples, result in less accurate CO results.[81] However, because the indirect Fick method is easier to perform and can be used at the bedside, it is more commonly used in clinical practice.[79]

1.5.1.2. Indicator dilution techniques

With this technique, an indicator (usually a dye) is injected into the right atrium and its concentration measured by means of a detector in or on the pulmonary artery.[77] CO is inversely proportional to the area under the curve, from a graph representing concentration *vs* time, as per the Stewart-Hamilton Equation:[77]

$$M = Q \int C\,(t)dt$$

Where:
M – injected indicator
Q – flow
C(t) – concentration that varies with time.

The main issue with this technique is the choice of indicator which needs to be stable, non-toxic and easily measurable.[77]

1.5.1.3. Thermodilution

Following the invention of the Swan-Ganz catheter in 1971, the thermodilution technique has been extensively used in clinical practice.[76, 78] The pulmonary artery catheter is inserted via the brachial, subclavian or jugular veins into the right atrium, ventricle and pulmonary artery, allowing injection, sampling, pressure and temperature recording.[25] With this procedure, a known amount (usually 5-10mL[25]) of a cold solution is injected into the right atrium through the proximal end of the pulmonary catheter and then detected distally by a thermistor.[71] Blood temperature changes allow the calculation of the area under the curve using a modified Stewart-Hamilton equation:[71]

$$CO = \frac{[VI * (TB - TI) * K1 * K2]}{\int \Delta TB(t)dt}$$

Where:
VI – injected volume
TB – blood temperature
T1 – injectate temperature
K1 – density factor
K2 – computation factor

In clinical practice this method is widely used[82] and correlates well with the dye dilution and Fick techniques.[71, 77] The thermodilution technique is therefore considered the gold standard for CO measurement.[71] The average of three to five CO thermodilution curves allows intermittent calculation of CO.[25] To appreciate clinically significant differences, there must be a 12-15% difference in consecutive CO measurements, reflecting the reproducibility of the technique.[82] The recently published recommendations from the International Working Group on Maternal Haemodynamics (IWGMH) suggest that the accuracy and precision of any such technique used to measure CO in pregnancy remain around 10-20%, which means that it will only detect changes in CO ranging from 15-30% (0.75-1.5L/min for a mean CO of 5L/min).[25]

1.5.2. *Minimally-invasive techniques*

Pulse-contour and pulse-power analysis and pressure-recording analytical methods use an arterial line and are based on the relationship between arterial pressure and SV.[25] The IWGMH advise validation in pregnancy of these techniques prior to use in clinical practice.[25]

Transoesophageal Doppler monitoring uses a Doppler probe inserted into the lower oesophagus and measures flow in the thoracic aorta.[25, 72] Blood flow between supra-aortic vessels and the descending aorta is not constant which can cause underestimation of CO.[72] The IWGMH advise validation in pregnancy.[25]

1.5.3. *Non-invasive techniques*

With the use of non-invasive techniques it is possible to study CO changes longitudinally before, throughout pregnancy and postnatally without the associated risks of invasive and minimally invasive techniques.[27]

1.5.3.1. Cardiac magnetic resonance imaging

The use of cardiac MRI (CMR) has been recognised as a reference technique for CO measurement in pregnancy and its use is mainly limited to women with complex cardiac conditions due to costs, availability and the need for an highly skilled professional to obtain and analyse the images.[25, 83] CMR does not require the use of ionising radiation and is therefore safe to use in the second and third trimesters of pregnancy.[25] Caution should be taken when used in the first trimester due to the heating of tissues by radiofrequency and exposure to a loud acoustic environment with a potential higher risk of vision loss in the offspring.[84] Obtaining high-resolution 3D images of the heart allows accurate and reproducible calculation of SV.[25, 85]

1.5.3.2. Transthoracic echocardiography

Transthoracic echocardiography (TTE) is considered to be non-invasive, safe and readily accessible in clinical practice and is commonly used to determine CO in the non-pregnant and in the pregnant population.[72, 86] There are multiple methods of assessing CO using TTE.[87] TTE using pulse-waved Doppler in the left ventricular outflow tract (LVOT) is a reference technique for measurement of CO in pregnancy and has been validated against invasive techniques.[86]

The *Teichholz* method uses the end-systolic (ESV) and end-diastolic volumes (EDV) from parasternal long-axis view (PLAX) during M-mode tracing to estimate SV, as the difference between ESV and EDV, and CO is the product of SV and HR.[25, 87] This method has several limitations as it depends on the shape of the LV (which can change during pregnancy) and is not recommended for use in pregnancy.[25, 87]

The *Simpson* method estimates SV and CO through an estimation of LV volume obtained from tracing the endocardial border in two orthogonal views at end-systole and end-diastole.[25, 87] This method is highly operator dependent and requires a specialist echocardiographer.[25]

The velocity-time integral (VTI) method is a well-established technique that has been validated outside pregnancy[88] A recent meta-analysis found only three studies (a total of 34 measurements) using TTE with the use of pulse-wave Doppler in the LVOT for validation against the thermodilution technique in pregnancy.[86, 89, 90] The results show excellent levels of agreement, little bias and limits of agreement and percentage error within the acceptable range: percentage error of 19.1% and intraclass coefficient (ICC) of 0.94 (95% CI [0.88-0.97]), suggesting that TTE is as good as the gold standard for use in pregnancy.[86]

For the cross-sectional area (CSA) of the LVOT the two-dimensional (2D) mode is used in the PLAX and the diameter of the LVOT measured on a frozen image in mid-systole up to 1cm from the annulus. The CSA is then obtained as:[25, 86]

$$LVOT_{CSA} = \pi(\frac{d}{2})^2$$

Where:
d – diameter of the LVOT

Measuring the LVOT is prone to higher intra- and inter-observer variability and therefore great care must be taken with this measurement, ensuring adequate zoom is used.[91] Following this, using the pulsed-wave Doppler mode in the 5-chamber apical view (A5C), the VTI of the aortic flow can be obtained by placing the cursor just proximal to the aortic valve leaflets.[25, 88] The SV is then calculated:

$$SV = LVOT_{CSA} * LVOT_{VTI}$$

And CO is simply:

$$CO = SV * HR$$

In CHD with an unrepaired shunt, CO is estimated from the pulmonary blood flow.[23] Pulmonary SV is calculated as[23]:

$$SVp = PA_{CSA} * PA_{VTI}$$

Where:

p – pulmonary

PA – pulmonary artery

CO (Qp) is then calculated as above for systemic CO (Qs), by multiplying SV and HR. The ratio of total pulmonary flow to total systemic flow (Qp/Qs) allows quantification of the shunt.[92] A Qp/Qs ratio of 1:1 is normal and indicates no shunt, a ratio >1:1 indicates a left-right shunt and a ratio <1:1 indicates a right-left shunt.[92]

TTE is non-invasive, readily available, safe to use in pregnancy and should be used as the reference technique against which other techniques are validated in pregnancy.[25, 86]

1.5.3.3. Impedance cardiography

Impedance cardiography (ICG) was first described by Kubicek *et al.*in 1966.[93] The principle behind this technique is that the impedance (Z) of the thorax (the resistance to alternating electrical current) depends on the amount of thoracic fluid.[94] Changes in the size and blood volume of the thoracic aorta with each cardiac cycle are responsible for most of the changes in thoracic impedance.[72] Assuming that the change in the amount of blood in the aorta with the cardiac cycle can be detected by changes in impedance, SV and CO can be measured by measuring changes in detected voltage.[94]

There are several ICG devices and each uses different equations and algorithms.[25] It has gained growing interest as most devices are easy to use, operator independent, non-invasive, reproducible, cost-effective and provide real time and continuous information.[25, 95] Studies have shown that CO measured by ICG

correlates with CO measured by other invasive and non-invasive techniques in the non-pregnant population but results are conflicting.[72, 87]

Kubicek *et al.*[93], commissioned by the National Aeronautics and Space Agency[72], described the technique using four electrodes (two around the neck and two around the abdomen) – please see Figure 1.4.

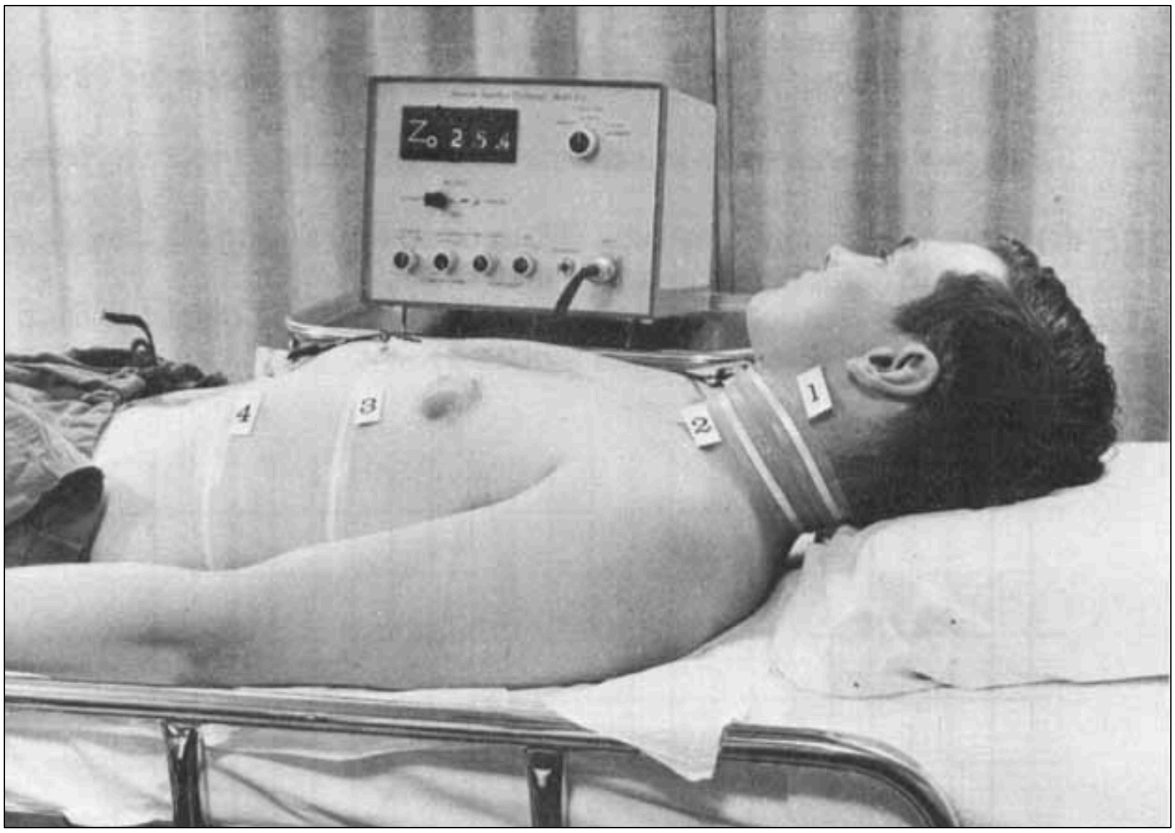


Figure 1.4 *– A photograph of the tape-on electrodes in place for impedance cardiography. It is important to maintain good separation between electrodes 1 and 2. The electrode configuration is used for obtaining 1) cardiac function data and 2) changes in the total fluid in the chest by observing Z_0 (the total impedance between electrodes 2 and 3). The Z_0 measurement has been found to be a very sensitive indicator of the development or reversal of such conditions such as pulmonary oedema, pulmonary congestion, and pleural effusion. Image reproduced with permission of the rights holders, John Wiley & Sons, Inc.* *© 2006 John Wiley & Sons, Inc.*

A sinusoidal alternating current of 4mA is transmitted between electrodes 1 and 4 and a voltage drop generated between electrodes 2 and 3, which is proportional to the product of the current times the magnitude of the impedance (Z_0 – baseline impedance) between electrodes 2 and 3. This voltage is then detected by amplifiers in the detection system. ICG detects pulse-synchronous changes in the thoracic cavity alongside an ECG.[72] Bioimpedance depends on the amount of body fluid: it decreases with increased fluid and vice-versa.[96] With each contraction, there is an increase in thoracic electrical

conductance given by the bioimpedance waveform, which is the derivative of the impedance over time (for a known alternating current of high frequency and low amplitude), ICG = dZ/dt.[72] dZ/dt is measured from zero to the most negative point (upward wave, please see Figure 1.5, D)[72, 93]

Stroke volume can then be calculated according to the formula:

$$\Delta V = \rho \frac{L^2}{Z_0^2} \mathrm{T}(\mathrm{dZ/dt})_{min}$$

Where:
ΔV – ventricular SV
ρ – electrical resistivity of the blood
L – mean distance between electrodes 2 and 3. To calculate the distance, an assumption is made that the chest is shaped in the form of a cylinder (electrically speaking).[97]
Z_0 – mean body impedance between electrodes 2 and 3
(dZ/dt) – the minimum value of dZ/dt occurring during a cardiac cycle
T – the ventricular ejection time obtained from the dZ/dt wave

Berstein *et al.*[97] changed Kubicek's equation by adding the patients weight, altering the assumed shape of the thorax to a truncated cone and by eliminating blood resistivity. The Sramek-Bernstein equation is as follows:[97]

$$SV = \delta * \frac{(0.17\ H)^3}{4.25} * \frac{\frac{dZ}{dt_{max}}}{Z_0} * LVET$$

Where:
δ – patient's weight
H – patient's height
LVET – left ventricular ejection time

When comparing different equations to measure SV with thermodilution techniques, the Sramek-Bernstein formula correlates the best.[72]

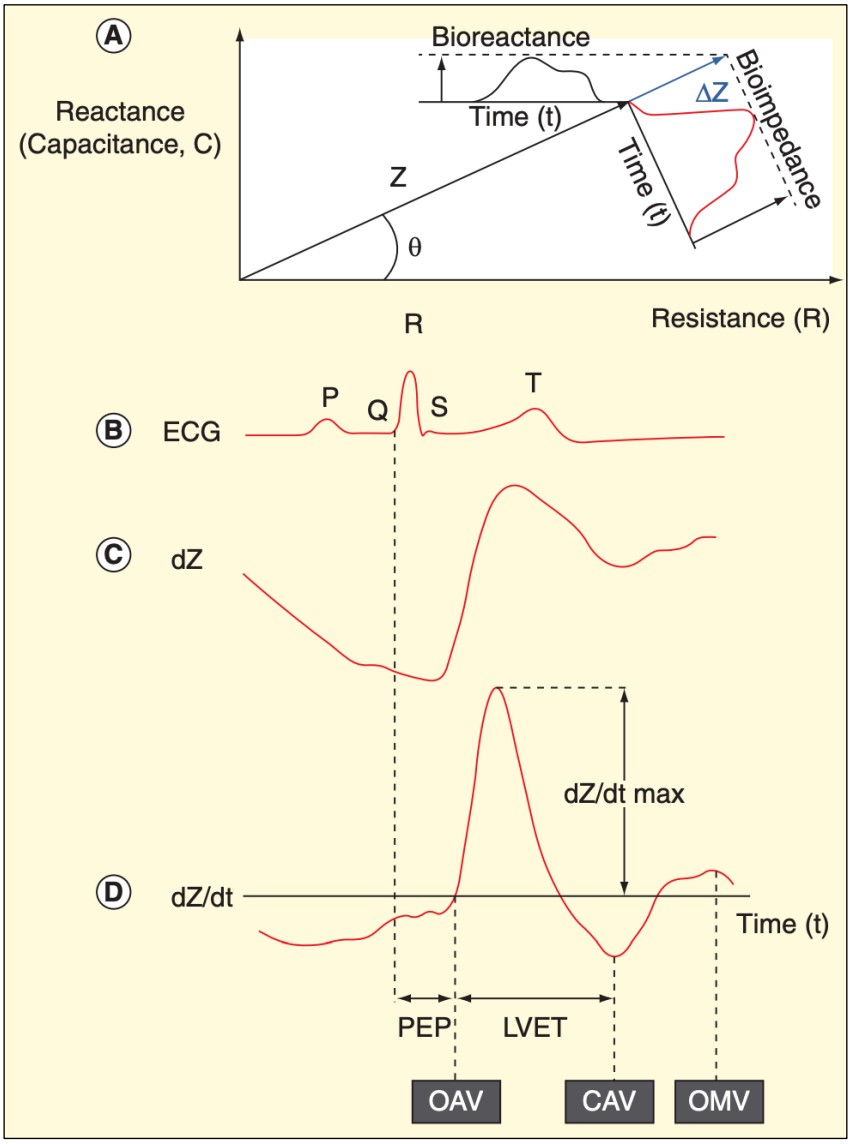


Figure 1.5 *– Impedance cardiography trace with a simultaneous ECG. At level A the constituents of the bioimpedance signal (dZ) are shown. They comprise a resistive and reactance component and the geometric sum of both make up the total impedance. At level B, a standard ECG trace is shown, synchronised to the thoracic impedance dZ (C) and the impedance cardiography signal, dZ/dt (D). CAV: closure of aortic valve; LVET: left ventricular ejection time; OAV: opening of aortic valve; OMV: opening of mitral valve; PEP: pre-ejection period. Image reproduced with permission of the rights holders, Taylor and Francis © 2013 Taylor and Francis, Copyright Clearance Centre.*

Modern ICG devices do not depend on baseline impedance (Z_0) and use waveform analysis to derive SV and all other derived parameters.[96] The PhysioFlow® PF07 Enduro™ (*Manatec Biomedical, France*) is a device that uses signal-morphology impedance cardiology (SM-ICG™), making it independent from Z_0. The equation for calculating SV has been modified to eliminate Z_0, blood resistivity and the distance between electrodes, parameters used in the Kubicek and Sramek-Bernstein equations.[98, 99] PhysioFlow® emits a high-frequency (75mHz) low amperage (1.8mA) alternating electrical current via electrodes.[99] Two sets of two electrodes (a pair of transmitting and a pair of sensing electrodes) are applied to the left side of the neck and along the xiphoid process, respectively (please

refer to Chapter 2), and an additional pair is used to obtain an ECG reading (V1 and V6 leads).[99] Positioning of the electrodes is not critical as PhysioFlow® does not depend on Z_0.[99] Signal quality is assessed by four waveforms: ECG and its first derivative (dECG/dt), the impedance waveform (ΔZ) and its first derivative (dZ/dt) – Figure 1.6.

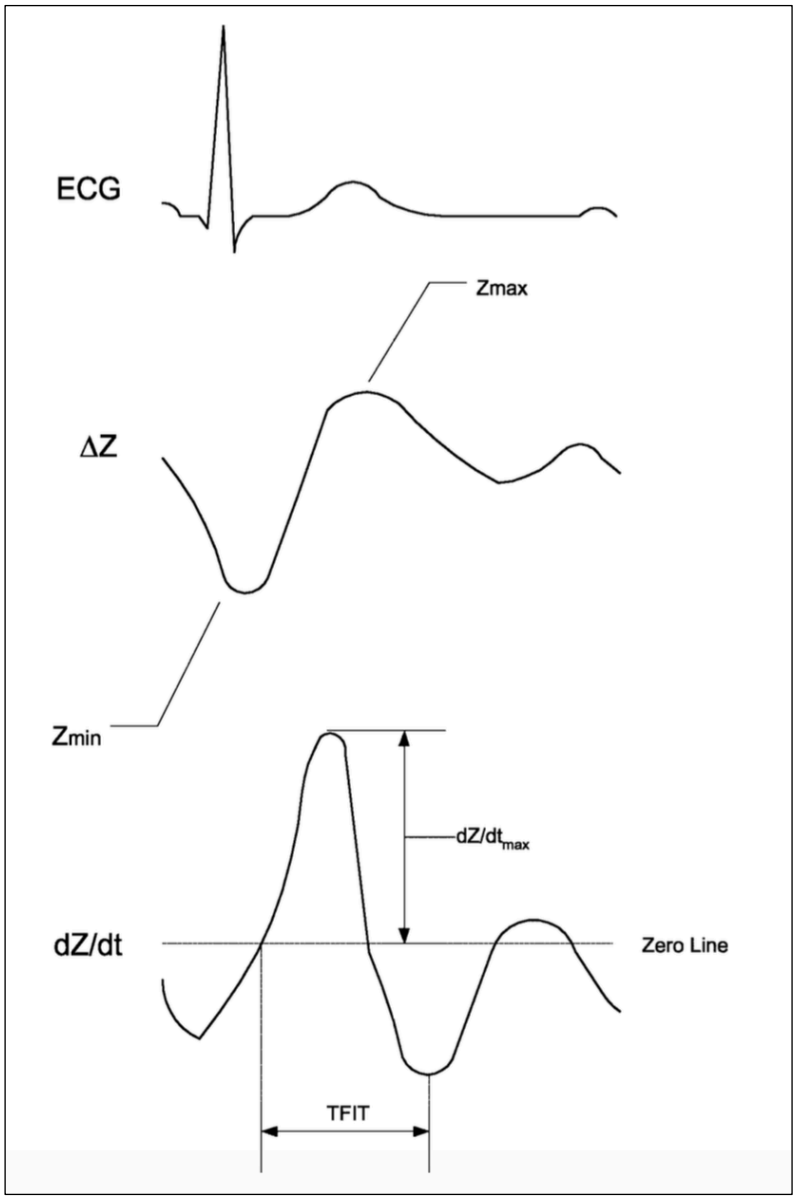


Figure 1.6 *– Waveforms obtained from PhysioFlow®. Image reproduced with permission of the rights holders, Springer-Verlag Berlin Heidelberg © 2000, Springer-Verlag Berlin Heidelberg.*

CO measurement by the PhysioFlow® device is based on the formula:

$$\dot{Q}_c = f_c * SV_i * BSA$$

Where:

$\dot{Q}_c$ – cardiac output, in l . min^{-1}

f_c – heart rate, in beats . min^{-1}, based on the R-R' interval from the dECG/dt which provides a more stable signal than the ECG itself

SV_i – stroke volume index, in ml . m^{-2} (SV divided by BSA)

BSA – body surface area (m^2), calculated according to the Haycock formula (BSA = 0.024265 * body mass $(Kg)^{0.5378}$ * height $(cm)^{0.3964}$.[99]

The first SV measurement is obtained over the course of 24 consecutive heart beats in the resting condition and retains the largest impedance variation during systole ($Z_{max} - Z_{min}$) and the largest rate of variation of impedance signal (dZ/dt_{max}, called the contractility index). The SV also depends on the thoracic flow inversion time (TFIT), which is measured on the first mathematical derivative of the impedance signal (please see Figure 1.6).[99]

PhysioFlow® has been validated for clinical use outside pregnancy.[99-102] Charloux *et al.*[99] compared CO measured by PhysioFlow® with the direct Fick method, at rest and at 6 min into a light exercise (10-50W) protocol, and demonstrated that the PhysioFlow® had acceptable clinical accuracy for both rest and exercise when compared to the gold standard, as well as a good coefficient of repeatability (0.94).[99] Richard *et al.*[100] measured CO at rest and during incremental exercise (starting at 20% of theoretical maximum power followed by 20-35W increments every minute up to maximum load tolerated) using Physioflow® and the direct Fick method. CO measurements using PhysioFlow® were repeated 3 days later to test for reproducibility.[100] The authors showed that PhysioFlow® provides clinically acceptable accurate measurements of CO during incremental exercise when compared to CO measurements obtained from the direct Fick method, and also report good reproducibility.[100] Broeder *et al.*[101] studied CO measurement with PhysioFlow® against the direct Fick method using the Stringer equation and with incremental exercise. The authors report no significant difference between the two methods with an r^2 of 0.99.[101] Robach *et al.*[102] validated PhysioFlow® against the cardio-green dilution

method and showed a mean bias of -0.4L/min (95% CI [4.83-4.01L/min]), which suggests that the CO measurements with PhysioFlow® were acceptable.[102]

PhysioFlow® has not been validated for use in pregnancy and there are only three reports of its use in pregnancy: Leroy *et al.*[103] used the PhysioFlow® to assess haemodynamic changes in the third trimester in women with preeclampsia, healthy controls and non-pregnant controls in three different positions: supine, left lateral and right lateral. The authors reported cardiac index (C_i) and TVR levels similar to those obtained by thermodilution techniques reported in the literature but did not specifically compare ICG with other methods in their study[103]; You-Ten *et al.*[104] used the PhysioFlow® to assess haemodynamics in women undergoing elective Caesarean section delivery under spinal anaesthesia. The authors reported that the continuous monitoring of SV, CO, C_i and TVR may provide an insight into the causes of hypotension during Caesarean delivery[104]; McLeod *et al.*[105] performed a randomised controlled trial comparing the impact on cardiovascular parameters of the administration of an oxytocin 5-unit bolus and placebo infusion *vs* oxytocin 5-unit bolus *vs* 30 min infusion at elective Caesarean section (n = 74), and measured maternal haemodynamics using the PhysioFlow®. The authors reported no haemodynamic differences between groups and, despite an increase in C_i and LV work index with oxytocin bolus, these parameters returned to normal within one hour and the additional oxytocin infusion did not affect maternal haemodynamics further.[105]

PhysioFlow® has also been used and validated in CHD.[106] A study comparing CO measurement at rest between PhysioFlow® and CMR in a population of patients with CHD (mean age 18 years old) showed good agreement between the techniques.[106] In this study, the subjects were of older age (compared to other similar studies), all patients had a biventricular heart and the authors excluded patients with significant aortic runoff lesions (aortic regurgitation, aortopulmonary shunt or patent ductus arteriosus), which is particularly important considering that ICG depends mainly on the blood flow of the aorta.[106] Legendre *et al.*[107] also concluded that PhysioFlow® was a "*highly reliable method*" in assessing SV and CO in patients with residual right outflow tract lesions following CHD repair. A previous report from Taylor *et al.*[108] showed poor accuracy of the PhysioFlow® compared to CMR in assessing

haemodynamics in a mixed population with CHD but this study was undertaken on a paediatric population with a median age of 2.8 years (interquartile range (IQR) 0.02-8.02) and median BSA of 0.54 m^2 (IQR 0.21-1.00). Another study by Taylor *et al.*[109] compared CO measurement using PhysioFlow® with the direct Fick technique and concluded that measurement of CO using PhysioFlow® was unreliable. The study population had a median age of 3.5 years (IQR 0.4-16.6), a median BSA of 0.62m^2 (IQR 0.31-1.71) and included patients with biventricular and univentricular circulations, and patients with cardiac shunts.[109]

ICG is ideal for use in pregnancy due to its non-invasive nature and ease of use (midwives could be trained to use it).[72] However, further research using ICG and in particular PhysioFlow® (due to its unique equation) is necessary if the device is to be validated for use in pregnancy, specifically in pregnant women with heart disease.[25]

1.6. The use of non-invasive cardiac output monitors in clinical practice and in the context of research

In pregnancy, which is associated with the aforementioned cardiovascular changes, there is growing interest in being able to measure haemodynamic parameters such as SV and CO non-invasively, in order to help with the early diagnosis of conditions such as PE or FGR and guide treatment.[25, 110] The methods and devices available for CO monitoring in pregnancy are: CMR, TTE, non-imaging continuous-wave Doppler, inert-gas rebreathing device, ICG, bioreactance, and non-invasive pulse-contour analysis.[25]

CMR is considered safe to use in the second and third trimester but its use is limited due to costs, availability and the need of highly skilled personnel to interpret the images.[25, 83] The role of CMR in the haemodynamic assessment of cardiovascular parameters in low-risk pregnancy was not established until the recent CHIRP study[85] was undertaken which established reference values for cardiac indices in pregnancy and the postpartum period. This study has established reference values from which future studies can be based on.

TTE is a well-established, readily available and non-invasive technique of CO measurement in pregnancy, commonly used in the clinical and research contexts and, has been suggested as the new reference standard for CO measurement in pregnancy.[25] However, the accuracy of TTE is highly dependent on the skill of the operator, and significant interobserver variability can occur, which can limit its use in research.[83]

The non-imaging continuous-wave Doppler USCOM 1A® device (*Uscom, Australia*) uses a transducer placed on the suprasternal notch to determine ascending aortic flow.[111] The USCOM® device has been used clinically in the non-pregnant population, mainly in the context of critical care.[112] This device has been validated for use in pregnancy in two studies[111, 113] and has shown good reproducibility but variable accuracy and precision, therefore prompting the IWGMH to recommend further validation studies.[25] Its main advantages are that it is easy to use and less operator dependent than TTE, albeit with the disadvantage that it does not account for possible changes in aortic dimension and compliance associated with pregnancy.[25]

For the inert-gas rebreathing technique, a mixture of nitrous oxide (blood soluble) and sulphur hexafluoride (blood insoluble) is administered through a closed breathing device and the relative levels of the two gases measured, allowing the estimation of CO based on the Fick principle.[25] This technique has been primarily used in the context of research[114] and is yet to be validated for use in pregnancy.[25] Its main advantage is that it is operator independent and its most significant limitation is that CO assumptions may not be valid in individuals with increased alveolar dead space or pulmonary oedema.[25]

The ICG and bioreactance techniques use electrodes to transmit a low amplitude high-frequency current to detect changes in impedance, in the case of ICG, or in phase shifts, in the case of bioreactance, as described earlier.[25] There are several ICG/bioreactance devices but validation studies have failed to show sufficient accuracy and precision for absolute CO values when compared to reference techniques.[25] However, these devices seem promising in studying trends in CO and therefore the IWGMH have recommended further validation studies.[25]

Finally, the non-invasive pulse-contour analysis estimates CO based on the relationship between arterial pressure and SV.[25] The arterial waveform is obtained from a variety of devices (oscillometric BP cuffs, volume clampfinger clips) which are operator independent and easy to use.[25] These devices have failed to show adequate accuracy and precision and also lack validation.

1.7. B-type natriuretic peptide in low-risk pregnancy and in pregnancy complicated by congenital heart disease

B-type natriuretic peptide (BNP) is secreted by cardiac myocytes mainly in response to changes in LV loading.[115] Myocyte stretch induced by pressure overload, volume expansion or increased myocardial wall stress, stimulates the secretion of the BNP pro-peptide which is then transformed into two main molecules: the NT pro-BNP (inactive) and BNP (bioactive).[115-117] Both molecules are released into the circulation and can be easily measured (many assays are available).[115] The physiological haemodynamic changes characteristic of pregnancy would, in the non-pregnant state, induce the release of BNP.[118] BNP has natriuretic, vasodilator and diuretic effects.[117] The concentration of BNP is higher in women than in men, increases with age and declining renal function, and decreases with increasing body mass index (BMI).[115]

Outside pregnancy, BNP can reliably discriminate between heart failure (HF) and other causes of dyspnoea, and predict the risk of sudden death in patients with established HF; however, it correlates poorly with levels of LV loading.[119, 120] The upper limits of normal in the non-acute setting are <35pg/mL for BNP and <125pg/mL for NT pro-BNP. In the acute setting, higher values are found and the cut-offs are: <100pg/mL for BNP and <300pg/mL for NT pro-BNP; patients with BNP values below these ranges are highly unlikely to have HF.[121] Diagnosing heart disease in pregnancy can be difficult as the normal symptoms of pregnancy can mimic those of heart decompensation or failure.[118] It is hypothesised that if the volume load of pregnancy is responsible for some of the cardiovascular events in pregnancy, BNP could play a role in predicting them.[120] Studies on BNP in pregnancy have shown conflicting results[122-124] and reference values for pregnancy are yet to be defined.[125] A recent study by Malhamé *et al.*[126] showed that despite higher baseline levels of BNP in pregnancy, optimal cut-off

values are similar to those used in the non-pregnant population. The authors suggest that BNP is useful to be used in pregnancy or postpartum period and that the optimal cut-off for diagnosing HF in pregnancy is <111 pg/mL. In many studies, BNP was measured to assess the strain on the LV secondary to HTN or PE.[127, 128] I have performed a review of BNP levels in pregnancy and included studies reporting data for healthy controls. Table 1.1 below summarises the results of the main studies performed in pregnancy/postpartum.

Table 1.1 – *Summary of studies of B-type natriuretic peptide (BNP) in pregnancy. CHT (Chronic Hypertension), CKD (Chronic Kidney Disease), GA (Gestational Age), PE (Preeclampsia), HTN (Hypertension).*

Study	Participants	Comment	Result
Conti-Ramsden *et al.*[127] 2019	21 controls 19 with PE 20 with CHT or CKD 19 with superimposed PE	Samples obtained in 3rd trimester for controls and at the time of presentation for other groups (GA: 26+6-37+6 weeks). BNP concentrations raised in PE compared to controls.	**BNP (ng/mL): Geometric Mean (95% CI)** 31.38 (95% CI 20.18-50.22) – PE 11.3 (95% CI 7.34-17.51) – controls
Mayama *et al.*[125] 2017	773 healthy pregnant women 58 non-pregnant	BNP levels markedly elevated in pregnancy and postpartum. Early elevation of BNP postpartum suggests that the abrupt postpartum changes have a higher impact than the gradual changes over the course of pregnancy.	**BNP (pg/mL): Mean (95% CI)** Non-pregnant 11.8 (95% CI 0-27.5) 35-37 weeks of gestation 17.9 (95% CI 0-44.7) Early postpartum (4 days postpartum) 42.5 (95% CI 0-112.6) 1 month postpartum 16.1 (95% CI 0-43.9)
Burlingame *et al.*[129] 2017	102 healthy pregnant women	First study to report longitudinal BNP/NT pro-BNP samples with echocardiographic data (6 time points). Isolated increase in BNP within 48 hours postpartum (physiological) with return to baseline within 6-12 weeks postpartum.	**Time point** / **BNP (pg/mL) Median (IQR)** / **NT Pro-BNP (pg/mL) Median (IQR)** 18-24 weeks: 26.3 (21.8-42.3) / 45.8 (32.4-83.4) 30-36 weeks: 24.7 (17.8-34.7) / 35.6 (22.4-56.0) Labour/Immediate postpartum: 36.4 (28.4-60.3) / 57.7 (34.9-93.3) 48h postpartum: 77 (31.2-108.6) / 107.5 (60.9-201.8) 6-12 weeks postpartum: 16.9 (14.3-33.2) / 49.1 (35.2-60.9) 6-12 months postpartum: 18.5 (15.8-25.7) / 40.75 (34.9-54.9)

Study	Participants	Comment	Result
Ozer *et al*.[130] 2017	29 healthy pregnant women	BNP levels increase during labour and in the postpartum period.	**NT pro-BNP (pmol/L): Mean ± SD** Active labour 5.19 ± 3.01 15 min postpartum 6.14 ± 3.48 3h postpartum 6.64 ± 4.26
Junus *et al*.[131] 2014	Women with early- and late-onset PE with matched controls GA: 25-31 weeks 22 controls, 18 PE GA: 35-40 weeks 14 controls, 20 PE	Levels of BNP higher in PE than in normal pregnancy. BNP levels higher in early-onset PE (not significant).	**BNP (pg/mL): Mean (IQR)** 25-31 weeks of gestation 48 (10-170) – controls 365 (14-9815) – PE 35-40 weeks of gestation 41 (14-172) – controls 176 (33-2547) – PE
Yurteri-Kaplan *et al*.[128] 2012	104 low-risk pregnant women (GA: 37-41 weeks)	BNP values at term in low-risk pregnancy are similar to those of non-pregnant controls.	**BNP (pg/mL)** Median: 20 (IQR 5-70) Mean ± SD: 46 ± 6
Hameed *et al*.[132] 2009	29 healthy pregnant women 25 non-pregnant	BNP in pregnancy double the non-pregnant controls. No change throughout pregnancy/ postpartum.	**BNP (pg/mL): Median (IQR)** Pregnant *vs* Non-pregnant 19 (0-143) vs 10 (10-37) Pregnant: 1st T: 20 (10-115) 2nd T: 18 (10-112) 3rd T: 26 (10-143) Postpartum: 18 (10-62)

Study	Participants	Comment	Result
Hamad *et al.*[124] 2009	35 with PE (Mean GA: 35 weeks) 30 healthy controls (Mean GA: 33 weeks)	Two time points: time of diagnosis of PE and 3-6 months postpartum. PE is associated with higher levels of BNP and these persist postpartum.	**BNP (pg/mL): Mean ± SD** Time of diagnosis: 46 ± 6 – controls 477 ± 152 – PE 3-6 months postpartum 61 ± 6 – controls 76 ± 16 – PE
Tihtonen *et al.*[133] 2007	19 with PE 15 with chronic HTN 26 healthy controls	Single assessment in 3rd trimester (GA: 27-39 weeks). Higher levels of NT pro-BNP in PE compared to chronic HTN or healthy subjects. In normal pregnancy, 31% had high levels of NT pro-BNP (>86pg/mL) but most within normal range.	**NT pro-BNP (pmol/L): Median (range)** 59 (40-130) – controls 98 (52-184) – chronic HTN 190 (87-1945) – PE
Resnik *et al.*[134] 2005	24 with severe PE 9 with mild PE 25 healthy controls	Four time points: 1st trimester, 2nd trimester, 3rd trimester and term. In normal pregnancy, BNP levels remain stable <20pg/mL suggesting that increased load in late pregnancy is handled without increase in left ventricular pressures. Severe PE is associated with higher BNP values compared to mild PE or healthy controls.	**BNP (pg/mL): Median (controls at 4 time points)** 1st T: 18.4 2nd T: 17.9 3rd T: 15.5 Term: 17.8 **BNP (pg/mL): Median (controls, mild PE, severe PE)** 17.8, 21.1, 101
Kale *et al.*[135] 2005	40 with PE 40 healthy controls	Higher levels of NT pro-BNP in PE compared to healthy subjects.	**NT pro-BNP (pg/mL): Mean ± SD** 74 ± 16.82 – controls 430 ± 28.91 – PE

Study	Participants	Comment	Result
Borghi *et al.*[122] 2000	40 with PE (Mean GA 28+3 weeks) 35 pregnant controls (Mean GA 30+6 weeks) 10 non-pregnant	Plasma levels significantly increased in the pregnant population. Higher levels in PE.	**BNP (pg/mL): Mean ± SD** 37.1 ± 10 – PE 21.5 ± 8.0 – pregnant 15.2 ± 5.0 – non-pregnant
Okuno *et al.*[136] 1999	35 with PE 35 normotensive controls	Two time points: time of diagnosis and 1 month postpartum. BNP remained within normal range (<20pg/ml) in the control group in pregnancy and postpartum. BNP was 7x higher in the PE group in pregnancy but returned to normal within 1 month postpartum.	**BNP (pg/mL): Mean ± SD** Pregnancy 48.75 ± 91.61 – PE 7.09 ± 8.36 – controls Postpartum 6.69 ± 4.99 – PE 8.44 ± 9.60 – controls
Itoh *et al.*[137] 1997	10 non-pregnant 30 pregnant (Mean GA 33+1 weeks) 28 with gestational hypertension (Mean GA 34+5)	Higher levels in pregnancy compared to non-pregnant subjects (not statistically significant). In pregnant women with gestational hypertension, BNP levels were significantly higher than in healthy pregnancy.	**BNP (pM): Mean ± SD** 1.8 ± 0.4 – non-pregnant 3.0 ± 0.5 – pregnant 15.1 ± 3.2 – gestational hypertension
Yoshimura *et al.*[138] 1994	Not available (abstract only)	Time points: pregnancy, 30 min after separation placenta and 5-72h postpartum Levels significantly higher at term compared to 1st trimester.	**BNP (pg/mL): mean ± SD** 1st T: 25 ± 2 Term: 49 ± 9 30min after placenta separation: 80 ± 25 5-72h postpartum: 116 ± 17

The role of BNP in CHD and pregnancy is not as well defined as it is in acquired heart disease.[116] In asymptomatic, non-pregnant patients with simple CHD (unrepaired atrial septal defect (ASD) and ventricular septal defect (VSD)), BNP and NT pro-BNP levels are slightly raised when compared to controls, which could be related to shunt severity and pulmonary artery pressures (data from systematic review).[117] In complex CHD, BNP levels are also increased when compared to healthy non-pregnant controls, even in asymptomatic patients, and correlated with end-diastolic RV dimensions and pulmonary regurgitation in patients with ToF and with RV function in patients with systemic RV (data from systematic review).[116]

Giannakoulas *et al.*[139] included 49 patients (of which 53% were women) with a variety of stable CHD (ToF, univentricular circulation, systemic RV, amongst other conditions) and concluded that BNP was a strong predictor of mortality (cut-off value of 78pg/mL). Bolger *et al.*[140] studied 53 patients with CHD (majority females) and 15 healthy controls and showed that BNP levels were seven times higher in CHD compared with healthy controls and that the levels correlated with NYHA functional class and systemic ventricular function. Hopkins *et al.*[141] showed a 12-fold increase in BNP levels in ten patients with cyanotic heart disease compared to controls.

An early study looking into BNP levels in pregnancy in women with cardiac disease[118] included 78 pregnant women (66 with cardiac disease [of these, 74% had CHD] and 12 controls) and showed that in the healthy controls BNP was low and did not increase during pregnancy (peak median: 35pg/mL, IQR 21-43pg/mL), whereas in women with cardiac disease levels were higher (peak median: 79pg/mL, IQR 51-152pg/mL) and highest in women with subaortic ventricular dysfunction (peak median: 137pg/mL, IQR 55-261pg/mL). No other differences in BNP levels were found in the remainder of the cardiac disease groups.[118] The same study reports cardiac adverse events in 13% of the women with cardiac disease, who had high BNP levels (peak median: 354pg/mL, IQR 299-805pg/mL); fetal or neonatal adverse events were not associated with BNP levels.[118]

As part of the ZAHARA II trial, investigators studied 213 pregnancies in 203 women with CHD. NT pro-BNP levels were available for 169 patients. Cardiovascular adverse events occurred in 10.3% of pregnancies and NT pro-BNP levels >128pg/mL at 20 weeks of gestation were predictive of events in later pregnancy with an odds ratio (OR) of 10.6 (95% CI [1.13-98.7], p = 0.039; negative predictive value (NPV) = 100%).[120] This is the only study that shows BNP has a predictive value for cardiac adverse events.

Further research with longitudinal assessment of BNP levels throughout pregnancy in low-risk women as well as in women with CHD is needed, specifically to investigate how BNP levels change throughout the different stages of normal pregnancy and in pregnant women with CHD.

1.8. Cytokines and cellular adhesion molecules in low-risk pregnancy and in pregnancy complicated by congenital heart disease

Pregnancy is a unique immunological state where the mother's immune system needs to be modified to tolerate an allogenic fetus but, at the same time, be able to protect the mother and the fetus from infection.[142] There is a skew towards a more prominent T helper cells (Th)2 response (interleukin (IL)-10) and a decrease in the Th1 response (IL-2, interferon (IFN)γ).[142]

Human trophoblast cells are invasive and are able to penetrate into myometrium, unless this process is halted.[143] In normal pregnancy, anti-inflammatory cytokines can promote adequate placentation, modulate trophoblastic invasion/differentiation, induce placental proliferation and antagonise proinflammatory cytokines.[142] Cytokine production can affect placentation by interfering with trophoblastic activity.[144] IL-6 has been associated with reduced fertility and a 50% reduction in viable implantation sites in mice; IL-11 has been associated with implantation failure and defective decidualisation; IL-1 is associated with normal implantation but affects offspring size in mice.[144] In the first trimester there seems to be an increase in growth factors (growth-regulated oncogene (GRO)α) and a decrease in inflammatory cytokines (IL-6), compared to non-pregnant controls.[145] In the third

trimester of pregnancy, there is an increase in proinflammatory cytokines such as IL-1β, IL-6 and IL-12[145]; this may relate to ripening of the cervix and to the onset of labour.[146]

Patients with CHD usually undergo surgery early in their lives but, despite the repair of their condition, some will still have residual haemodynamic abnormalities.[147] This can cause disruption of normal oxygenation and smooth blood flow in the pulmonary vessels.[148] Patients with CHD show similar patterns of neurohormonal activation as seen in patients with heart failure but data on cytokines is limited.[149] It is suggested that hypoxia can stimulate immune activation[149] and this immune activation can be responsible for late complications in CHD. Patients with coarctation of the aorta, in particular, are known to have higher circulating levels of proinflammatory cytokines and adhesion molecules.[150] Regions of disturbed oscillatory flow induce upregulation of proinflammatory adhesion molecules.[151] The mechanism by which endothelial cells respond to fluid flow and fluid sheer stress is likely to be the frictional force that occurs in the cell surface.[152]

Nassef *et al.*[149] showed significantly higher levels of inflammatory cytokines (IL-6 and tumour necrosis factor (TNF)α) in patients with CHD (even higher levels in those with cyanotic CHD) and these levels correlated with disease severity and the degree of remodelling. Similar observations were made by Sharma *et al.*[153] TNFα has pleiotropic effects on inflammation, immunity, cell proliferation, differentiation and apoptosis and its synthesis comes from T-cells, granulocytes, macrophages, mast cells, natural killer cells, smooth muscle cells, keratinocytes, non-immune endothelial cells and fibroblasts.[148] The expression of TNFα correlates with the degree of cardiac dysfunction and, in patients with heart failure, antagonising the actions of TNFα improves cardiac function; the stimulus for its production is, however, unknown.[154] IL-6 is a pleiotropic factor that plays a role in the acute phase response to infection but also has both pro- and anti-inflammatory properties which can lead to chronic inflammation, endothelial cell dysfunction and fibrinogenesis, and affect autoimmunity.[148]

Zorzanelli *et al.*[147] showed a correlation between clinical parameters and cytokine profile in young adults undergoing surgical repair of CHD (shunt lesions). GROα and IL-17E correlated with patient

age and pulmonary hypertension severity.[147] GROα plays an important role in angiogenesis and the authors observed high levels in early life followed by an exponential decrease with age; a decrease in concentration of GROα contributes to impaired vascular growth in patients with cardiac shunts.[147]

Wang *et al.*[148] measured the expression of ghrelin, TNFα and IL-6 in 63 children with CHD (cyanotic and acyanotic) against 28 controls. The expression of these were statistically significantly higher in the CHD group (both cyanotic and acyanotic) compared with the controls. Patients with higher ghrelin levels had worse heart failure and disorders of weight gain.[148] TNFα (which contributes to progression of CHD) was increased in CHD and IL-6 was also raised, contributing to the increased risk of cardiovascular disease as it plays a role in atherothrombosis.[148] Yilmaz *et al.*[155] found similar results. Ghrelin is secreted by X- and A-cells found in the stomach and drives the release of growth hormone which regulates growth, weight, nutrition intake and energy intake/output.[148] Ghrelin also affects compensatory myocardial hypertrophy via vasodilatory activity and sympathetic nerve stimulation.[148]

In patients with CHD, it seems that in addition to TNFα and IL-6, IL-10, IL-12 and IL-18 play important roles on the cachectic process.[156] Noori *et al.*[156] showed increased levels of TNFα, IL-6 and IL-18 in patients with CHD when compared to controls, but no difference with regards to IL-10 or IL-12. IL-10 is a pleiotropic anti-inflammatory cytokine whose main function is to supress proinflammatory cytokines (IL-1, IL-6, IL-12 and TNFα).[142] IL-12 is produced by the antigen presenting cells and regulates the immune response of natural-killer cells.[156]

Cheung *et al.*[157] measured the expression of IL-6, IL-8, IL-10 and TNFα in patients undergoing surgery with one group having ischaemic preconditioning – there were no differences in the levels of cytokines between the two groups. Madhok *et al.*[158] showed significant elevation of IL-6, IL-8 and IL-10 levels post corrective surgery with a return to pre-operative levels soon after; they also showed that patients with higher levels of IL-8 had greater need for inotropic support, which suggests that the inflammatory response from IL-8 has a direct effect on oxygen delivery to the tissues.[158]

Moutafi *et al.*[159] studied patients with repaired CoA and concluded that levels of proinflammatory cytokines were raised, which is consistent with an early inflammatory activation process, but levels of intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) were not different between the study population and controls.[159] Brili *et al.*[150] studied the effect of atorvastatin on endothelial function and showed a significant reduction in the circulating levels of IL-1 and VCAM with treatment. IL-1 is a proinflammatory cytokine affecting multiple tissues and IL-1β, specifically, has been shown to inhibit human endometrial stroma decidualisation, and also to be pro-atherogenic.[144] IL-1β stimulates the production of ICAM and VCAM from endothelial cells lining the walls of the coronary arteries.[160]

The role of inflammation in the pathophysiology of pulmonary hypertension has been studied extensively[161] and IL-6 seems to play a role in pulmonary artery remodelling.[147, 161] Administration of IL-6 to rats induces pulmonary hypertension and increases hypoxia-induced pulmonary hypertension.[161] Duncan *et al.*[161] measured circulating cytokines in a population of paediatric patients with pulmonary hypertension and showed that IL-6, IL-10 and monocyte chemoattractant protein (MCP)-1 predicted adverse events better than haemodynamic parameters alone. The authors also showed that IL-6 and vascular endothelial growth factor (VEGF) were specifically associated with adverse events, while IL-6 and endothelial growth factor (EGF) were correlated with pulmonary artery pressures.[161]

IFNγ is mainly produced by natural-killer cells and it is proinflammatory, playing an important role in autoimmune and chronic inflammatory diseases.[162] Levels of IFNγ, TNFα, IL-1, IL-6, IL-8, IL-10 and IL-12 are raised in patients with pulmonary hypertension and control cell proliferation, migration and differentiation.[163]

Current protein arrays allow for multiplex analysis of samples with high sensitivity, specificity and precision using small sample size volumes.[161] These arrays could prove to be invaluable in investigating expression of cytokines in patients with CHD during pregnancy as, to date, and to my knowledge, no studies have been performed.

1.9. The effect of beta-blockers on cardiac adaptation to pregnancy, response to exercise, fetal growth, B-type natriuretic peptide concentrations and on cytokine and cellular adhesion molecules concentrations

Beta-blockers are often used in patients with CHD and these can have an effect on the cardiac adaptation to pregnancy, response to exercise, fetal growth, circulating levels of BNP and circulating levels of cytokines and CAMs.[55, 164-166]

The neurohormonal activation in patients with CHD which occurs secondary to a reduction in blood flow to the kidneys and skeletal muscle, result in limited exercise capacity and worsening of the cardiac function in the long term.[164] Beta-blockers (as well as other agents such as angiotensin-converting enzyme inhibitors) are used in the treatment of heart failure with the aim to reverse such changes.[139] Patients on beta-blocker therapy have lower resting and peak exertional heart rates and therefore careful interpretation of exercise testing results is needed.[167] Diller *et al.*[168] showed that chronotropic incompetence is present in up to 62% of patients with adult CHD and predicted an increased mortality risk. However, beta-blockers were not associated with survival in this study.[168] Khan *et al.*[169], on the other hand, demonstrated that chronotropic response was independently associated with death in those treated with metoprolol or atenolol. Chronotropic incompetence is also associated with adverse pregnancy outcomes in pregnant women with CHD such as PE, preterm delivery and SGA.[170]

The use of beta-blockers correlates negatively with birthweight.[55] The ROPAC database[171] showed significantly lower birth weights in those taking beta-blockers (lower by an average of 270g) although it is yet to be established if the cause for the lower birth weight is related to the use of beta-blockers or to the CHD lesion itself requiring the use of beta-blockers. Cauldwell *et al.*[57] suggested that the use of beta-blockers is associated with lower birth weight even after correcting for individual CHD lesions.

Silverman *et al.*[165] showed that beta-blocker use was associated with higher levels of NT-proBNP in patients with heart failure whose ejection fraction was ≥50%. Khand *et al.*[172] showed that after initiating treatment with beta-blockers in patients with heart failure, BNP levels did not decrease as expected,

despite improvement in other haemodynamic parameters. Davis *et al.*[173] suggest that BNP levels increase with beta-blockade in heart failure due to increased secretion of BNP secondary to longer cardiac filling times and its negative inotropic effect. The authors also suggest reduced renal clearance of BNP following beta-blockade although they could not explain the mechanism behind this. The increased BNP levels were not related to deterioration in the clinical status.[173]

Beta-blockers can also affect circulating levels of cytokines, as demonstrated by Ohtsuka *et al.*[166] with significant reductions in the circulating levels of TNFα and IL-10 after initiating beta-blocker therapy. Steptoe *et al.*[174] also demonstrated a reduced inflammatory response with beta-blockade, with reduced levels of IL-6 and IL-1 (a proinflammatory cytokine) in response to mental stress.

1.10. Hypotheses and Aims

Hypothesis 1: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably measure CO at any trimester.*

Aim: To investigate CO measured by ICG and compare this to CO measured by conventional TTE at each of the three trimesters in low-risk pregnancy: 10-14 weeks of gestation (Visit A), 18-22 weeks of gestation (Visit B) and 30-34 weeks of gestation (Visit C).

Hypothesis 2: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*

Aim: To obtain three CO measurements at each visit (A, B and C): rest (T1), immediately after exercise (T2) and after recovery (T3), and directly compare the CO measures obtained from TTE with the average CO measurement obtained from ICG during the time of VTI acquisition.

Hypothesis 3: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably measure CO at any trimester.*

Aim: To investigate CO measured by ICG and compare this to CO measured by conventional TTE at each of the three trimesters in pregnancy complicated by CHD: 10-14 weeks of gestation (Visit A), 18-22 weeks of gestation (Visit B) and 30-34 weeks of gestation (Visit C).

Hypothesis 4: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*

Aim: To obtain three measurements at each visit (A, B and C): rest (T1), immediately after exercise (T2) and after recovery (T3) and directly compare the CO measures obtained from TTE with the average CO measurement obtained from ICG during the time of VTI acquisition.

Hypothesis 5: *There is a second trimester drop in BP in normal low-risk pregnancy and this is not demonstrated in pregnant women with CHD.*

Aim: To investigate longitudinally the maternal BP in low-risk pregnant women and compare this to the BP of pregnant women with CHD.

Hypothesis 6: *In pregnancy, there is a progressive increase in the HR with advancing gestation.*

Aim: To investigate longitudinally the maternal HR in low-risk pregnant women and compare this to the HR of pregnant women with CHD.

Hypothesis 7: *In pregnancy, there is a decrease in peripheral oxygen saturations (SpO_2) with advancing gestation.*

Aim: To investigate longitudinally the maternal SpO_2 in low-risk pregnant women and compare this to the SpO_2 of pregnant women with CHD.

Hypothesis 8: *In pregnancy, there is a progressive increase in CO until 34 weeks of gestation.*

Aim: To investigate longitudinally the maternal haemodynamics (SV and CO) as assessed by measurement of cardiac output with ICG and TTE of low-risk women.

Hypothesis 9: *In pregnancies complicated by heart disease, there is a suboptimal increase in CO and SV and/or a suboptimal decrease in TVR.*

Aim: To investigate longitudinally the maternal haemodynamics as assessed by measurement of CO and SV with ICG and TTE and investigate differences in BP between pregnant women with CHD and low-risk pregnant women.

Hypothesis 10: *Fetal growth is related to maternal CO and impaired cardiovascular adaptation to pregnancy can lead to defective placentation and, therefore, FGR.*

Aim: To obtain a measure of EFW (as defined by the study protocol), BW and BW centiles, and correlate these with maternal haemodynamics: (a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 11: *Increased UtA PI in the third trimester is associated with lower EFW and, consequently, with lower BWs and associated BW centiles.*

Aim: To obtain a measure of UtAD PI in the third trimester (between 30-34 weeks of gestation) and correlate this with EFW in the third trimester (between 30-34 weeks of gestation), BW and BW centiles.

Hypothesis 12: *Defective placentation can be secondary to cardiovascular maladaptation in pregnancy and there is an association between UtAD PI, CO and MAP.*

Aim: To correlate UtAD indices with: (a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 13: *Increased UAD PI is associated with lower EFW, BW and BW centiles.*

Aim: To obtain a measure of UAD in the third trimester (between 30-34 weeks of gestation) and correlate these with EFW, BW and BW centiles, as well as with the maternal haemodynamics: (a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 14: *CPR in the third trimester is a surrogate marker for placental insufficiency and a low CPR is associated with high UtAD PI and lower EFW, BW and BW centiles.*

Aim: To correlate CPR with EFW, BW and BW centiles and with the maternal haemodynamics: a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 15: *Pregnancy is associated with fluid overload which stimulates the release of BNP. However, the levels of BNP do not change significantly across the three trimesters of pregnancy.*

Aim: To obtain a measure of BNP concentration at each of the three trimesters of pregnancy (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation) and establish if there is a difference between the different trimesters of pregnancy in low-risk pregnancy and in pregnant women with CHD.

Hypothesis 16: *Pregnancy is associated with fluid overload which causes release of BNP and suboptimal increase in CO/SV and/or suboptimal decrease of TVR in CHD is associated with higher levels of BNP.*

Aim: To obtain a measure of BNP concentration in low-risk pregnancy and in pregnant women with CHD and establish if there is a difference between the two groups in each of the studied trimesters. To correlate the haemodynamic parameters with levels of maternal BNP, measured longitudinally, in low-risk women and in women with CHD.

Hypothesis 17: *There is a change in the women's immune profile throughout pregnancy.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, at the three trimesters of pregnancy (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation).

Hypothesis 18: *Higher circulating levels of proinflammatory cytokines and cellular adhesion molecules may cause inadequate spiral artery remodelling, placental ischaemia and/or insufficiency and, therefore, causes FGR and preterm birth.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, and correlate circulating levels with BW, BW centiles and gestational age.

Hypothesis 19: *In pregnancy complicated by CHD, cardiac function is related to the circulating levels of cytokines and cellular adhesion molecules.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, and correlate circulating levels with CO at rest and with ΔCO.

Chapter 2.
Materials and Methods

Chapter 2. Materials and Methods

2.1. Study documentation and research approvals

I created all the study documents necessary to obtain the required approvals for this study. These included:

- Research protocol
- Study logo
- Participant information sheet (PIS): women with congenital heart disease (Appendix 7), women without heart disease – low-risk pregnancy (Appendix 8), women with SGA babies (Appendix 10) and for participation in Part 2 (Appendix 11)
- Informed consent forms (ICF): participation in Part 1 (Appendix 9) and Part 2 (Appendix 12)
- Invitation letter (Appendix 13)
- Advertisement recruitment posters: women with low-risk pregnancy and women with pre-existing heart disease in pregnancy (Appendix 14) and women with SGA babies (Appendix 15)
- Data collection sheet (Appendix 16)

For the study protocol, I performed a review of the literature on haemodynamic changes in normal (low-risk) pregnancy, as well as in pregnancy complicated by heart disease; methods used in assessing these dynamic changes in pregnancy, with a focus on ICG (PhysioFlow®) and its relevance for use in pregnancy (particularly in women with cardiac disease). The study protocol was reviewed by *Professor Mark R Johnson*, *Dr Sonya Babu-Narayan* and *Professor Philip Steer* (my supervisors). According to the study protocol, three groups of women were to be included in the study: women with low-risk pregnancy, women with pre-existing heart disease and women with SGA babies. Due to time constraints, only women in the low-risk and pre-existing heart disease groups were recruited.

In addition to creating the study documents listed above, I initially completed application forms to obtain sponsorship from CW. I was then advised to apply for sponsorship with Imperial College London instead, for which I had to complete new application forms. The Integrated Research Application

System (IRAS), Schedule of Events and Statement of Activities forms were completed to obtain favourable opinion from the Research Ethics Committee (REC) and the Health Research Authority (HRA), and support from the National Institute for Health Research Clinical Research Network (NIHR CRN). The approval documents can be found in the appendices (Appendix 1 for sponsorship letter, Appendix 2 for HRA approval letter, Appendix 3 for REC favourable opinion letter, Appendix 4 for confirmation of capacity and capability at CW, Appendix 6 for NIHR CRN support letter). Funding was obtained from *Borne Charity* (Appendix 5).

A summary of the applications and approval dates are listed in Table 2.1 below.

Table 2.1 *– Summary of research approvals for the HDiP – MCAFO Study. CPMS (Central Portfolio Management System), CW (Chelsea and Westminster Hospital NHS Trust Foundation), HRA (Health Research Authority), IRAS (Integrated Research Application System), NIHR CRN (National Institute for Health Research Clinical Research Network), REC (Research Ethics Committee)*

Action	Entity/ID	Date of Submission/ Approval
Sponsorship Application	CW	Submitted: 15 February 2017 Rejected: 07 March 2017
Sponsorship Application	Imperial College London (17IC3946)	Submitted: 03 April 2017 Granted: 04 May 2017
IRAS Application	IRAS ID: 222431	Submitted: 25 May 2017
NIHR CRN Portfolio Application	CPMS ID: 35127	Submitted: 05 June 2017 Granted: 04 October 2017
REC Application	REC Ref: 17/LO/0970	Granted: 29 June 2017
HRA Application	-	Granted: 14 July 2017
Capacity and Capability confirmation form CW	C&W17/098	Granted: 03 August 2017

2.2. Set-up of clinical room for study procedures

A study specific room was set up at CW with an echocardiography machine, an ergometer cycle and a Physioflow® Enduro™ (*Manatec Biomedical, France*) device to perform the study visits and procedures (Figure 2.1).



Figure 2.1 *– Clinical room used for the exercise test for the HDiP – MCAFO Study (left) and PhysioFlow® Enduro™ device and PF-50™ AgCl electrodes (right).*

2.2.1. *Echocardiography machine*

The iE33 xMatrix – Diamond Select Ultrasound System (*Philips Ultrasound, USA*) was used throughout the study. This machine was equipped with a S5-1 transducer (frequency 1-5MHz) and a 3-lead ECG Grabber AAMI cable set (*Philips Ultrasound, USA*).

I arranged for the machine to be serviced and negotiated a service contract covering the study period. Installation of internet ports to allow connection of the echocardiography machine and uploading of images to the Trust's network was also arranged by me.

2.2.2. *Ergometer cycle*

A Kettler E3 Ergometer Cycle (*Kettler GmbH, Germany*) was used throughout the study. This cycle had an operator regulated resistance of 25-4000 watts, adjustable saddle (horizontal and vertical

adjustments), gravity pedals and a maximum user weight of 150kg. I purchased and assembled the ergometer cycle and arranged servicing with Clinical Engineering.

2.2.3. *PhysioFlow® Enduro™*

The Physioflow® Enduro™ (*Manatec Biomedical, France*) device has CE and FDA approval. I was trained on the equipment and its software by a company's representative. A few 'dry runs' were performed to test the equipment.

2.3. Participant recruitment

This was a prospective study conducted at CW from August 2017 to May 2019. Potentially eligible low-risk participants were screened from CW's pregnancy booking clinic lists. CW runs 13 booking clinics per week, with an average of 200 bookings per week. Potentially eligible participants with pre-existing heart disease were screened from the Joint Cardiac Obstetric Medicine Clinic at CW. This clinic runs on a weekly basis and an average of 12 patients are seen at the clinic per week.

Low-risk pregnant women were approached by me or a member of the research team and asked if they had any interest in participating. Participants with pre-existing heart disease were approached solely by me. If they were interested, participants were given the relevant PIS and a short explanation about the study. They were then allowed sufficient time to consider participation (which was at the participant's discretion). If they agreed to participate, written informed consent was obtained by signing the ICF. A copy of the ICF was given to each participant, another copy was filed in the site file and the original filed in the medical notes. Women who needed extra time to consider participation were given my contact details (email and phone number) and asked to contact me whenever ready.

After signing the ICF, participants were given a consecutive study number coded as LR (low-risk) or CHD (congenital heart disease) followed by a number (LR001 – LRXXX and CHD001 – CHDXXX) and the first visit was scheduled. The first participant was recruited to the study on 23rd Aug 2017.

Participants were screened for inclusion in the trial according to the following eligibility criteria:

Inclusion criteria

- Able to provide informed consent
- Women of reproductive age: 18 to 60 years-old
- No history or symptoms of congenital or acquired heart disease and no known chronic medical co-morbidity for the low-risk pregnancy group
- Presence of any structural congenital heart disease for the cardiac disease group
- Carrying a singleton pregnancy
- Able to attend the visit at the gestations specific the study windows (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation)

Exclusion criteria

- Unable to read and speak English to a fluency level adequate for the full comprehension of procedures required in participation and consent
- Currently participating in a clinical trial with an investigational drug
- Any condition that, in the investigator's opinion, compromised the participant's ability to meet protocol requirements

2.4. Study visits

Table 2.2 below illustrates the study visits.

Table 2.2 *– Time point of visits for the HDiP – MCAFO study.*

Planned Visits	Date
A (10-14 weeks of gestation)	Same day as booking appointment, *booking scan* or at participant's convenience
B (18-22 weeks of gestation)	Same day as *anomaly scan* or at participant's convenience
C (30-34 weeks of gestation)	Same day as *growth scan* (extra scan arranged by research team) or at participant's convenience

For both the low-risk pregnancy group the CHD groups, the following data were collected at each visit, after confirming the informed consent form had been signed and remained valid:

Study visit A

After the booking scan (if on the same day of the study visit), participant's eligibility was checked against the criteria set in the protocol. Study data were recorded on a data collection sheet (Appendix 16), which included the participant's study number, date of the visit, visit time point, estimated date of delivery (EDD, which was based on crown-rump length (CRL) obtained during the first trimester ultrasound), gestational age (GA) at time of the visit (which was also based on CRL obtained from the first trimester ultrasound), height and weight at booking and arm circumference. Details of age, race[1], smoking status, use of medication, gravidity and parity and, in the case of participants with CHD, the type of CHD lesion and detailed description of the lesion were also collected in the same visit, BMI and target HR ([220-age] * 0.7) calculated. After collecting these data, a blood sample was taken and the time of collection recorded in the data collection sheet – details of the blood sample collection procedure are described in section 2.7. If the participant did not provide consent for blood sampling, this was documented. Following the blood sample collection, haemodynamic studies, as described below in section 2.5, were started.

Study visit B

After the *anomaly scan* (if on the same day of the study visit), and similarly to study visit A, participant's study number, date of the visit, visit time point, EDD, GA at time of the visit, height and weight at booking and arm circumference were recorded on the data collection sheet. After collecting this information, a blood sample was taken (as per section 2.7), followed by haemodynamic studies (as described in section 2.5).

[1] **Racial groupings**: Asian (East Asian and South Asian), Black (Afro-Caribbean, Central and South African, African American), Middle-Eastern (Iranian, Iraqi, Lebanese and Turkish), White (Mediterranean, North African and White European) and Mixed (any combination of the other groups)

Study visit C

At study visit C, for women in the low-risk group, an extra *growth scan* and Doppler studies were arranged between 30-34 weeks of gestation. For women in the CHD group, a visit was arranged at the same time interval. However, an extra scan was not arranged – study data available is from *growth scans* performed as part of routine standard of care.

After the *growth scan*, and similarly to study visits A and B, participant's study number, date of the visit, visit time point, EDD, GA at time of the visit, height and weight at booking and arm circumference were recorded on the data collection sheet. After collecting this information, a blood sample was taken (as per section 2.7), followed by haemodynamic studies (as described in section 2.5).

2.5. Haemodynamic studies

Submaximal aerobic exercise in pregnancy is considered safe for both mother and the fetus, in the absence of any contraindications.[175] There is limited evidence on the effects of vigorous exercise in pregnancy but Beetham *et al.*[176] reported no adverse events. Maximal heart rate is one of the commonest values used in medicine and physiology as a criterion for achieving maximal exertion, and can be estimated by the formula 220-age.[177] Seventy percent of the predicted maximal heart rate is considered by the *American College of Sports Medicine* as vigorous and high-intensity exercise, hence this was selected as the target heart rate to be achieved by women in the study. The Canadian Guideline for physical activity throughout pregnancy[178] also defines vigorous exercise as 60-80% of the heart rate reserve. Table 2.3 summarises all the procedures performed at each visit during the haemodynamic studies. A detailed description will follow below.

Table 2.3 *– Summary of haemodynamic studies' procedures at each of the trimesters (A, B and C). BP (blood pressure; mm of mercury, mmHg), HR (heart rate; beats per minute, bpm), ICG (impedance cardiography; PhysioFlow® Enduro™), SpO_2 (peripheral oxygen saturations; %), TTE (transthoracic echocardiography; T1 – time point one (readings taken at rest); T2 – time point two (reading taken immediately after exercise and as soon as participant was able to transfer from ergometer cycle to couch); T3 – time point three (readings taken once HR decreased to at least ±20% of baseline readings, after recovery).*

Time	Procedures
0 min	Arrive and Rest
5 min (T1)	ICG Attached Baseline readings: -HR -BP -SpO_2 -ICG -TTE
After baseline readings	Exercise (ergometer cycle) – to increase HR by 70% of predicted by age (minimum) and keep above 70% of predicted for 1 minute
Immediately after exercise (T2)	Readings: -HR -BP -SpO_2 -ICG -TTE
Recovery (T3)	Readings: -HR -BP -SpO_2 -ICG -TTE

After arrival into the study room, participants were asked to change into a hospital gown and rest for at least five minutes. Following the rest period, skin preparation was performed as per manufacturer's instructions[179]: the areas required for electrode placement were cleaned with an alcohol preparation pad (2% Chlorhexidine in 70% alcohol, Clinell®, *GAMA Healthcare Ltd., UK*) and dried with a paper towel; the same areas were then rubbed with skin preparation abrasive gel (Nuprep®, *Weaver and Company, USA*), using gauze, until the skin turned bright pink. Following confirmation that the electrodes were within the use by date, Physioflow® PF-50™ AgCl electrodes were connected to Physioflow® cables before placing them on the skin. The covered electrodes were peeled and applied

with the central gel pad touching the skin first (application lifting the sides of the electrodes); the electrodes were then flushed against the skin to make sure there were no air bubbles or wrinkles present.

Six Physioflow® PF-50™ AgCl electrodes were placed on the following areas of the body (Figure 2.2): Z1 (white) was placed on the left lateral triangle of the neck (vertical line running down from the earlobe), Z2 (blue) on the left lateral triangle of the neck below and slightly overlapping Z1 – participants were asked to look ahead and keep a neutral neck position whilst Z1 and Z2 were placed, and cables were attached to the participant's right shoulder using 3M white Micropore™ tape; ECGV1 (red) was placed mid sternum – the V1 cable was attached to the participant's abdomen using 3M white Micropore™ tape; ECGV6 (orange) was sited on the same position as V6 for 12-lead ECG (mid-axillary line, 5th intercostal space); Z3 (green) was positioned on the left paraspinal at the point of reflection of the xiphoid process on the spine and Z4 (black) on the left paraspinal below Z3 (or overlapping).

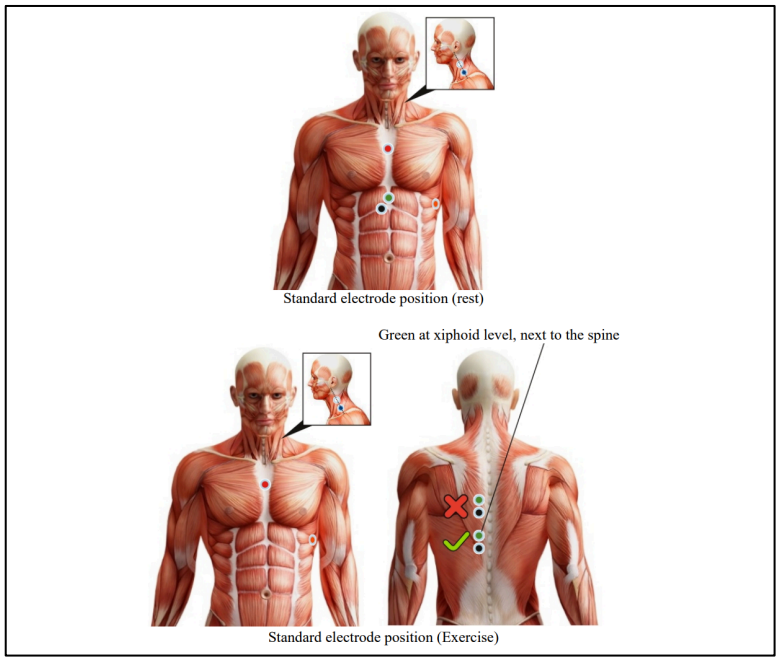


Figure 2.2 *– Sites for electrode placement as per PhysioFlow® manufacturer's instructions for rest and exercise testing. The locations for exercise testing were used. Image reproduced with permission of the rights holders, Manatec Biomedical © 2017 Manatec Biomedical.*

In participants with a pacemaker or an implantable cardioverter defibrillator, electrode placement was different, with Z1 and Z2 on the opposite side of the implant placement, ECGV1 on the opposite side as ECGV6, Z3 on the xiphoid process and Z4 lateral to Z3 on the right side (Figure 2.3).

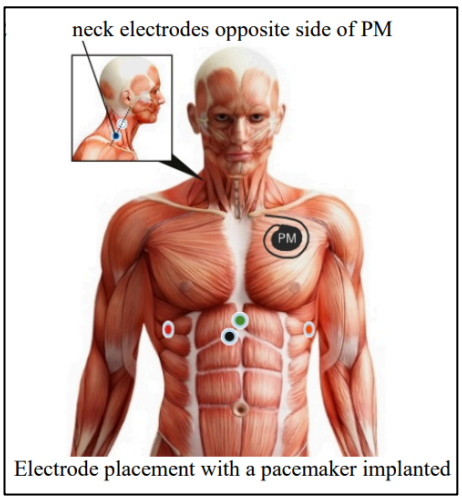


Figure 2.3 *– Electrode placement in participants with a pacemaker or implantable cardioverter defibrillator. Image reproduced with permission of the rights holders, Manatec Biomedical © 2017 Manatec Biomedical.*

Following confirmation that no cables were touching or running over any of the other electrodes, the participant was asked to stand up, rotate their neck and torso left and right to ensure mobility was maintained, and adjustments were made if needed. Batteries were inserted into the device and it was turned on. The participants were asked to lie down in left lateral decubitus with at least a 15-degree left lateral tilt to avoid aorto-caval compression, as per IWGMH guidance[25] and a 15-degree head elevation.

The mid-upper arm circumference of the right arm was measured using a measuring tape (midpoint between the olecranon process and the acromion with the arm on extension). A blood pressure cuff was placed on the right arm (if contra-indicated, the left arm was used instead). A standard blood pressure cuff was used for mid upper arm circumferences up to 32cm and a large cuff used for mid upper arm circumference between 32 and 42cm. Blood pressure was measured using an Omron® MIT Elite device (*Omron Healthcare, Japan*), an automated device using inflationary oscillometry which has been validated for use in pregnancy and in PE.[180, 181] This device has also achieved the highest possible grade (A/A), as assessed by the British Hypertension Society protocol.[181] The participant's blood pressure

was recorded on the data collection sheet and the device disconnected from the cuff; the cuff was left in situ for easy access for the subsequent measurements. The FPX50DL pulse oximeter (*Ana Wiz Ltd, UK*) was placed on the right index finger and the heart rate and peripheral oxygen saturations were recorded on the data collection sheet.

The Physioflow® software (version 2.7.3) was launched with a Bluetooth antenna (30m range) plugged into a USB port. Once the Bluetooth signal from the PhysioFlow® was identified, a new episode was created by pressing '*Create New Participant'* and the participant details entered: study number, study visit (A, B or C), date of birth, height and weight (at booking), and gender. Booking weight was used for all three visits as there is little increase in the chest dimensions with increased weight in pregnancy and, due to the unique pattern of weight gain, there is no need to correct for body surface area.[72] By indexing all measurements to pre-pregnancy body surface area, all measurements start at the same baseline.[25] CO is a highly variable parameter that changes in response to changes in SV and/or HR and also depends on body size, metabolic rate, advancing age and advancing gestational age.[25] All of these factors can impact on the correct interpretation of absolute CO values measured throughout pregnancy. Indexing the absolute CO values to either BSA, pre-pregnancy BSA or to specific pregnancy CO reference values for the studied device is advocated by some to overcome such limitations.[25] The limitation of indexing to maternal BSA is that it does not accurately reflect pregnancy specific body changes occurring secondary to the increase in the size of the uterus, amniotic fluid and maternal composition of fat and muscle. In addition to this, due to the increase in basal metabolic rate in pregnancy (by 1.5 fold), indexing to BSA will under correct CO. Indexing for prepregnancy BSA allows for all measurements to start at the same baseline but does not take into account changes occurring throughout pregnancy. The most accurate way to use indexing is to do so by using specific pregnancy CO values for the studied device. However, for many devices such "reference values" do not exist.[25] We opted to not use indexed CO values as, firstly, for the purpose of validation it was not important since we were calculating ΔCO; and secondly, we studied trends across trimesters and therefore each individual acted as their own control.

Following this, the '*Next*' button is pressed and the options '*Measurement averaging type in time (10 seconds)*', '*Measurement mode (exercise test)*' and '*Number of cycles for calibration set at 30 cycles*' were selected, as recommended by the manufacturer. Participants were asked to keep still, not to talk and avoid taking deep breaths during the calibration period. Once a signal of good quality (full green bars) was identified, the 'proceed to calibration' command was activated. The baseline BP was recorded (as described above) and, once calibration had been completed, the '*Proceed to real time monitoring*' command activated, which, as the command name itself suggests, initiates the real time recording. I then waited for between two to five minutes for signal stabilisation and during this time I entered the participant details (Study ID number and date of birth) on the echocardiography machine. An additional three ECG electrodes were placed (as per standard locations) to provide a timing ECG signal for the TTE machine.

The Guidelines from the British Society of Echocardiography were used to obtain the TTE images. Images were obtained by a senior cardiac physiologist, or by me under their supervision. The senior cardiac physiologist and I (whilst operating the PhysioFlow®) were blinded to the measurements obtained from the other method. Any abnormality detected was noted by the cardiac physiologist and appropriate action taken.

The left parasternal long-axis view (PLAX) was obtained for measurement of the LVOT (and TTE images stored for offline review). Following this, the apical 5-chamber (A5C) view was obtained to acquire the LVOT VTI. Once the view was deemed appropriate, the PhysioFlow® software was labelled using: '*Add mark*' button for 'START ECHO T1', T1 being the first time point (baseline reading at rest). The Doppler VTI waveforms were then obtained (at least three good quality and, ideally, consecutive waveforms). Once this was completed, the PhysioFlow® software was again labelled: '*Add mark*' button for 'STOP ECHO T1'. Images were again stored for offline analysis.

The participant then transferred themselves to the stationary ergometer cycle. Non-weight-bearing exercise is preferred to weight-bearing exercise in pregnancy, as per IWGMH recommendations,

because it reduces the risk of falls and the potential influence of gestational weight gain on exercise performance[175], hence the choice of cycle ergometer. The PhysioFlow® software was labelled with 'TX TO BIKE' to note the transfer, and the participants were told not to start the exercise until instructed to. Time was allowed for the HR spike (the result of transfer from supine to sitting position) to return to baseline before exercise was started and the software labelled with 'START EXERCISE'. Participants were told the starting and target HR and that they would be informed once they had achieved the target HR. Participants were allowed to choose the speed and resistance used on the ergometer cycle. Once the target HR was achieved, this was labelled on the PhysioFlow® as 'ACHIEVED HR' and participants were asked to maintain the HR above this level for at least 60 seconds. During this time, when it was considered feasible, a reading of LVOT VTI was obtained and this was labelled on the software as 'UPRIGHT CO'.

At the end of this time, participants were asked to transfer back to the couch and into the same position as for baseline readings. A pulse oximeter was placed on their right index finger and their HR and peripheral oxygen saturations recorded. As soon as feasible, the A5C view was obtained on TTE, 'START ECHO T2' marked on PhysioFlow® software and LVOT VTI pulse wave Doppler obtained as before. T2 was the second time point for comparison. As soon as this was completed, 'STOP ECHO T2 was marked on the Physioflow® software and a second measure of BP obtained.

Participants were allowed to rest and recover from exercise until the HR returned to ±20% of baseline readings. If the HR had not returned to those levels within 5 minutes, a reading (T3) was taken anyway. The A5C view was obtained once more, the PhysioFlow® software labelled 'START ECHO T3', LVOT VTI pulse wave Doppler obtained too and PhysioFlow® software labelled marked 'STOP ECHO T3'. The last reading of SpO_2 and BP was obtained and recorded, and 'END EXAM' marked on Physioflow® software.

An illustration of the PhysioFlow® software and a depiction of how measurements described above were obtained for the validation of the PhysioFlow® against TTE (Chapter 3) can be found below (Figures 2.4 and 2.5).

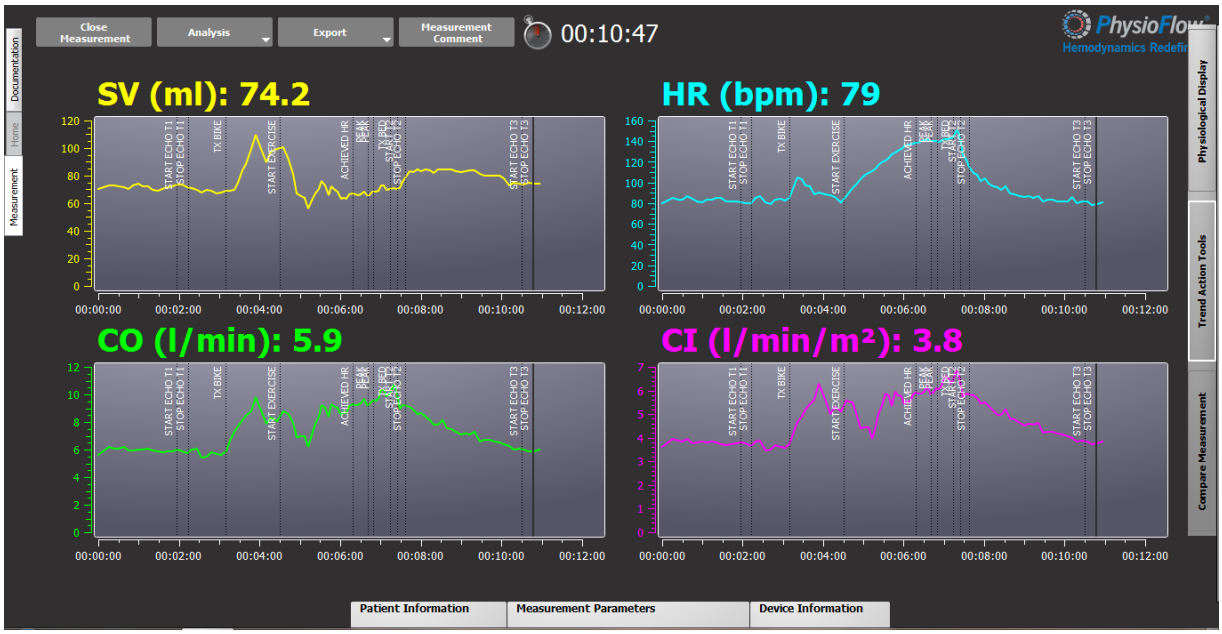


Figure 2.4 *– Representation of the screen on the PhysioFlow® software illustrating four parameters (user's choice) being continuously measured over time: C_i (cardiac index), CO (cardiac output), HR (heart rate) and SV (stroke volume) in participants having TTE (transthoracic echocardiography).*

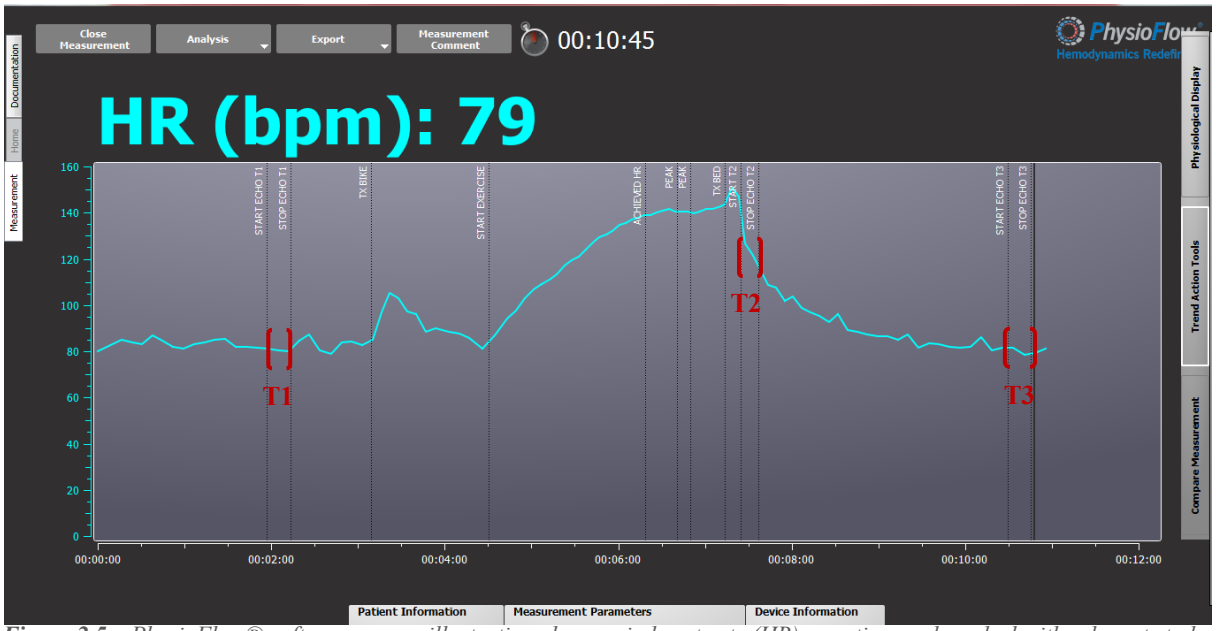


Figure 2.5 *– PhysioFlow® software screen illustrating changes in heart rate (HR) over time and marked with relevant study procedures' labels for participants undergoing transthoracic echocardiography. T1 – average HR between start and end of transthoracic echocardiography (TTE) at rest; T2 – average HR between start and end of TTE immediately after exercise; T3 – average HR between start and end of TTE in the recovery phase. Corresponding measurements of cardiac output (CO), stroke volume (SV) and average signal quality were also taken.*

On occasion, TTE was not available. However, these participants still underwent the same study procedures as described before. To allow comparison between participants who had PhysioFlow®, specific time points were selected and analysed (Figures 2.6 and 2.7).



Figure 2.6 *– Representation of the screen on the PhysioFlow® software illustrating four parameters (user's choice) being continuously measured over time: C_i (cardiac index), CO (cardiac output), HR (heart rate) and SV (stroke volume) in participants not having TTE (transthoracic echocardiography).*

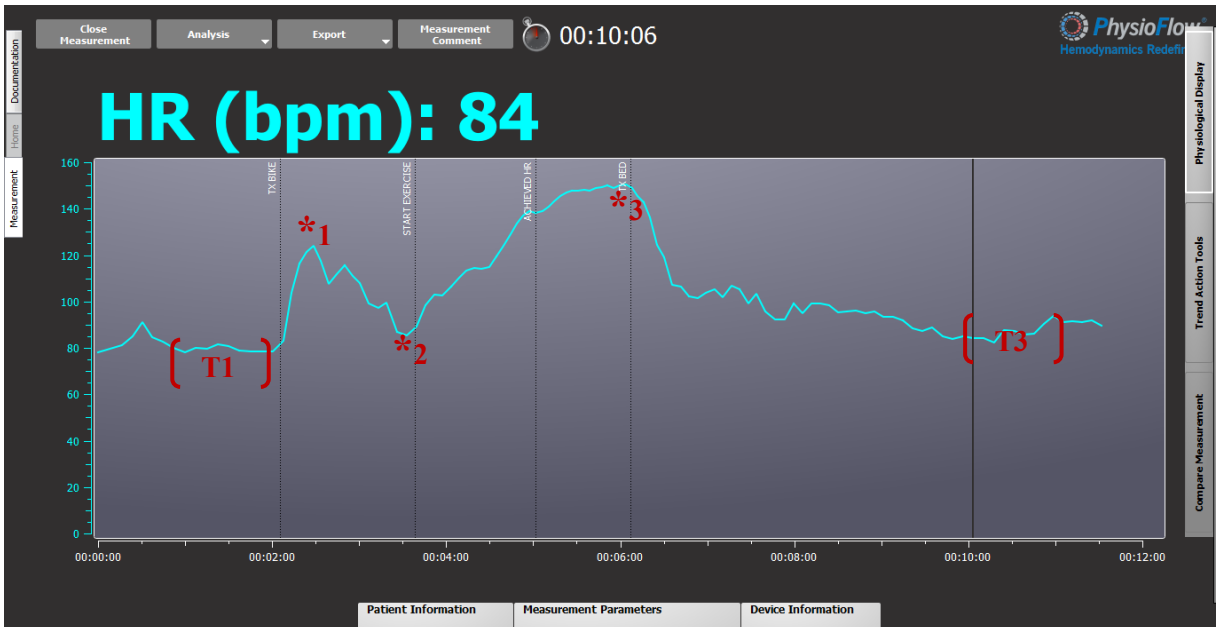


Figure 2.7 *– PhysioFlow® software screen illustrating changes in heart rate (HR) over time and marked with relevant study procedures' labels for participants undergoing impedance cardiography only. T1 – time point one (rest); T3 – time point three (after recovery); *1 – Maximum HR after transfer to cycle; *2 – Pre-exercise HR; *3 – Peak exercise HR.*

Table 2.4 summarises the measurements extracted offline from Physioflow® and TTE. Some of these measurements were used for direct comparison between Physioflow® and TTE, whilst others for comparison of haemodynamic parameters between participants. To enable direct SV, HR and CO measurement comparison between Physioflow® and TTE, an average of the several measurements recorded by ICG between the start and the finish of the TTE was used. The signal quality, averaged over that time, was also obtained.

Table 2.4 *– Offline measurements extracted from PhysioFlow® and transthoracic echocardiography (TTE). CO (cardiac output), HR (heart rate), SV (stroke volume), VTI (velocity-time integral). T1 – time point one (readings taken at baseline), T2 – time point two (readings taken immediately after exercise and after participant transfers to couch); T3 – time point three (readings taken after the participant's HR has returned to below ±20% of the initial baseline HR).*

Time point	Physioflow®	TTE
Before T1	-	LVOT on PLAX
T1	SV, HR, CO and signal quality: at the start, average (between start and end of T1) and end.	HR, VTI, SV, CO: 3xDoppler waves obtained. Measurements averaged.
***1: Max HR after transfer to cycle**	SV, HR, CO and signal quality	-
***2: Pre-exercise HR**	SV, HR, CO and signal quality	-
Upright HR/CO	SV, HR, CO and signal quality	HR, VTI, SV, CO: 3xDoppler waves obtained. Measurements averaged.
***3: Peak exercise HR**	SV, HR, CO and signal quality	-
T2	SV, HR, CO and signal quality: at the start, average (between start and end of T2) and end.	HR, VTI, SV, CO: 3xDoppler waves obtained. Measurements averaged.
T3	SV, HR, CO and signal quality: at the start, average (between start and end of T3) and end.	HR, VTI, SV, CO: 3xDoppler waves obtained. Measurements averaged

2.6. Growth scans and Doppler studies

Two senior sonographers performed (and reported) all the scans using the GE Voluson™ S10 Expert (*General Electric Company, USA*), equipped with a transabdominal C4-8-D probe (frequency 8-2 MHz).

Low-risk participants were asked to attend between 30-34 weeks of gestation for a fetal growth scan. GA was confirmed based on the agreed EDD. A measure of the head circumference (HC), abdominal circumference (AC) and femur length (FL) were obtained to calculate the EFW, which was automatically calculated by the ultrasound machine using the Hadlock formula.

For the HC, a measurement of the biparietal diameter (BPD) and occipitofrontal diameter (OFD) were first obtained, as per guidelines.[182, 183] A cross-sectional view of the fetal head was obtained at the level of the thalami, ensuring there was a symmetrical view of both ventricles, a continuous midline echo representing the falx cerebri broken in half by the cavum septi pellucidi and that no cerebellum was visualised. The callipers were positioned to measure from an "outer-to-outer" manner at the widest part of the skull and using an angle which was perpendicular to the falx cerebri to obtain the BPD. For the OFD, callipers were positioned in the middle of the bone echo and a measurement performed from the occipital to the frontal bone. The ultrasound machine automatically calculated the HC through the formula: HC = 1.62 (BPD + OFD). For the AC, a transverse section of the fetal abdomen was obtained, ensuring this was as circular as possible, that the umbilical vein was at the level of the portal sinus, a stomach bubble was visible and no kidneys were visualised. The AC was measured directly with ellipse callipers from the outer skin line. For the FL, the femur was identified and both ends of the ossified metaphysis visualised. Callipers were positioned at the ends of the ossified diaphysis and the length of the diaphysis measured.

Following fetal biometry, the Doppler studies were performed according to the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) guidelines.[184] For uterine artery Doppler (UtAD), using a transabdominal approach, the probe's ultrasound beam was directed into the lower quadrant of the abdomen angled medially and then moved laterally, until the uterine artery was seen crossing the external iliac artery. The Doppler gate was placed 1cm downstream from the crossover point and the Doppler waveform obtained, trying to achieve complete alignment with the blood flow. The same procedure was performed for the contralateral side. For the umbilical artery Doppler (UAD), a free loop of cord was identified, the umbilical artery identified using colour Doppler and the sample volume placed over this area. For the middle cerebral artery (MCA) Doppler, an axial section of the brain, which included the thalami and the sphenoid bone wings, was obtained and magnified. Colour flow mapping was used to identify the circle of Willis and the proximal MCA. The pulsed-wave Doppler gate was placed in the proximal third of the MCA and the angle between the ultrasound beam and direction of blood flow kept to as close to 0^{o} as possible.

2.7. Blood samples for B-type natriuretic peptide and Multiplex assays

2.7.1. *Sample collection and storage*

Following skin preparation as per CW's guidance, two samples (2 * 6mL) of peripheral blood were taken into a BD Vacutainer® K2EDTA tube (*Becton, Dickinson and Company, USA*). The blood was immediately stored at 4º Celsius and processed within 2 hours of collection, in accordance with the Department's Standard Operating Procedure number 1 '*Protocol for preparation of PBMC from whole blood'*: spun for 10 minutes at 800g at 4º Celsius on a centrifuge *VWR Mega Star 600R (VWR International, USA*). Two to three (depending on the volume of plasma obtained) 1.5mL *TubeOne*® microcentrifuge tubes (*STARLAB, UK*) were labelled on the cap and on the side with the participant's study number (LRXXX or CHDXXX) and initials, and with the date. Plasma was then aliquoted (1-1.2ml on each microcentrifuge tube) and immediately stored at -80º Celsius in a pre-labelled box inside a freezer. A sample from a positive control (participant from a cardiac ward with known elevated BNP) was collected two weeks prior to processing the samples and then processed and stored in the same way as the rest of the samples.

2.7.2. *Human BNP ELISA assay*

The ab193694 – BNP Human enzyme-linked immunosorbent assay (ELISA) Kit (*Abcam plc, UK*) was used for the quantitative assessment of Human BNP in the plasma. The detection range for this assay is 14-1000 pg/mL and the sensitivity is 14pg/mL. The inter-assay variability is less than 12% and the intra-assay variability is less than 10%. Well effects have not been documented for this assay.

2.7.2.1. Reagent preparation

Reagents and standards were equilibrated at room temperature as per manufacturer's instructions. The 5X Diluent Assay B (5-Bromo-5-nitro-1,3-dioxane) was diluted 5-fold with distilled water and stored for future use as 1X Assay Diluent B. The wash buffer solution was prepared by diluting 20mL of the 20X Wash Buffer Concentrate into 380mL of distilled water to yield 400mL of wash buffer. The

Detection Antibody BNP (biotinylated anti-Human BNP) was briefly centrifuged and 100µL of 1X Assay Diluent B was added to the vial to prepare a detection antibody concentrate. The detection antibody concentrate was then diluted 80-fold with 1x Diluent Assay B. The 1X HRP-Streptavidin (Glycerol) Solution was prepared by gently centrifuging the 200X HRP-Streptavidin concentrate and pipetting up and down to mix gently. This was then diluted 200-fold using the 1X Assay Diluent B.

2.7.2.2. Standard preparation

The vial containing the Human BNP Standard was gently centrifuged and 400µL of Assay Diluent C (5-Bromo-5-nitro-1,3-dioxane) added to the vial to obtain a 50ng/mL Stock Standard. This preparation was gently mixed. The following standards were prepared using the following dilutions (Table 2.5):

Table 2.5 *– Standard dilution preparation for Human BNP ELISA assay.*

Standard Number	Sample diluted	Volume diluted (µL)	Volume of diluent used (µL)	Starting concentration (pg/mL)	Final concentration (pg/mL)
1	Stock Standard	20	980	50,000	1,000
2	Standard 1	300	300	1,000	500
3	Standard 2	300	300	500	250
4	Standard 3	300	300	250	125
5	Standard 4	300	300	125	62.5
6	Standard 5	300	300	62.5	31.25
7	Standard 6	300	300	31.25	15.63
8	None	-	300	0	0

2.7.2.3. ELISA Assay

Samples were removed from the freezer and slowly thawed overnight at 4º Celsius. As the samples still had ice the following morning, they were brought to room temperature on the day of the assay and were nor diluted for the assay.

Low-risk participants with both longitudinal CO data from the Physioflow® (CO data from PhysioFlow® for all three visits) and longitudinal samples (plasma samples from all three visits) were selected (n = 39), and an extra four participants with longitudinal samples but without longitudinal CO data were added: a total of 43 participants with longitudinal plasma samples. To use up the remaining available spaces on the last BNP plate, an extra nine participants who only had CO data and plasma samples at two trimesters were chosen at convenience (four participants with data and samples in trimesters A and B, one with data and samples in trimesters B and C, and four with samples and data in trimesters A and C).

The standards, positive control and samples were added to each plate in duplicate. Longitudinal samples were analysed on the same plate. The plate layout for all plates was planned ahead of the experiment. An example of how standards, samples and controls were distributed on plate 1 can be found below (Table 2.6). The same positive control was used in all 6 plates. After subtracting the wells occupied by the standards in duplicate, the blanks and the positive control, each plate could accommodate longitudinal samples for 13 participants in duplicate (13 participants * 2 (duplicate) * 3 (three time points) = 78 wells + 8 standards (including blank) * 2 (duplicate) + positive control * 2 (duplicate = 96 wells).

For women with CHD, there were only two participants with complete longitudinal samples. The rest of the participants either had samples at one or two trimesters. In total, there were 5 samples for trimester A, 19 for trimester B and 21 for trimester C.

Table 2.6 *– Distribution of standards, control and samples on BNP plate 1. All other plates followed a similar distribution. ST – Standard; +C – positive control; Pt – participants; a, b or c – timepoint samples collected (a:10-14 weeks of gestation, b: 18-22 weeks of gestation, c: 30-34 weeks of gestation); A and B – sample and its duplicate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST 1A	ST 1B	Pt 1aA	Pt 1aB	Pt 1bA	Pt 1bB	Pt 1cA	Pt 1cB	Pt 9aA	Pt 9aB	Pt 9bA	Pt 9bB
B	ST 2A	ST 2B	Pt 2aA	Pt 2aB	Pt 2bA	Pt 2bB	Pt 2cA	Pt 2cB	Pt 9cA	Pt 9cB	Pt 10aA	Pt 10aB
C	ST 3A	ST 3A	Pt 3aA	Pt 3aB	Pt 3bA	Pt 3bB	Pt 3cA	Pt 3cB	Pt 10bA	Pt 10bB	Pt 10cA	Pt 10cB
D	ST 4A	ST 4B	Pt 4aA	Pt 4aB	Pt 4bA	Pt 4bB	Pt 4cA	Pt 4cB	Pt 11aA	Pt 11aB	Pt 11bA	Pt 11bB
E	ST 5A	ST 5B	Pt 5aA	Pt 5aB	Pt 5bA	Pt 5bB	Pt 5cA	Pt 5cB	Pt 11cA	Pt 11cB	Pt 12aA	Pt 12aB
F	ST 6A	ST 6B	Pt 6aA	Pt 6aB	Pt 6bA	Pt 6bB	Pt 6cA	Pt 6cB	Pt 12bA	Pt 12bB	Pt 12cA	Pt 12cB
G	ST 7A	ST 7B	Pt 7aA	Pt 7aB	Pt 7bA	Pt 7bB	Pt 7cA	Pt 7cB	Pt 13aA	Pt 13aB	Pt 13bA	Pt 13bB
H	BLANK A	BLANK B	Pt 8aA	Pt 8aB	Pt 8bA	Pt 8bB	Pt 8cA	Pt 8cB	Pt 13cA	Pt 13cB	+C A	+C B

One hundred microlitres of standard (x8), sample and a positive control were added to the wells of a 96-well plate. For this experiment, I used a total of 6 plates which were left to incubate overnight at 4º Celsius with gentle shaking. The following morning, the solutions were discarded and the plate washed four times – washing was performed by adding 300µL of wash buffer using a multi-channel pipette. After the last wash, plates were inverted and gently tapped against dry paper towels. Following this step, 100µL of Detention Antibody BNP solution was added to each well and incubated for one hour at room temperature with gentle shaking. This solution was then discarded and washed as described above. One hundred microlitres of the HRP-Streptavidin solution was then added to each well followed by a 45-minute incubation at room temperature with gentle shaking. This solution was then discarded and washed as described above. The TMB (3,3', 5,5' – tetramethylbenzidine) One-Step solution was then added to each well and allowed to incubate for 30 minutes, in the dark, at room temperature with gentle shaking. Finally, 50µL of the Stop Solution (Sulphuric acid) was added to each well to allow change of colour. Plates were immediately read on a 450nm plate reader.

Results were analysed using the ELISA analysis website (https://elisaanalysis.com/app, ElisaKit.com©, Pty Ltd, 2012, Australia). The mean absorbance for each set of duplicate standards, samples and controls was calculated by subtracting the average blank absorbance.

2.7.3. *Repeat Human BNP ELISA assay with different dilutions*

Due to inaccurate results from the initial assay (please see Chapter 6), the assay was repeated, using the same methods described above, but testing for different dilutions. For the dilutional pilot assay test, I used two samples from low-risk participants (LR) and one sample from a patient with congenital heart disease (CHD). Samples were selected at random and were tested in duplicate. A total of six strips (each with eight wells) were used. The plate layout for dilutional testing was as below (Table 2.7).

Table 2.7 *– Pilot dilution test for BNP ELISA assay. A and B – sample and its duplicate; CHD – congenital heart disease group; LR – Low-risk group; Pt – participant; ST – Standard.*

	1	2	3	4	5	6
A	ST 1A	ST 1B	Pt 1A – LR NEAT	Pt 1B – LR NEAT	Pt 2A – LR 1:5	Pt 2B – LR 1:5
B	ST 2A	ST 2B	Pt 1A – LR 1:2	Pt 1B – LR 1:2	Pt 2A – LR 1:10	Pt 2B – LR 1:10
C	ST 3A	ST 3A	Pt 1A – LR 1:4	Pt 1B – LR 1:4	Pt 1A – CHD NEAT	Pt 1B – CHD NEAT
D	ST 4A	ST 4B	Pt 1A – LR 1:5	Pt 1B – LR 1:5	Pt 1A – CHD 1:2	Pt 1B – CHD 1:2
E	ST 5A	ST 5B	Pt 1A – LR 1:10	Pt 1B – LR 1:10	Pt 1A – CHD 1:4	Pt 1B – CHD 1:4
F	ST 6A	ST 6B	Pt 2A – LR NEAT	Pt 2B – LR NEAT	Pt 1A – CHD 1:5	Pt 1B – CHD 1:5
G	ST 7A	ST 7B	Pt 2A – LR 1:2	Pt 2B – LR 1:2	Pt 1A – CHD 1:10	Pt 1B – CHD 1:10
H	BLANK A	BLANK B	Pt 2A – LR 1:4	Pt 2B – LR 1:4	NOT USED	NOT USED

Results were analysed using the ELISA analysis website (https://elisaanalysis.com/app, ElisaKit.com©, Pty Ltd, 2012, Australia).

2.7.4. *ARCHITECT Ci8200 BNP*

A different assay was later used to retest the same samples due to issues with the stability of the protein used in the first assay, the ab193694 – BNP Human ELISA Kit (please see Chapter 6). The ARCHITECT Ci8200 BNP (*Abbott, USA*), a chemiluminescent microparticle immunoassay, was used and read on the ARCHITECT iSystem (*Abbott, USA*). The measurement range for this assay is 10-5000pg/mL.

Samples were thawed and brought to room temperature on the day of testing, briefly centrifuged and any cryoprecipitate from protein denaturation was removed with a standard pipette. The samples were then transferred to compatible tubes (200µL) and these were labelled with appropriate barcodes so that the concentration could be read on the ARCHITECT iSystem (*Abbott, USA*). The samples were read in batches (40 samples at a time).

2.7.5.*Bio-Plex® Multiplex Immunoassay*

Bio-Plex® Multiplex Immunoassay (*Bio-Rad Laboratories, Inc., USA*) custom-made plates were used to test the expression of IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin, GROα, ICAM and VCAM. All cytokines and ICAM/VCAM were tested using a single 12.5µL sample from each of the selected participants.

2.7.5.1. Preparation

The Bio-Plex® system was started to allow for warm-up for 30 minutes. Whilst waiting, the assay buffer, wash buffer and sample diluent were brought to room temperature, as well as the samples, after being thawed overnight at 4^{o} Celsius. The Bio-Plex® system was calibrated according to the software's instructions.

One vial of cytokine standard was reconstituted in 250µL of diluent, vortexed for 5 seconds and incubated on ice for 30 minutes. Another vial of diabetes standard was reconstituted in 250µL of diluent, vortexed for 5 seconds and incubated on ice for 30 minutes. To make up the first standard (S1), 64µL of each standard was mixed with 72µL of standard diluent to a total of 200µL. Standards and blanks were then diluted 4-fold as below (Table 2.8). Solutions were vortexed for 5 seconds between liquid transfers.

Table 2.8 *– Standard dilution preparation for Multiplex Immunoassay.*

Standard Number	Sample diluted	Volume diluted (µL)	Volume of diluent used (µL)
1	Reconstituted standard	128 (64+64)	72
2	Standard 1	50	150
3	Standard 2	50	150
4	Standard 3	50	150
5	Standard 4	50	150
6	Standard 5	50	150
7	Standard 6	50	150
8	Standard 7	50	150
Blank	-	-	150

The coupled beads were vortexed for 30 seconds, diluted in 1x Bio-Plex buffer using 288µL of the 20xbeads (seven) and 575µL of the 10x beads (one, total volume = 2,591µL), 3,269µL of the assay buffer to yield a total volume of 5,750µL. These were then protected from light with aluminium foil.

2.7.5.2. Layout

Due to budget constraints only three plates were used, therefore I decided to analyse all samples from pregnant participants with congenital heart disease (n= 45) alongside matched (by GA, n = 45) samples from low-risk pregnant women. All samples were run in duplicate. On each 96 well plate, 18 wells were used for standards and blanks (9 * 2). After inclusion of the standard and blank wells and the selected

study samples, 54 wells were left unoccupied so these were used to analyse the longitudinal samples of nine participants with low-risk pregnancy (9 * 3 (3 trimesters), in duplicate = 54). An example of the plate layout is presented in Table 2.9.

Table 2.9 *– Example of the layout of a 96-well plate used for the Bio-Plex Multiplex Immunoassay. A, B and C – trimester of sample (A – 10-14 weeks of gestation, B 18-22 weeks of gestation, C – 30-34 weeks of gestation); CHD – congenital heart disease group; LR – low-risk group; ST – Standard. All samples in duplicate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST 1A	ST 1B	BLANK	BLANK	LR 3B	LR 3B	CHD6B	CHD6B	LR9B	LR9B	LR 12C	LR 12C
B	ST 2A	ST 2B	CHD1A	CHD1A	CHD4A	CHD4A	CHD6C	CHD6C	CHD9C	CHD9C	CHD 13B	CHD 13B
C	ST 3A	ST 3A	LR 1A	LR 1A	LR 4A	LR 4A	LR 6A	LR 6A	LR 9C	LR 9C	CHD 13C	CHD 13C
D	ST 4A	ST 4B	CHD2A	CHD2A	CHD5B	CHD5B	LR 6B	LR 6B	CHD 10B	CHD 10B	LR 13B	LR 13B
E	ST 5A	ST 5B	LR 2A	LR 2A	LR 5B	LR 5B	LR 6C	LR 6C	LR 10B	LR 10B	LR 13C	LR 13C
F	ST 6A	ST 6B	CHD3A	CHD3A	CHD5C	CHD5C	CHD8C	CHD8C	CHD 11C	CHD 11C	CHD 14B	CHD 14B
G	ST 7A	ST 7B	LR 3A	LR 3A	LR 5C	LR 5C	LR 8C	LR 8C	LR 11C	LR 11C	LR 14B	LR 14B
H	ST 8A	ST 8B	CHD3B	CHD3B	CHD6A	CHD6A	CHD9B	CHD9B	CHD 12C	CHD 12C	CHD 15B	CHD 15B

2.7.5.3. Assay

The diluted beads were vortexed for 10-20 seconds and 50µL was added to each well. As beads were being added manually with single pipetting, the beads were vortexed every time a column was filled. Following this, the plates were washed twice with 100µL of the wash buffer. Samples, standards and blanks were vortexed and added to each well as per the layout above. Plasma used for human cytokine assay were diluted 1:4 and ICAM/VCAM diluted 1:100 with sample diluent, as per manufacturer's instructions, within the wells, and using the sample diluent (25µL of sample to 75µL of diluent for human cytokine assay and a consecutive dilution of 10µL of sample in 30µL of sample diluent followed by 5µL of this first dilution, diluted in 120µL of sample diluent, for ICAM/VCAM). The plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, to incubate for 30 minutes. When 10 minutes of incubation were left, the detection antibodies were vortexed for 5 seconds and diluted 1x:300µL of detection antibody in 2,700µL of

detection antibody diluent to yield a total volume of 3,000µL. The plates were then washed 3 times with 100µL of the wash buffer. The diluted detection antibodies were vortexed and 25µL added to each well. The plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, and allowed to incubate for 30 minutes. The Bio-Plex Manager protocol software was prepared and the standard values from S1 entered on the software. With 10 minutes of incubation time left, the 100x SA-PE was vortexed for 5 seconds and diluted: 60µL of 100x SA-PE diluted with 5,940µL of assay buffer to yield 6,000µL of volume. The plates were once again washed three times with 100µL of the wash buffer. The beads were resuspended in 125µL of the assay buffer. The plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, for 30 seconds. Plates were uncovered and placed on the reader using the appropriate settings.

2.8. Delivery data

Study participants' delivery data was collected, including GA at delivery, onset of labour (spontaneous or induction of labour (IOL) and indications for IOL where applicable), mode of delivery (MOD) and indications for assisted delivery/ Caesarean section, and estimated blood loss (EBL). Sex of the newborn, BW and BW centile (according to the Aberdeen Maternity and Neonatal Databank nomograms) data were also collected.

The Aberdeen Maternity and Neonatal Databank[185] was used to develop nomograms[186], which allow precise quantification of birth centiles and population-based comparisons.[187, 188] These widely used nomograms take into account parity, GA at delivery, sex of the newborn and BW[186]. Two charts (male and female) exist (Figure 2.8). Using a ruler, a line is drawn from the BW to the GA at delivery using the appropriate nomogram (first born *vs* later born) and the BW centile is recorded as a continuous variable.

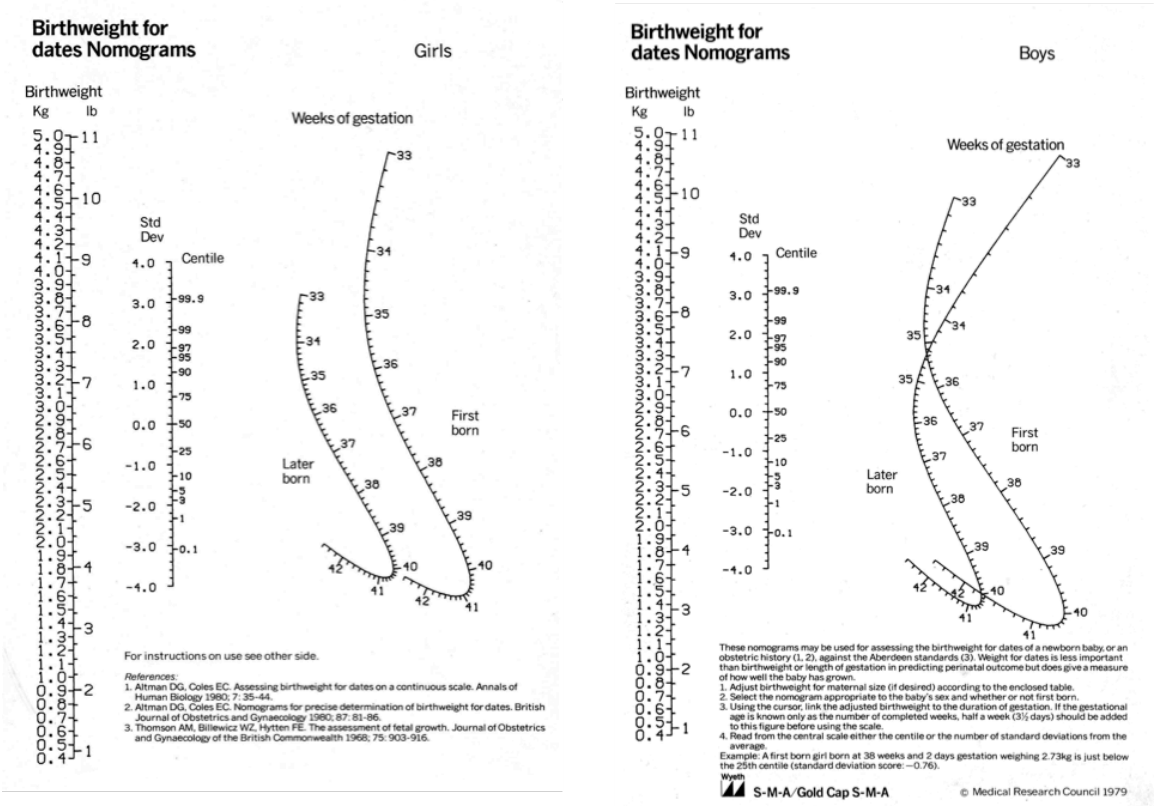


Figure 2.8 *– Girls and boys nomograms for the assessment of birtheight centiles according to parity against the Aberdeen standards.[186] Image reproduced with permission of the rights holders, John Wiley and Sons © 2005, John Wiley and Sons*

2.9. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26.0, 2019 (*IBM Corp., Armonk, New York, USA*) and Microsoft® Excel for MAC, version 16.30, 2019 (*Microsoft Corp., Redmond, WA, USA*).

The Kolmogoroff–Smirnoff, the Shapiro-Wilk tests and visual inspection of the histograms and Q-Q plots were used to assess normality of the distribution of the data. Mann-Whitney, Chi-square tests or Fisher exact, as appropriate, were performed to examine the differences in the demographic characteristics between the examined populations. Bonferroni adjustments were performed where necessary. The adjusted significance level after the Bonferroni correction is $1 – (1 – \alpha)^{1/n}$, were α is the type I error and n is the number of measurements.[189] Bonferroni correction reduces the chance of a type I error at the expense of increased type II errors.[190] We decided to use the Bonferroni correction when multiple testing was used, as we were testing specific hypotheses and not purely undertaking unplanned

exploratory testing, and also because we wanted to avoid type I errors as much as possible. For each individual Chapter, different analyses were performed. A more extensive detailed explanation of the statistical methods used for each chapter can be found under the methodology section of each individual chapter. Below is a brief summary of specific statistical analysis by chapter.

Chapter 3

Agreement of echocardiographic measurements between operators was assessed using correlation coefficient, linear regression and intraclass coefficient (ICC). Mean CO and standard deviation (SD) were calculated at each trimester and time point and for each device. Independent t-test was used to test for differences between CO measurement between echocardiography and ICG. Agreement in absolute terms was evaluated using correlation and Bland-Altman plots. Upper and lower limits of agreement (LOA) were calculated as Mean ± 2*SD and the percentage errors were calculated as 2 * SD of the mean difference divided by the mean of measurements. Agreement was considered to be good if bias (LOA) was low and the percentage error < 30%.[191] Agreement between trending capabilities of the TTE and PhysioFlow® were acquired by the use of a 4-quadrant (4Q) plot with concordant analysis, with calculation of angular bias and radial limits of agreement as proposed by the IWGMH and other authors.[25, 94, 191, 192] For this, three pairs of relative ΔCO were obtained per participant: $[(CO_1 - CO_2)/CO_1] * 100\%$ for each time point – $T_1 - T_2$, $T_2 - T_3$, $T_1 - T_3$. A central exclusion zone was defined for a change of 15% and a concordance rate >90% was assumed to show a reliable trending ability. Data showing more than 150% variation in CO were also excluded for the low-risk population. Angular bias was calculated as the arctangent function (Atan2) of (Xi/Yi) * ((180) / (π – 45)) and radial LOA were inferred from the SD of the mean thetas (ϑ) calculated from the arctangent function as 95% CI (Mean ± 2 * SD). Good trending ability was defined as angular bias ≤ ±5° and radial limits of agreement ≤ ±30°.

Chapter 4

Mean ± SD were calculated at each time point and for each device if data followed a Gaussian distribution; Median (min-max) were calculated if data did not follow a Gaussian distribution. Trends

between trimesters in each of the study groups were assessed using Paired t-test for Gaussian data and Wilcoxon matched-pair signed-rank for non-Gaussian data. Differences between the two study groups were analysed using Mann-Whitney U tests.

Chapter 5

Spearman-rank correlation (r_s) was used to evaluate correlation between EFW, AC/EFW, BW and BW centile with CO, ΔCO and MAP. Doppler indices were presented as median (min-max). Spearman-rank correlation (r_s) was also used to evaluate correlations between EFW, AC/EFW, BW and BW centile, CO, ΔCO and MAP with all Doppler indices.

Chapter 6

Non-parametric tests (Mann-Whitney, Spearman-rank, Wilcoxon-rank) were used to assess differences across trimesters and across populations. Due to the nature of the assay used, values below 10pg/mL were recorded as <10pg/mL. Samples were therefore categorised in three different groups: 1 – BNP expression < 20.0pg/mL; 2 – BNP expression 20.0-39.9 pg/mL and 3 – BNP expression ≥40 pg/mL. Where appropriate, BNP levels <10pg/mL, were given a value of 5pg/mL to allow statistical analysis. Cardiac function was indirectly measured by PhysioFlow® and calculated as the percentage increase in CO and SV between the resting and exercise measurements ΔCO/SV (([T2-T1] / T2) * 100%)

Chapter 7

Pearson and Spearman-rank tests were used, where appropriate, to study the correlations between cytokine/cellular adhesion molecules (CAM) expression and the variables of interest. Independent t–test, paired t-test and repeated measures ANOVA were used to assess the longitudinal changes of maternal parameters.

Chapter 3.

Maternal cardiac output trending:

validation of impedance cardiography (PhysioFlow®) against transthoracic echocardiography in low-risk pregnancy and in pregnancy complicated by congenital heart disease

Chapter 3. Maternal cardiac output trending: validation of impedance cardiography (PhysioFlow®) against transthoracic echocardiography in low-risk pregnancy and in pregnancy complicated by congenital heart disease

3.1. Introduction

Important haemodynamic changes occur during pregnancy, which are discernible during the cycle of conception and become more marked as pregnancy progresses.[193] These changes are essential to the establishment of adequate utero-placental flow and fetal growth.[2, 193] CO, the volume of blood pumped into the aorta per minute and the product of SV and HR, increases by 30-50% of the pre-pregnancy values by 24 weeks of gestation and these levels are maintained until term.[22, 26] There are discrepancies in the literature regarding the magnitude and the pattern of the increase in CO throughout pregnancy, mainly after the second trimester.[22, 24] A large meta-anlysis[24], which included nearly 1500 women from pre-pregnancy to the late postpartum period, showed that CO increases in a non-linear fashion and peaks (31% above non-pregnant values) in the early third trimester. TVR behaves in an inverse pattern, decreasing by up to 30% and reaching a nadir at around 28 weeks.[22, 24] The physiological haemodynamic changes of pregnancy are usually well tolerated.[2] However, if there is haemodynamic maladaptation, this can lead to maternal and fetal morbidity such as PE and FGR.[193] Being able to measure cardiac indices, such as CO changes during pregnancy, could help identify women at risk of these complications.[83] Women with heart disease are also known to have a blunted cardiovascular adaptation to pregnancy.[33] Heart disease in pregnancy is the main cause of maternal death in the western world[3] and having an accurate and non-invasive means of measuring CO could be of considerable assistance in the management of these women.

The gold standard for CO measurement is the direct Fick method, using a pulmonary artery catheter.[71] Due to its invasive nature and associated complications, this technique is rarely used in pregnancy.[25] TTE using the pulsed-wave Doppler VTI method to measure SV has been validated against the thermodilution method in pregnancy and is therefore considered the gold standard in pregnancy.[86] It is safe, non-invasive and readily available in clinical practice.[25, 86] However, TTE only provides CO values

on a heartbeat by heartbeat basis and requires a qualified clinician to make the measurements.[25] In more complex situations, where other non-invasive techniques have not provided an adequate measurement, CMR can be used after the first trimester.[3] Interest in ICG has been growing recently, as most of the devices used to make the measurements are simple to use, operator independent, non-invasive, reproducible, cost-effective and provide real time measurements on a continuous basis, over many heartbeats.[25, 95] Some studies have shown that CO measured by ICG correlates with CO measured by other invasive and non-invasive techniques in the non-pregnant population, whilst others have not found a close relationship.[72, 87] The PhysioFlow® PF07 Enduro™ (*Manatec Biomedical, France*) is a device that uses signal-morphology impedance cardiology (SM-ICG™) and does not depend on baseline impedance (Z_0).[96, 99] The PhysioFlow® has been validated for clinical use in the non-pregnant population and showed good correlation with invasive methods.[99-102] It has been used to measure SV and CO in pregnancy[103-105] but has yet to be validated by comparison with other methods. PhysioFlow® has been shown to accurately estimate SV in a population with CHD, against CMR.[106] To date, studies of ICG have not shown it to be of sufficient accuracy and precision (different devices with different equations) to replace other invasive and non-invasive methods.[25] However, even though ICG might not provide an accurate measure of the absolute CO, it might still be able to provide sufficient information on relative changes through time (the measurement of relative changes between the various stages of pregnancy) and to be useful for monitoring during labour or Caesarean section.[25]

3.2. Hypotheses and Aims

Hypothesis 1: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably measure CO in any trimester.*

Aim: To investigate CO measured by ICG and compare this to CO measured by conventional TTE at each of the three trimesters in low-risk pregnancy: 10-14 weeks of gestation (Visit A), 18-22 weeks of gestation (Visit B) and 30-34 weeks of gestation (Visit C).

Hypothesis 2: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*

Aim: To obtain three CO measurements at each visit (A, B and C): rest (T1), immediately after exercise (T2) and after recovery (T3), and directly compare the CO measures obtained from TTE with the average CO measurement obtained from ICG during the time of VTI acquisition.

Hypothesis 3: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably measure CO at any trimester.*

Aim: To investigate CO measured by ICG and compare this to CO measured by conventional TTE at each of the three trimesters in pregnancy complicated by CHD: 10-14 weeks of gestation (Visit A), 18-22 weeks of gestation (Visit B) and 30-34 weeks of gestation (Visit C).

Hypothesis 4: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*

Aim: To obtain three measurements at each visit (A, B and C): rest (T1), immediately after exercise (T2) and after recovery (T3) and directly compare the CO measures obtained from TTE with the average CO measurement obtained from ICG during the time of VTI acquisition.

3.3. Methodology

3.3.1. *Study design and participant recruitment*

Study design and participant recruitment are described in detail in Chapter 2.

3.3.2. *Study procedures*

Demographic data was collected on the first visit and included age, race, booking height, weight, and BMI, smoking status, gravidity (including history of previous miscarriages or termination of pregnancy), parity, EDD (based on CRL from first trimester ultrasound), GA at the time of the visit,

use of medication and, in the case of participants with CHD, the type of CHD lesion and detailed description of the lesion.

After a 5-minute rest period, the PhysioFlow® was sited according to the manufacturer's instructions using six electrodes (please refer to Chapter 2 for detailed instructions).[179] Women were asked to lie in the left lateral position, the right arm circumference was measured to allow appropriate BP cuff selection prior to measuring the BP in the right arm (unless specifically contra-indicated by structural anomaly, in which case the left arm was used). The PhysioFlow® device was then calibrated according to the manufacturer's instructions and real time monitoring initiated. Three extra electrodes were sited to provide a timing ECG signal for the TTE machine. Measurement of the LVOT was performed using the PLAX view. Then, the A5C view was obtained to acquire the LVOT VTI (at least three consecutive good quality waves for each time point). Measurements were continuous for the ICG and obtained at three time points from the TTE: T1 – rest, T2 – immediately after exercise and T3 – after recovery. For participants deemed suitable (where signal quality was adequate during exercise and where the breasts did not interfere with acquisition of good quality VTI in the upright position), an extra measurement (LVOT VTI) was taken after the target heart rate was achieved and before the end of the exercise, in the upright position. For the purpose of later direct comparison, a marker was inserted into the PhysioFlow® recording when the acquisition of the Doppler VTI was taking place. The CO from TTE was measured by ultrasound as an average over several cardiac cycles at each of the time points and an average measurement of three good quality Doppler VTI waves was used. After acquisition of T1, the participants were asked to exercise on an ergometer cycle to achieve at least 70% of the predicted maximum heart rate (calculated as [220-age] * 0.7), and once this was achieved, to continue above this level for at least 60 seconds after which they immediately transferred to the left lateral position and the second TTE measurement took place (T2, marked on PhysioFlow® software). Women were then allowed to rest and once their HR was ±20% of the T1 HR, a third TTE measurement was taken (T3, marked on PhysioFlow® software).

The cardiac physiologist was blinded for the ICG measurements and the researcher operating the ICG was blinded for the TTE measurements. All data were analysed offline. All ICG measurements were performed and extracted by me – SV, HR, CO and signal quality. Where the signal quality was below 80%, the measurement was excluded from analysis. TTE measurements were mostly performed by a senior cardiac physiologist (80% of the measurements) and some were performed by me (20% of the measurements), after training and after confirming the agreement and reproducibility between my own measurements and those of the cardiac physiologist.

3.3.3. *Measurements and statistical analysis*

The CSA of the LVOT was calculated as: $\pi * (LVOT/2)^2$. Three consecutive and good quality Doppler waves were measured and an averaged VTI used. CO was then calculated as $LVOT_{CSA} * LVOT_{VTI} *$ HR (calculated from the R-R' interval). Graphs from the PhysioFlow® were also analysed offline. The average SV, CO, HR and signal quality from the three time points (average between the start and end of T1, T2 and T3) were extracted.

Further data were collected after delivery, including GA at delivery, onset of labour (spontaneous or IOL and indications for IOL), MOD and indications for assisted delivery or Caesarean section (where applicable), BW and BW centile according to the Aberdeen Maternity and Neonatal Databank nomograms,[186] sex of the newborn and EBL.

Statistical analyses were performed using IBM SPSS Statistics, version 26.0, 2019 (*IBM Corp., Armonk, New York, USA*) and Microsoft® Excel for MAC, version 16.30, 2019 (*Microsoft Corp., Redmond, WA, USA*). The Kolmogoroff–Smirnoff and the Shapiro-Wilk tests were used to assess normality of the distribution of the data, as well as visual inspection of the histograms and Q-Q' normality plots. Agreement of echocardiographic measurements between operators was assessed using the Pearson correlation coefficient, linear regression and ICC. Mean CO and SD were calculated for each device at each time point. Independent t-test was used to test for differences in CO measurement

between echocardiography and ICG. Agreement in absolute terms was evaluated using correlation and Bland-Altman plots. Upper and lower limits of agreement (LOA) were calculated as mean ± 2 * SD and the percentage errors were calculated as 2 * SD of the mean difference divided by the mean of measurements. Agreement was considered to be good if bias (LOA) was low and the percentage error < 30%.[191] Agreement between trending capabilities of the TTE and PhysioFlow® were assessed by the use of a 4-quadrant (4Q) plot with concordant analysis, with calculation of angular bias and radial limits of agreement, as proposed by the IWGMH and other authors.[25, 94, 191, 192] For this, three pairs of relative ΔCO were obtained per participant: $[(CO_1 – CO_2)/CO_1] * 100\%$ for each time point – $T_1 – T_2$, $T_2 – T_3$, $T_1 – T_3$. A central exclusion zone was defined for a change of 15% or less to allow for random error due to imprecision, and a concordance rate >90% was assumed to show a reliable trending ability. Data showing more than 150% variation in CO within the same modality were also excluded. Angular bias was calculated as the arctangent function (Atan2) of (Xi/Yi) * ((180)/(π – 45)) and radial LOA were inferred from the SD of the mean thetas (ϑ) calculated from the arctangent function as 95% CI (Mean ± 2 * SD). Good trending ability was defined as angular bias ≤ ±5° and radial limits of agreement ≤ ±30°.

3.3.4. *Sample size*

Two studies[194, 195] in non-pregnant individuals demonstrated a concordance rate of 95% between ICG and TTE with a 95% CI and required 73 measurements to achieve significance. We included 50 low-risk women or more in each trimester, each with CO measurements by both devices, at three time intervals (T1, T2 and T3), thus enabling comparison over at least 150 measurements of CO at each trimester. We included 19 women with CHD with a total of 66 measurements of CO by both devices across the three trimesters.

3.4. Results

Data were available for analysis from 94 low-risk participants and for 26 participants with CHD. Some participants were only seen once (at one of the trimesters: A, B or C), some were studied twice (either at trimester A and B, A and C or B and C) and others were seen at all three trimesters (A, B and C).

TTE measurement of CO was not available at all visits. Complete longitudinal TTE data (ABC) was available for 14 low-risk participants and complete longitudinal PhysioFlow® data was available for 45 low-risk participants (Table 3.1). Complete longitudinal TTE data (ABC) was only available for one participant with CHD and complete longitudinal PhysioFlow® data was available for only two participants with CHD (Table 3.2).

Table 3.1 – *Number of participants for whom echocardiographic and PhysioFlow® data were available at each of the trimesters in the low-risk cohort: A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation. Categories are exclusive; 89 participants with echocardiographic data and 94 with Physioflow® data.*

Trimester	Echocardiogram, n	PhysioFlow®, n
A	15	11
B	8	2
C	12	2
AB	9	6
AC	12	15
BC	19	13
ABC	14	45

Table 3.2 – *Number of participants for whom echocardiographic and PhysioFlow® data were available at each of the trimesters in the CHD cohort. A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation. Categories are exclusive; 27 participants with echocardiographic data and 28 with Physioflow® data.*

Trimester	Echocardiogram, n	PhysioFlow®, n
A	1	1
B	9	7
C	9	8
AB	-	-
AC	1	1
BC	6	9
ABC	1	2

For the purpose of validation of the PhysioFlow® against TTE for trend analysis, I analysed each of the trimesters separately and included only the participants for whom I had simultaneous data on both TTE and PhysioFlow® (Tables 3.3 and 3.10).

3.4.1. *Trending analysis of PhysioFlow® against TTE in low-risk pregnancy*

For the trending analysis of PhysioFlow® against TTE in the low-risk group, at least 50 participants with data from PhysioFlow® and TTE were selected per trimester (Table 3.3). After excluding the participants whose PhysioFlow® measurements had a signal quality <80%, data from 94 participants with measurements from at least one trimester were analysed. The maternal characteristics, obstetric history and outcomes and fetal outcomes are described in Table 3.4.

TTE measurements were performed by two different operators. The results of the analysis of agreement of TTE measurements between operators are described in Table 3.5. The mean ± SD CO at each trimester and time point are presented in Table 3.6, whilst the percentage increases and decreases of CO between time points (T1, T2 or T3) at each trimester is described in Table 3.7. Table 3.8 shows the results of the percentage errors calculation.

Table 3.3 – *Data available for validation analysis (simultaneous data from transthoracic echocardiogram and PhysioFlow®) at each trimester. A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation.*

Trimester	Echocardiogram and PhysioFlow® data, n
A	50
B	50
C	57

Table 3.4 – *Maternal characteristics, obstetric history and pregnancy outcomes of low-risk women included in the validation of PhysioFlow® against transthoracic echocardiography for trending analysis (participants whose PhysioFlow® measurements had a signal quality <80% were excluded). N = 94*

Maternal Characteristics	
Age, median (min – max)	33 (24 – 51) <35 (n, %) – 53 (56%) ≥35 (n, %) – 41 (44%)
Race (n, %)	White – 78, 83% Black – 2, 2.1% Asian – 7, 7.4% Middle-Eastern – 4, 4.3% Mixed – 3, 3.2%
Weight at booking, kg, median (min – max)	62.0 (43.0 – 102.0)
BMI at booking, kg/m^2, median (min – max)	22.5 (18.0 – 35.3)
Smoking status, n (%)	Never – 82 (87.2%) Stopped after conception – 4 (4.3%) Stopped <1year before conception – 3 (3.2%) Stopped >1year before conception – 5 (5.3%)
Obstetric Characteristics	
Gravidity, n (%)	G1 – 49 (52.1%) G2 – 24 (25.5%) ≥G3 – 21 (22.4%)
Parity, n (%)	P0 – 59 (62.8%) ≥P1 – 35 (37.2%)
Previous Miscarriage, n (%)	No – 74 (78.7%) Yes – 20 (21.3%)
Previous Termination of Pregnancy, n %)	No – 83 (88.3%) Yes – 11 (11.7%)
Mode of Delivery, n (%)	Spontaneous vaginal delivery (SVD) – 46 (48.9%) Instrumental vaginal delivery – 13 (13.8%) Ventouse delivery – 5 (5.3%) Forceps delivery – 8 (8.5%) Caesarean section (CS) – 35 (37.2%) Emergency CS – 21 (22.3%) Elective CS – 14 (14.9%)
Gestational Age at delivery, weeks+days, median (min – max)	39+5 (20+0 – 42+0)
Sex of the newborn, n (%)	Male – 54 (57.4%) Female – 40 (42.6%)
Birthweight, g, median (min – max)	3444.0 (2360 – 5220)‡ (n = 93)
Birthweight centile, %, median (min – max)	65.0 (3.0 – 100.0)
Estimated blood loss, mL, median (min – max)	400 (100 – 2800)

‡ N = 93 Missing value for one participant due to late miscarriage at 20 weeks of gestation. Only CO data from visit A used.

Table 3.5 – *Association of the transthoracic echocardiographic measurements between both operators. N = 30 (low-risk participants). CO – cardiac output; HR – heart rate; LVOT – left ventricular outflow tract; R-R' – interval between consecutive R waves; SV – stroke volume; VTI – velocity time integral. Significance level: p <0.05.*

	Pearson's Correlation Coefficient, ***r***	*Linear Regression,* r^2	*Intraclass Correlation Coefficient,* ***ICC***	**p value***
LVOT	0.900	0.811	0.854	0.18
HR (R-R')	0.990	0.989	0.993	0.17
LVOT VTI	0.990	0.981	0.989	0.45
SV	0.985	0.989	0.992	0.13
CO	0.987	0.993	0.996	0.64

*Paired sample t-test/Wilcoxon signed-rank test, as appropriate.

Table 3.6 – *Cardiac output (CO, L/min) as measured by transthoracic echocardiography (TTE) and impedance cardiography (ICG) at each trimester (A – 10-14 weeks' gestation, B – 18-22 weeks' gestation, C – 30-34 weeks' gestation), at each of the time points (T1 [rest], T2 [immediately after exercise] and T3[after recovery]) and in the upright position, in low-risk participants. Data presented as mean ± standard deviation. Significance level: p <0.05.*

Trimester	**Time point**	**CO_{TTE}** (L/min)	**CO_{ICG}** (L/min)	**p value**
A	T1	N = 50 4.3 ± 0.9	N = 77 6.1 ± 1.3	<0.001***
	T2	N = 50 9.6 ± 1.8	N = 50 10.8 ± 2.8	0.017*
	Upright	N = 28 10.4 ± 1.4	N = 26 12.0 ± 3.5	0.031*
	T3	N = 50 5.1 ± 1.0	N = 77 7.2 ± 1.4	<0.001***
B	T1	N = 50 4.4 ± 0.7	N = 66 6.1 ± 1.1	<0.001***
	T2	N = 50 9.5 ± 1.6	N = 50 10.5 ± 2.7	0.86
	Upright	N = 39 10.7 ± 2.1	N = 39 12.1 ± 2.7	0.007**
	T3	N = 50 4.9 ± 0.9	N = 66 7.1 ± 1.5	<0.001***
C	T1	N = 57 4.2 ± 1.1	N = 75 6.2 ± 1.1	<0.001***
	T2	N = 57 8.8 ± 2.5	N = 57 10.4 ± 2.5	0.002**
	Upright	N = 37 11.1 ± 2.5	N = 37 11.8 ± 2.5	0.16
	T3	N = 57 4.5 ± 1.1	N = 75 7.4 ± 1.5	<0.001***

*p<0.05, **p<0.01, ***p<0.001

Table 3.7 – *Variation in cardiac output (CO, L/min) from T1 (rest) to T2 (immediately after exercise) and from T2 to T3 (after recovery) as a percentage increase or decrease at each of the trimesters (A – 10-14 weeks' gestation, B – 18-22 weeks' gestation, C – 30-34 weeks' gestation). TTE – transthoracic echocardiogram; ICG – impedance cardiography.*

Trimester	Time point	CO_{TTE} (L/min)	Variation CO_{TTE} (%)	CO_{ICG} (L/min)	Variation CO_{ICG} (%)
A	T1	4.3 ± 0.9		6.1 ± 1.3	
	T2	9.6 ± 1.8	123% ↑	10.8 ± 2.8	77% ↑
	T3	5.1 ± 1.0	88% ↓	7.2 ± 1.4	50% ↓
B	T1	4.4 ± 0.7		6.1 ± 1.1	
	T2	9.5 ± 1.6	116% ↑	10.5 ± 2.7	72% ↑
	T3	4.9 ± 0.9	94% ↓	7.1 ± 1.5	48% ↓
C	T1	4.2 ± 1.1		6.2 ± 1.1	
	T2	8.8 ± 2.5	110% ↑	10.4 ± 2.5	68% ↑
	T3	4.5 ± 1.1	96% ↓	7.4 ± 1.5	41% ↓

Table 3.8 *– Percentage errors in the low-risk cohort for each of the trimesters (A – 10-14 weeks' gestation, B – 18-22 weeks' gestation, C – 30-34 weeks' gestation) and time points (T1 – rest, T2 – immediately after exercise, T3 – after recovery).*

Percentage Errors	T1	T2	T3
A	52.8%	56.9%	38.1%
B	46.2%	46.0%	47.5%
C	54.9%	70.8%	50.8%

The Bland-Altman plots for comparison of the absolute values of CO measurements (in L/min) for both techniques at each of the trimesters (A, B and C) and at each of the visit time points (T1, T2 and T3) for the low-risk participants, are presented below in Figures 3.1 – 3.9. The red line represents the mean, the dark lines represent the upper and lower limits of agreement (LOA). The scatter plots for the absolute CO values measured by both techniques with a fit line at total are presented below in Figures 3.10 – 3.18, with the correspondent correlation coefficient (*r*) and p value.

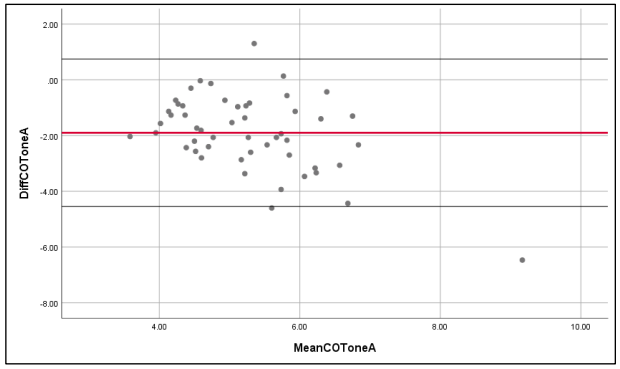


Figure 3.1 *– Bland-Altman plot for cardiac output (CO) measured at T1 (rest) in the first trimester (A). Mean ± SD: -1.9 ± 1.3. Upper LOA: 0.8, Lower LOA -4.5.*

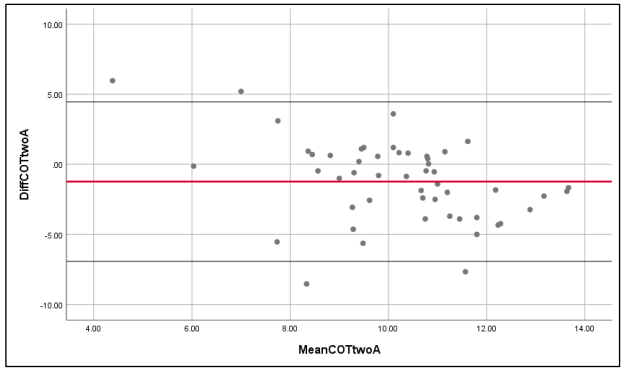


Figure 3.2 *– Bland-Altman plot for cardiac output (CO) measured at T2 (immediately after exercise) in the first trimester (A). Mean ± SD: -1.2 ± -2.9. Upper LOA: 4.5, Lower LOA -6.9.*

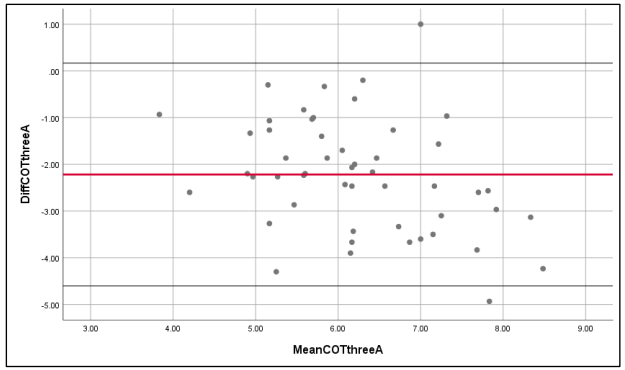


Figure 3.3 *– Bland-Altman plot for cardiac output (CO) measured at T3 (after recovery) in the first trimester (A). Mean ± SD: -2.2 ± 1.21. Upper LOA: 0.17, Lower LOA -4.6.*

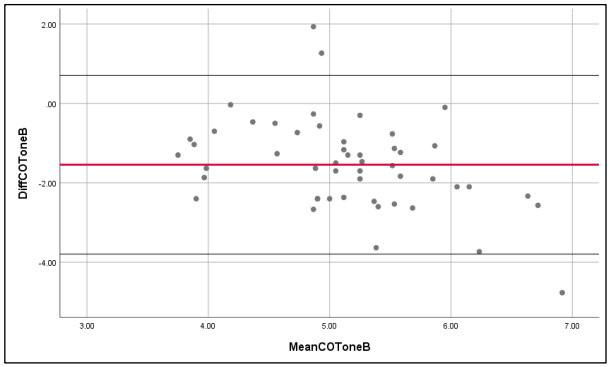


Figure 3.4 *– Bland-Altman plot for cardiac output (CO) measured at T1 (rest) in the second trimester (B). Mean ± SD: -1.5 ± 1.1. Upper LOA: 0.7, Lower LOA -3.8.*

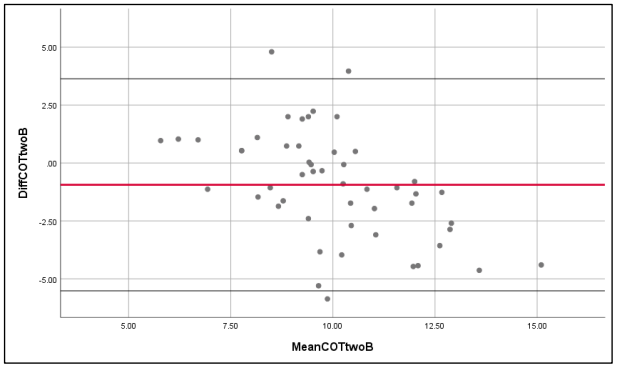


Figure 3.5 *– Bland-Altman plot for cardiac output (CO) measured at T2 (immediately after exercise) in the second trimester (B). Mean ± SD: -0.9 ± 2.3. Upper LOA: 3.6, Lower LOA -5.5.*

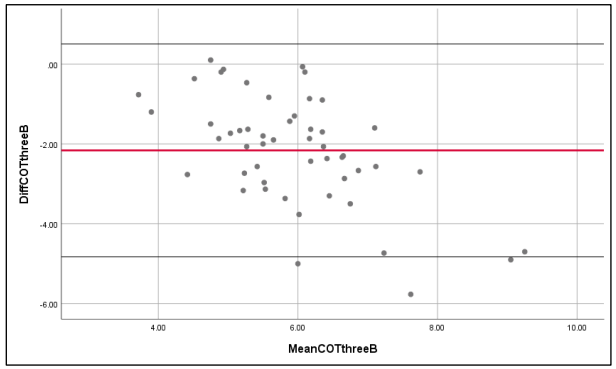


Figure 3.6 *– Bland-Altman plot for cardiac output (CO) measured at T3 (after recovery) in the second trimester (B). Mean ± SD: -2.2 ± 1.4. Upper LOA: 0.5, Lower LOA -4.8.*

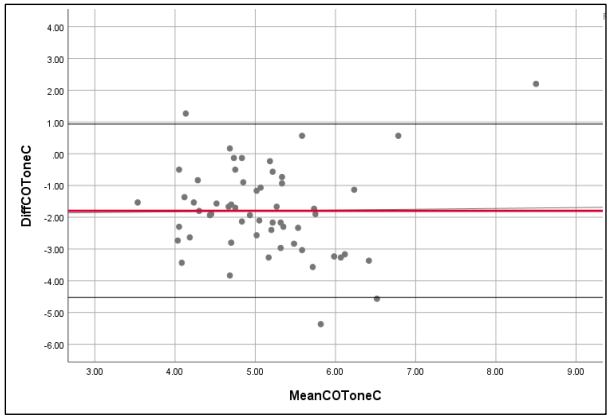


Figure 3.7 *– Bland-Altman plot for cardiac output (CO) measured at T1 (rest) in the third trimester (C). Mean ± SD: -1.8 ± 1.4. Upper LOA: 0.9, Lower LOA -4.5.*

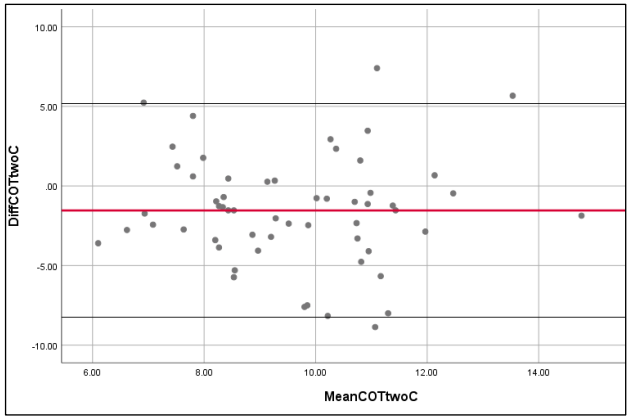


Figure 3.8 *– Bland-Altman plot for cardiac output (CO) measured at T2 in the third trimester (C). Mean ± SD: -1.5 ± 3.4. Upper LOA: 5.2, Lower LOA -8.2.*

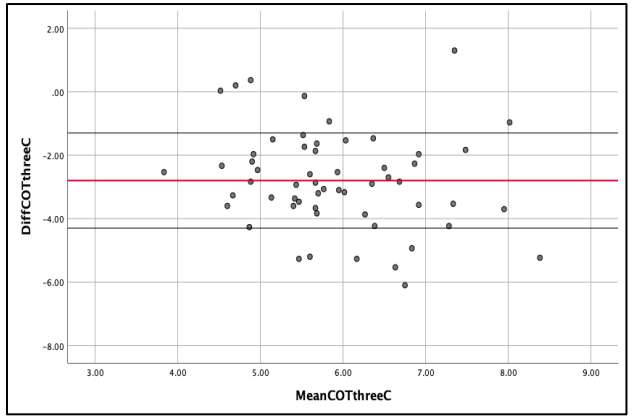


Figure 3.9 *– Bland-Altman plot for cardiac output (CO) measured at T3 in the third trimester (C). Mean ± SD: -2.8 ± 1.5. Upper LOA: -1.3, Lower LOA -5.1.*

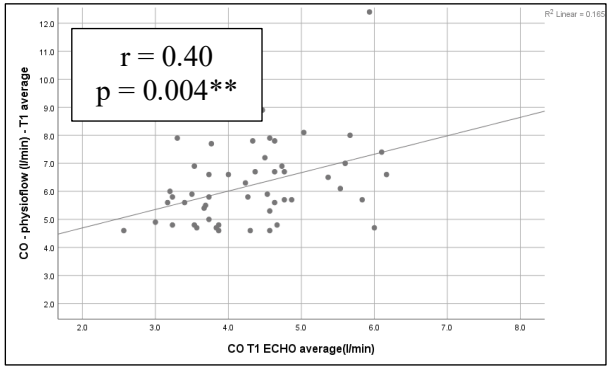


Figure 3.10 *– Scatter plot of cardiac output (CO) measured by both techniques at T1 (rest) in the first trimester (A) with fit line at total. r – correlation coefficient.*

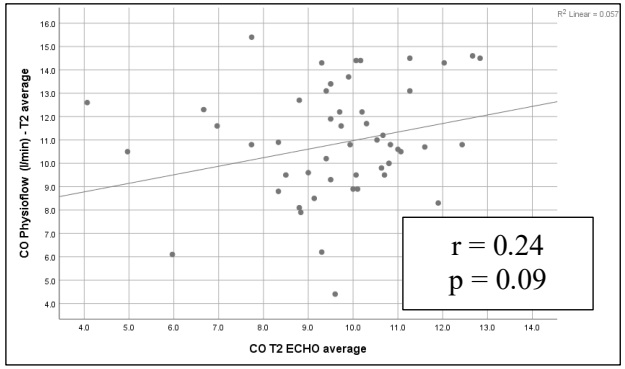


Figure 3.11 *– Scatter plot of cardiac output (CO) measured by both techniques at T2 (immediately after exercise) in the first trimester (A) with fit line at total. r – correlation coefficient.*

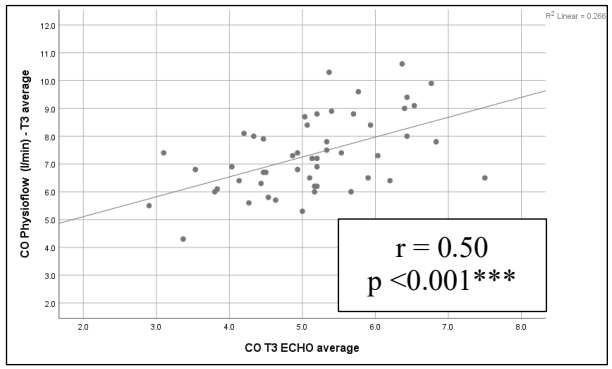


Figure 3.12 *– Scatter plot of cardiac output (CO) measured by both techniques at T3 (after recovery) in the first trimester (A) with fit line at total. r – correlation coefficient.*

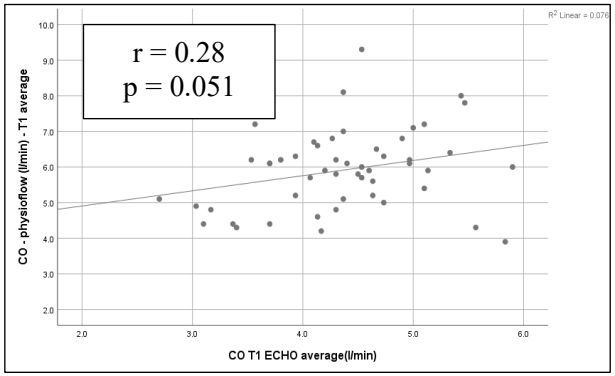


Figure 3.13 *– Scatter plot of cardiac output (CO) measured by both techniques at T1 (rest) in the second trimester (B) with fit line at total. r – correlation coefficient.*

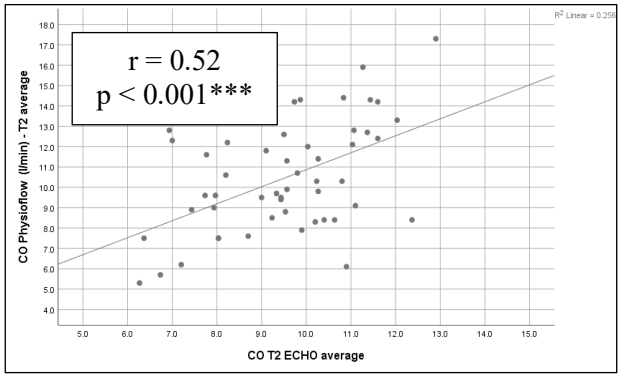


Figure 3.14 *– Scatter of cardiac output (CO) measured by both techniques at T2 (immediately after exercise) in the second trimester (B) with fit line at total. r – correlation coefficient.*

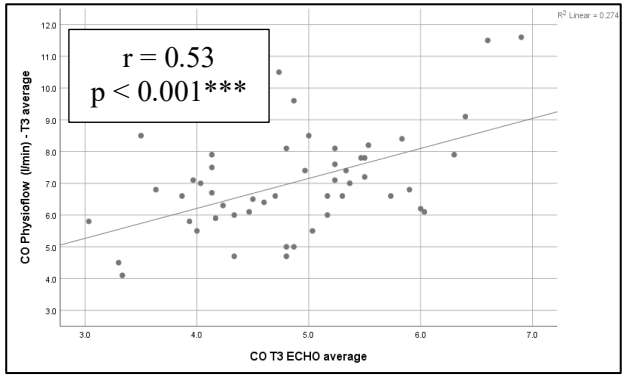


Figure 3.15 *– Scatter plot of cardiac output (CO) measured by both techniques at T3 (after recovery) in the second trimester (B) with fit line at total. r – correlation coefficient.*

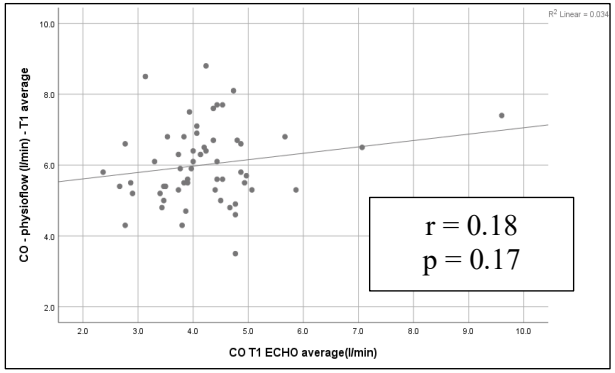


Figure 3.16 *– Scatter plot of cardiac output (CO) measured by both techniques at T1 (rest) in the third trimester (C) with fit line at total. r – correlation coefficient.*

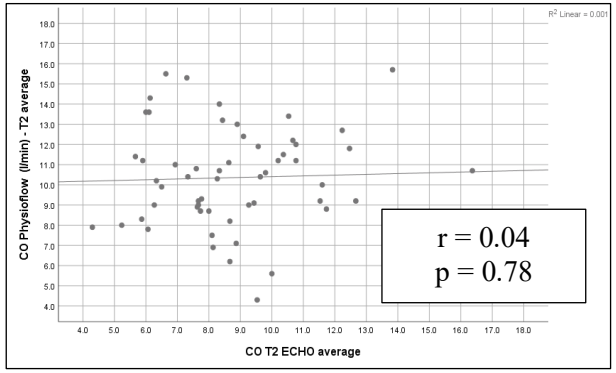


Figure 3.17 *– Scatter plot of cardiac output (CO) measured by both techniques at T2 (immediately after exercise) in the third trimester (C) with fit line at total. r – correlation coefficient.*

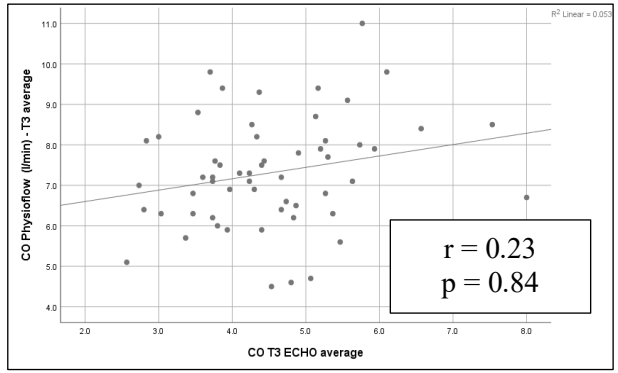


Figure 3.18 *– Scatter plot of cardiac output (CO) measured by both techniques at T3 (after recovery) in the third trimester (C) with fit line at total. r – correlation coefficient.*

The CO trending ability of the PhysioFlow® against TTE in the low-risk group was assessed by the use of 4-quadrant (4Q) plots, which are illustrated below in Figures 3.19 – 3.22. In the first two plots, different central exclusion zones were defined (10% or 15%). In addition to these, in Figures 3.21 and 3.22, large variations of CO (>150%) were also excluded. A summary of the concordance analysis is presented in Table 3.9.

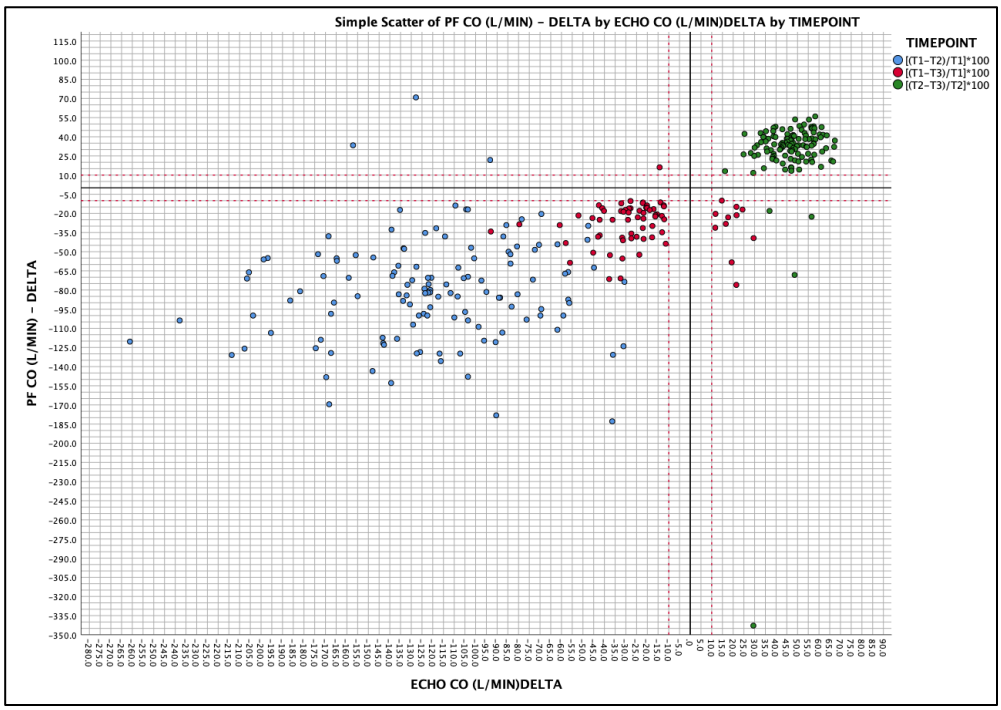


Figure 3.19 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using a* ***central exclusion zone of 10%*** *in the low-risk cohort. The concordance rate is 94.0% (300/319). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

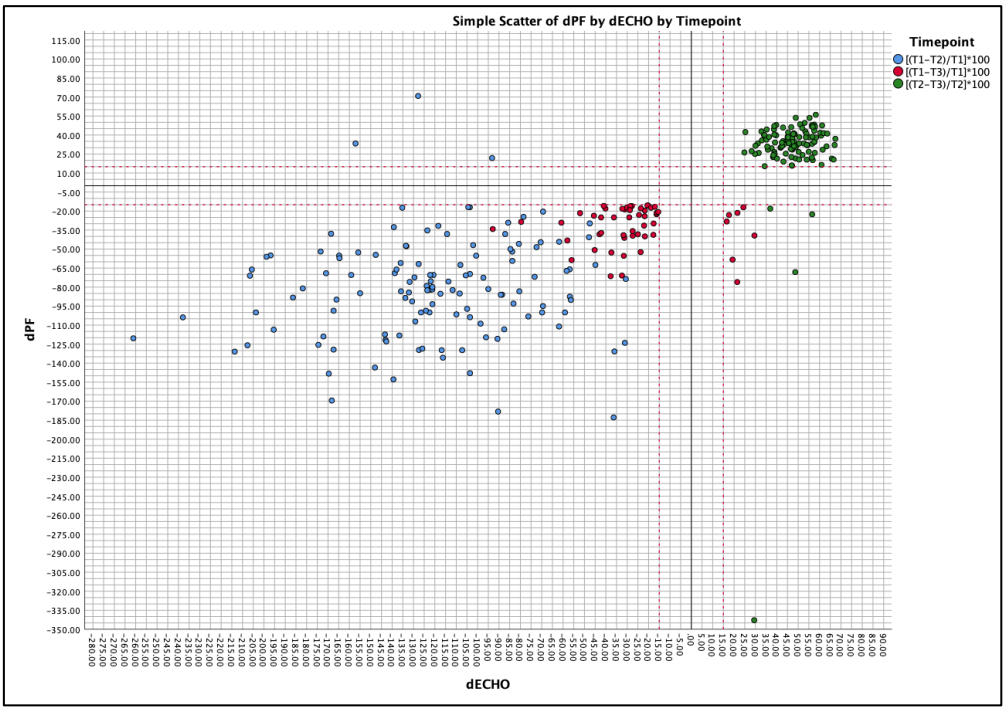


Figure 3.20 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using a* ***central exclusion zone of 15%*** *in the low-risk cohort. The concordance rate is 95.3% (281/295). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

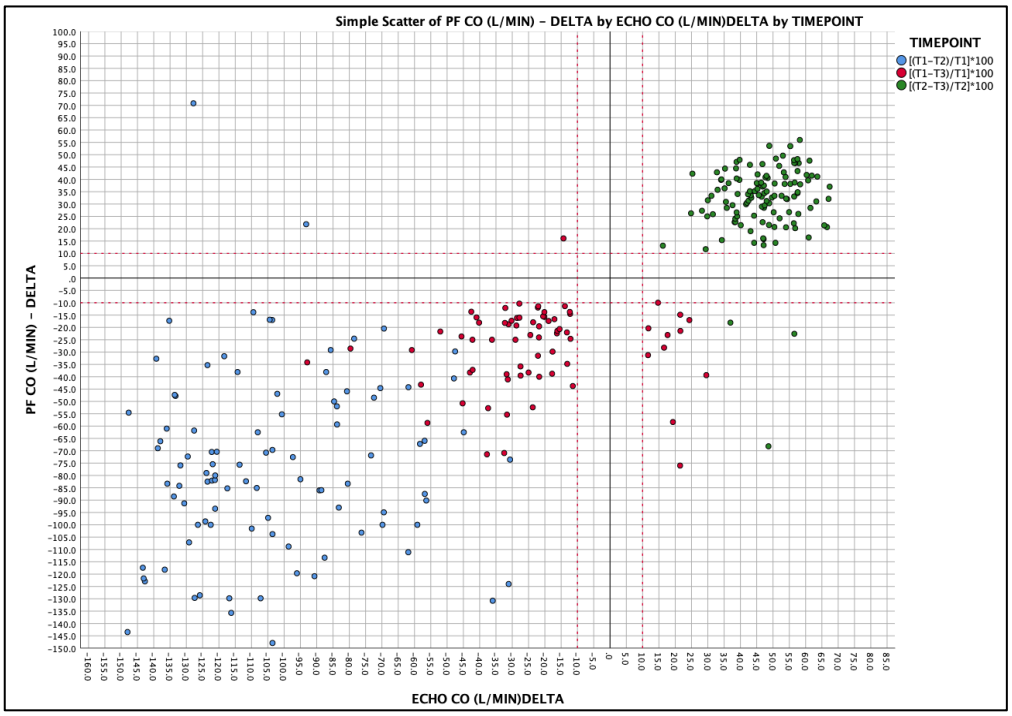


Figure 3.21*– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using a* ***central exclusion zone of 10% and excluding larger variations (>150%)****, in the low-risk cohort. The concordance rate is 94.4% (271/287). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

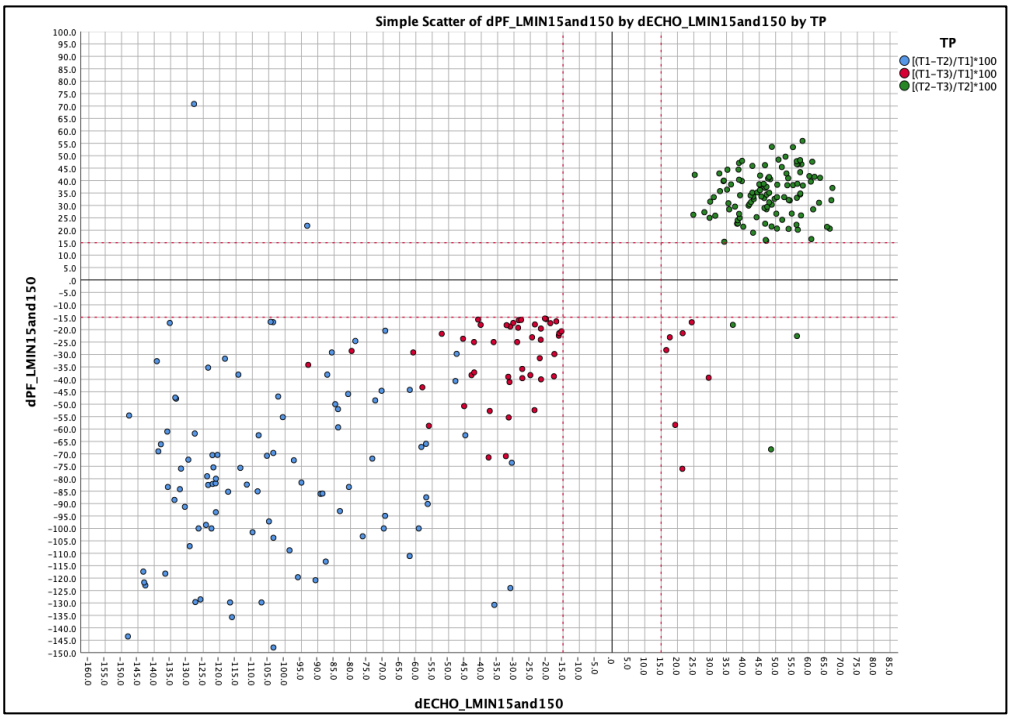


Figure 3.22 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using a* ***central exclusion zone of 15% and excluding larger variations (>150%)*** *in the low-risk cohort. The concordance rate is 95.4% (251/263). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

Table 3.9 – *Concordance analysis of the trending ability of PhysioFlow® tested against transthoracic echocardiogram with 4-Q plots using concordance rates as well as angular bias and radial limits of agreement from transformation of cartesian data into polar data. Data for different central exclusion zones as well as exclusion of >150% variation in cardiac output in the low-risk cohort. 4-Q – 4-quadrant plots.*

4-Q Plot Analysis	Concordance rate	Angular Bias (Mean of ϑ)	Standard Deviation of ϑ	Radial limits of Agreement*
10% Central Exclusion Zone	94.0%	5.2º	±6.7º	[-7.9, 18.6]
15 % Central Exclusion Zone	95.3%	5.1º	±6.7º	[-8.3, 18.5]
10% Central Exclusion Zone + **Exclusion >150%**	94.4%	4.6º	±6.6º	[-8.6, 17.8]
15% Central Exclusion Zone + **Exclusion >150%**	95.4%	4.5º	±6.7º	[-8.9, 17.9]

*Extrapolated from the 95% CI of the angular bias.

3.4.2. *Trending analysis of PhysioFlow® against TTE in pregnant women with CHD.*

For the trending analysis of PhysioFlow® against TTE in the CHD group, participants with data from PhysioFlow® and TTE were selected (Table 3.10). After excluding the participants whose PhysioFlow® measurements had a signal quality <80%, data from 19 participants with measurements from at least one trimester were analysed. Their maternal characteristics, obstetric history and outcomes are described in Table 3.11 and type of cardiac lesion in Table 3.12.

CO was measured by ICG and TTE. The mean ± SD CO were calculated for trimesters B and C and at each time point (Table 3.13). Table 3.14 shows the results of the percentage errors calculation.

Table 3.10 – *Data available for validation analysis at each trimester. A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation.*

Timepoint	Echocardiogram and PhysioFlow® data, n
A	4
B	18
C	19

Table 3.11 *– Maternal characteristics, obstetric history and pregnancy outcomes of pregnant women with CHD included for the validation of PhysioFlow® against echocardiography, for trending analysis. N = 19 (after PhysioFlow® measurements with signal quality <80% were excluded). N = 19.*

Maternal Characteristics	
Age, median (min - max)	32 (25 – 39)
Race, n (%)	White – 18, 95% Asian – 1, 5%
Weight at booking, kg, median (min – max)	65.0 (50.0 – 88.0)
BMI at booking, kg/m^2, median (min – max)	23.5 (17.4 – 34.4)
Smoking status, n (%)	Never – 17 (90%) Stopped >1y before conception – 1 (5%) Smoking – 1 (5%)
Use of medication, n (%)	None – 12 (63.2%) Beta-blockers – 4 (21.0%) Bisoprolol, Labetalol Other – 3 (15.8%) Aspirin, Low-molecular-weight heparin, metformin, thyroxine
Obstetric Characteristics	
Gravidity, n (%)	G1 – 9 (48%) G2 – 5 (26%) ≥G3 – 5 (26%)
Parity, n (%)	P0 – 11 (57%) ≥P1 – 8 (43%)
Previous Miscarriage, n (%)	No – 5 (26%) Yes – 14 (74%)
Previous Termination of Pregnancy, n %)	No – 19 (100%)
Mode of Delivery, n (%)	Spontaneous vaginal delivery (SVD) – 6 (31.6%) Instrumental vaginal delivery – 3 (15.8%) Ventouse delivery – 1 (5.3%) Forceps delivery – 2 (10.5%) Caesarean section (CS) – 10 (52.6%) Emergency CS – 5 (26.3%) Elective CS – 5 (26.3%)
GA at delivery, weeks+days, median (min – max)	39+1 (34+3 – 40+2)
Sex of the newborn, n (%)	Male – 10 (53%) Female – 9 (47%)
Birthweight, g, median (min – max)	2943.0 (1970 – 3760)
Birthweight centile, %, median (min – max)	30.0 (7.0 – 90.0)
Estimated blood loss, mL, median (min – max)	597 (100 – 1800)

Table 3.12 – *Type of cardiac lesions of participants with congenital heart disease. AS – aortic stenosis; ASD – atrial septal defect; BAV – bicuspid aortic valve; CoA – coarctation of the aorta; PDA – patent ductus arteriosus; r – repaired; ToF – Tetralogy of Fallot; TCPC – total cavopulmonary connection; TGA – transposition of the great arteries; VSD – ventricular septal defect. N = 19.*

CHD lesion	N
rTOF	**7**
rCoA	**5**
rCoA only	*1*
rCoA + BAV +rVSD	*1*
rCoA + BAV	*3*
VSD	**3**
Unrepaired VSD	2
rVSD + rPDA + BAV	1
AS + BAV	**1**
Fontan TCPC	**1**
Ebstein's Anomaly	**1**
TGA (arterial switch)	**1**

Table 3.13 – *Cardiac output (CO, L/min) as measured by transthoracic echocardiography (TTE) and impedance cardiography (ICG) for trimesters B and C (B – 18-22 weeks' gestation, C – 30-34 weeks' gestation) at each of the time points (T1 [rest], T2 [immediately after exercise] and T3[after recovery]) for the CHD cohort. Data presented as mean ± standard deviation. Data from first trimester (A) excluded from analysis as n = 4. Significance level <0.05.*

Trimester	Time point	CO_{TTE} (L/min)	CO_{ICG} (L/min)	p value
B	T1	N = 16 5.4 ± 2.7	N = 18 6.2 ± 1.1	0.005**
	T2	N = 16 9.9 ± 3.8	N = 16 9.8 ± 2.6	0.60
	T3	N = 16 5.7 ± 2.7	N = 18 7.2 ± 1.3	0.040*
C	T1	N = 17 5.3 ± 1.7	N = 20 6.5 ± 1.0	0.007**
	T2	N = 17 9.6 ± 2.8	N = 17 10.6 ± 2.4	0.12
	T3	N = 17 6.0 ± 1.8	N = 20 7.7 ± 1.2	0.81

*p<0.05, **p<0.01, ***p<0.001

Table 3.14 *– Percentage errors for trimesters B and C (B – 18-22 weeks' gestation, C – 30-34 weeks' gestation) at each of the time points (T1 – rest, T2 – immediately after exercise, T3 – after recovery) in the CHD cohort. Data from first trimester (A) excluded from analysis as n = 4.*

Trimester	T1	T2	T3
B	95.2%	46.2%	81.0%
C	57.6%	75.4%	72.0%

The Bland-Altman plots for absolute values of CO measurement (in L/min) for both techniques for trimesters B and C and at each of the visit time points (T1, T2 and T3) for the CHD cohort, are presented in Figures 3.23 – 3.28. The red line represents the mean, the dark lines represent the upper and lower limits of agreement (LOA). The scatter plots for the absolute CO values measured by both techniques with a fit line at total are presented in Figures 3.29 – 3.34, with the correspondent correlation coefficient (r) and p value. Data from first trimester (A) were excluded from analysis as n = 4.

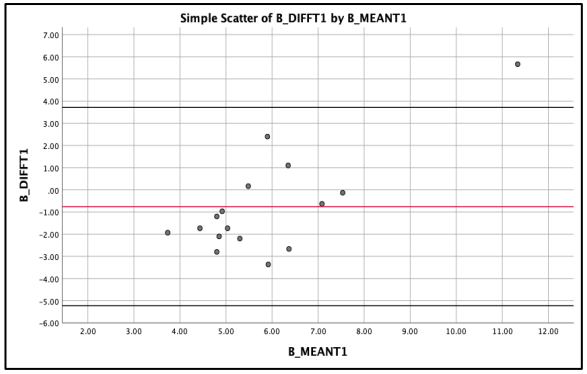


Figure 3.23 *– Bland-Altman plot for cardiac output (CO) measured at T1 (rest) in the second trimester (B) in the CHD cohort. Mean ± SD: -0.7 ± 2.3. Upper LOA: 3.8, Lower LOA -5.2.*

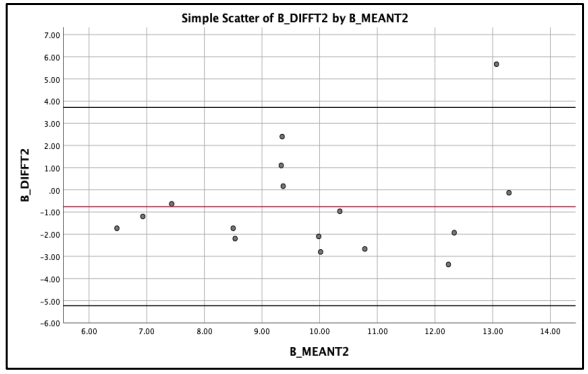


Figure 3.24 *– Bland-Altman plot for cardiac output (CO) measured at T2 (immediately after exercise) in the second trimester (B) in the CHD cohort. Mean ± SD: -0.7 ± 2.3. Upper LOA: 3.8, Lower LOA -5.2.*

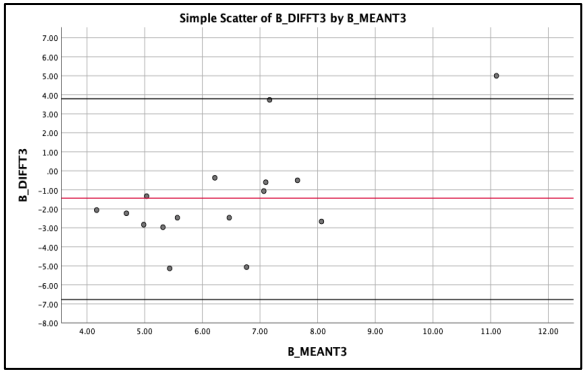


Figure 3.25 *– Bland-Altman plot for cardiac output (CO) measured at T3 (after recovery) in the second trimester (C) in the CHD cohort. Mean ± SD: -1.4 ± 2.7. Upper LOA: 3.8, Lower LOA -6.8.*

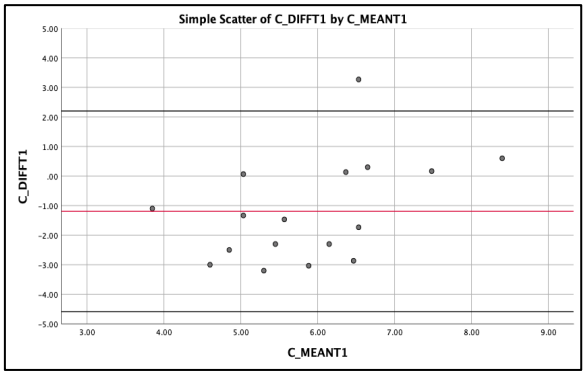


Figure 3.26 *– Bland-Altman plot for cardiac output (CO) measured at T1 (rest) in the third trimester (C) in the CHD cohort. Mean ± SD: -1.2 ± 1.7. Upper LOA: 2.2, Lower LOA -4.6.*

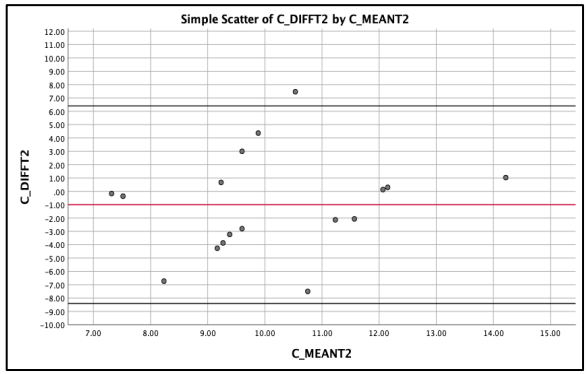


Figure 3.27 *– Bland-Altman plot for cardiac output (CO) measured at T2 (immediately after exercise) in the third trimester (C) in the CHD cohort. Mean ± SD: -1.0 ± 3.8. Upper LOA: 6.4, Lower LOA -8.4.*

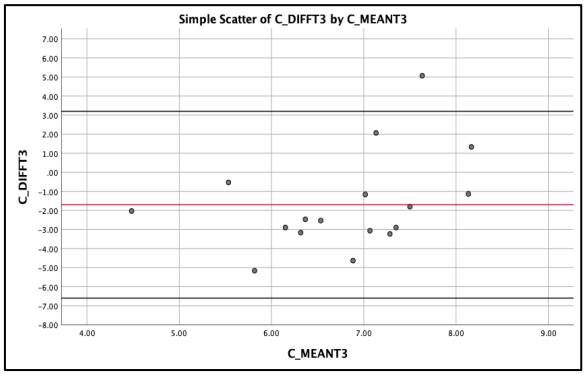


Figure 3.28 *– Bland-Altman plot for cardiac output (CO) measured at T3 (after recovery) in the third trimester (C) in the CHD cohort. Mean ± SD: -1.7 ± 2.5. Upper LOA: 3.2, Lower LOA –6.6.*

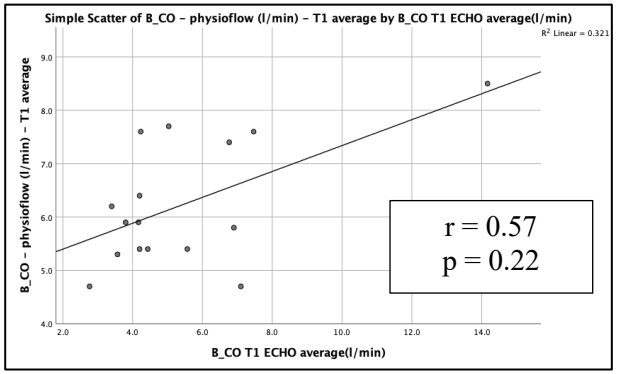


Figure 3.29 *– Scatter plot of cardiac output (CO) measured by both techniques at T1 (rest) in the second trimester (B) in the CHD cohort, with fit line at total. r – correlation coefficient.*

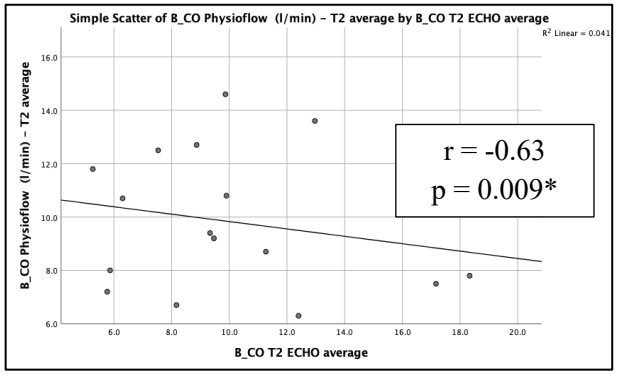


Figure 3.30 *– Scatter plot of cardiac output (CO) measured by both techniques at T2 (immediately after exercise) in the second trimester (B) in the CHD cohort, with fit line at total. r – correlation coefficient.*

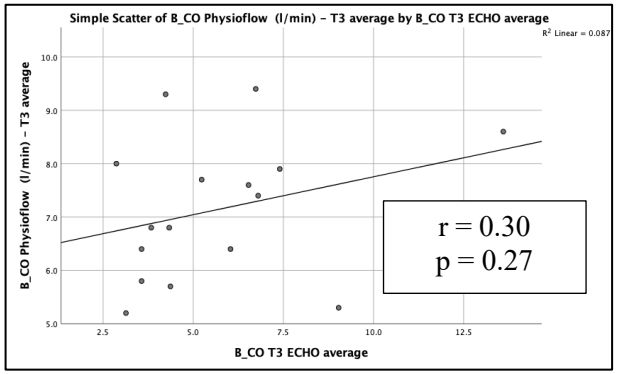


Figure 3.31 *– Scatter plot of cardiac output (CO) measured by both techniques at T3 (after recovery) in the second trimester (B) in the CHD cohort, with fit line at total. r – correlation coefficient.*

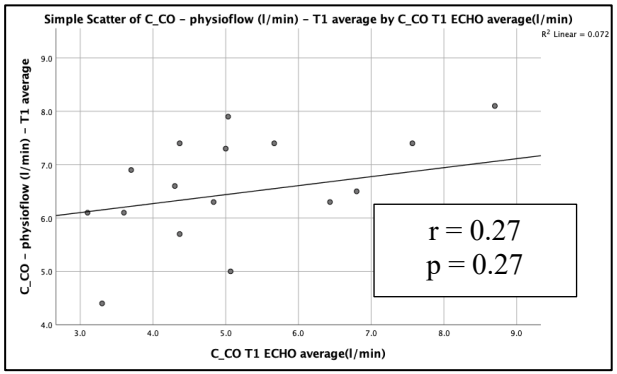


Figure 3.32 *– Scatter plot of cardiac output (CO) measured by both techniques at T1 (rest) in the third trimester (C) in the CHD cohort, with fit line at total. r – correlation coefficient.*

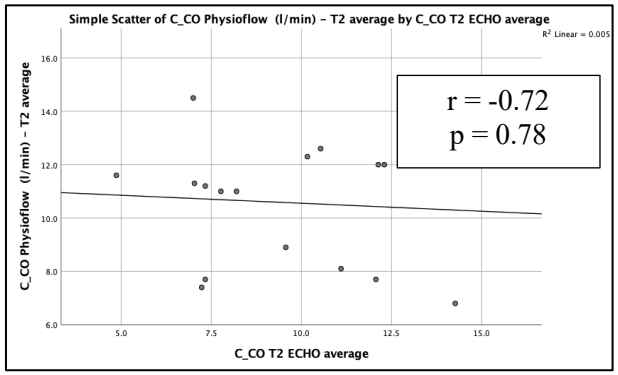


Figure 3.33 *– Scatter plot of cardiac output (CO) measured by both techniques at T2 (immediately after exercise) in the third trimester (C) in the CHD cohort, with fit line at total. r – correlation coefficient.*

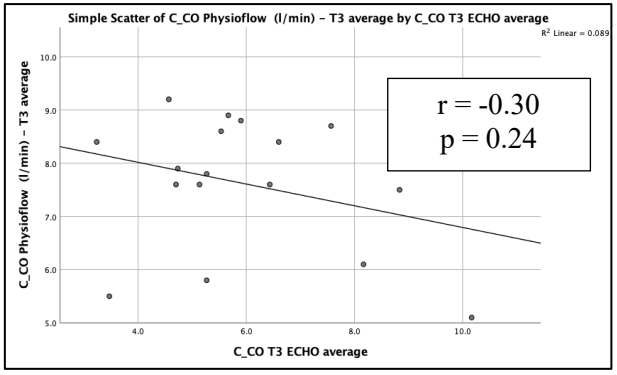


Figure 3.34 *– Scatter plot of cardiac output (CO) measured by both techniques at T3 (after recovery) in the third trimester (C) in the CHD cohort, with fit line at total. r – correlation coefficient.*

The CO trending ability of the PhysioFlow® against TTE in the CHD group was assessed by the use of 4Q plots, which are illustrated below in Figures 3.35 – 3.38. In the first two plots, different central exclusion zones were defined (10% or 15%). In addition to these, in Figures 3.37 and 3.38, large variations of CO (>150%) were also excluded. A summary of the concordance analysis is presented in Table 3.15.

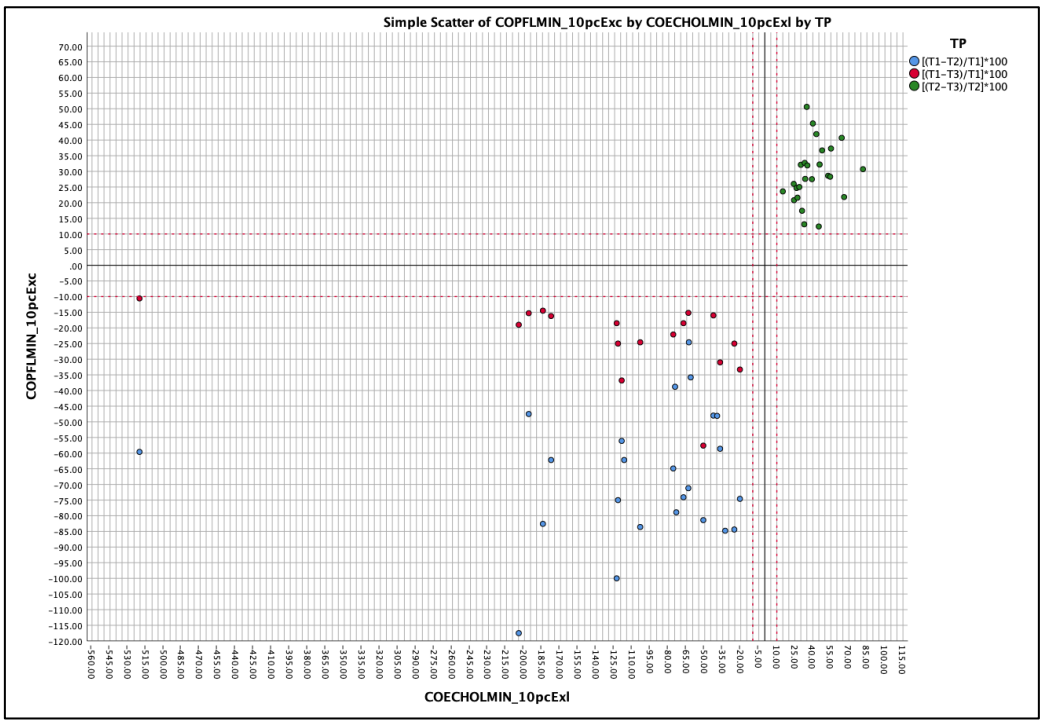


Figure 3.35 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using* ***a central exclusion zone of 10%*** *in the CHD cohort. The concordance rate is 100.0% (66/66). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery*

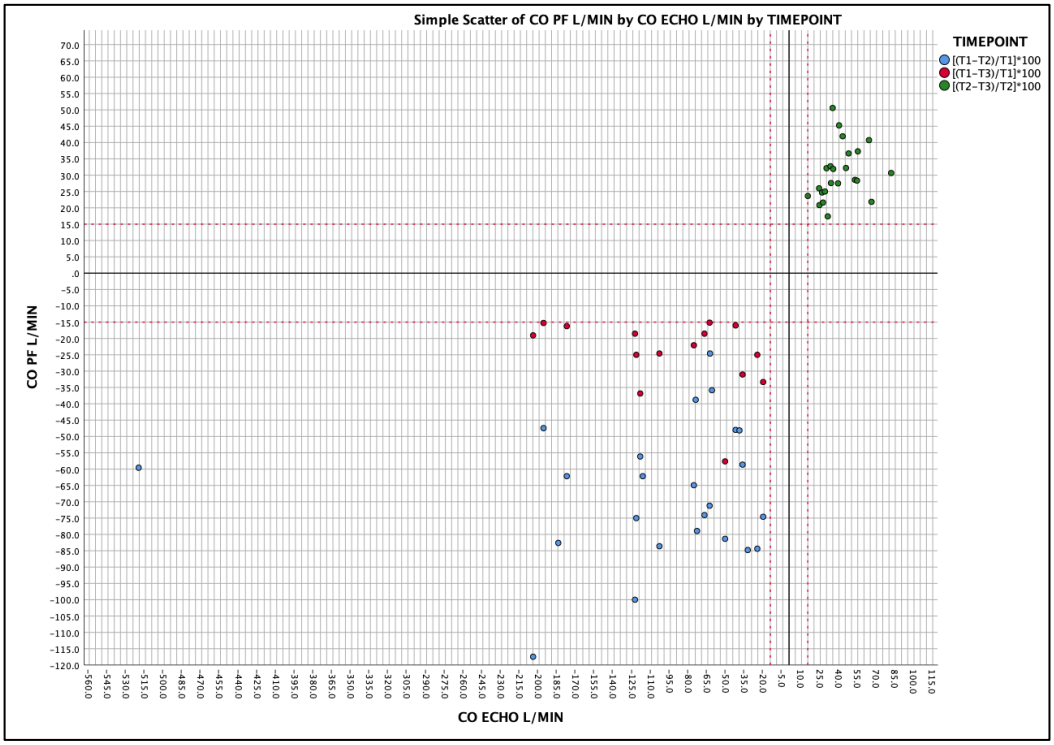


Figure 3.36 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality and using a* ***central exclusion zone of 15%*** *for the CHD cohort. The concordance rate is 100.0% (62/62). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

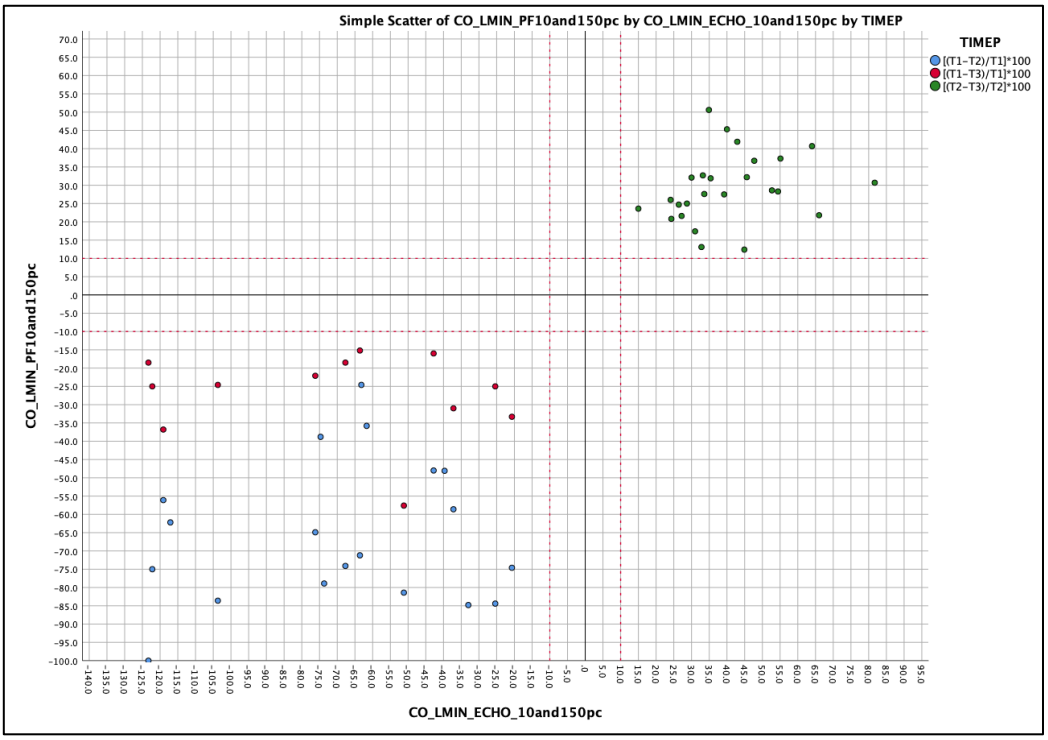


Figure 3.37 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality, using* ***a central exclusion zone of 10% and excluding large variations in CO of >150%****, for the CHD cohort. The concordance rate is 100.0% (56/56). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

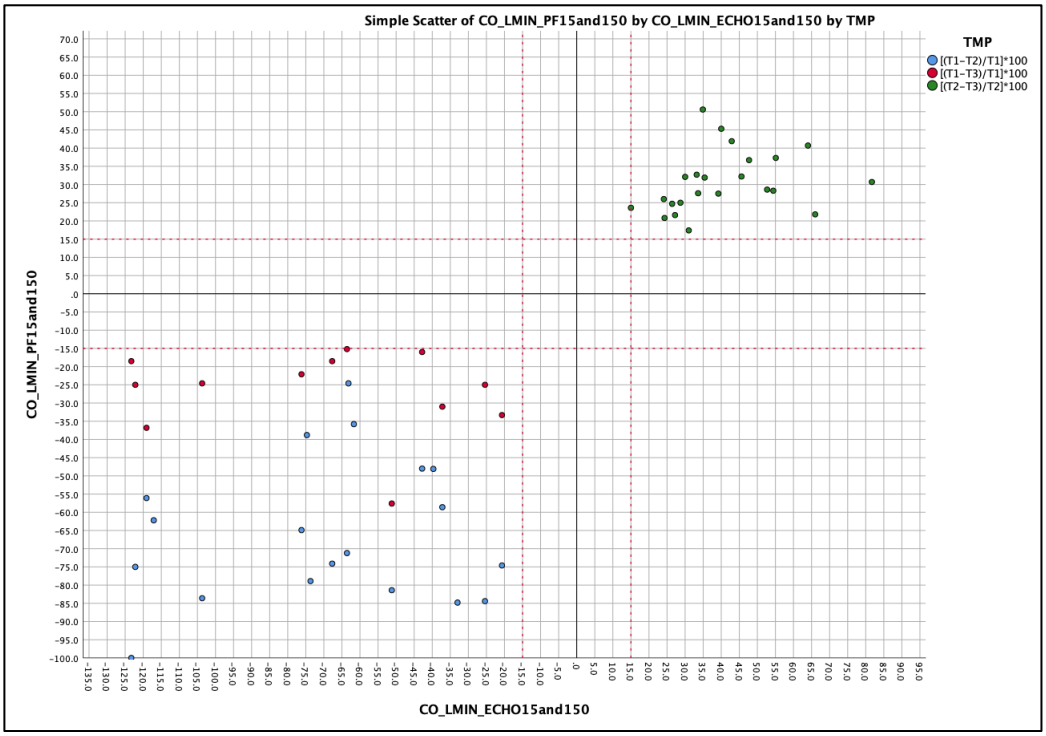


Figure 3.38 *– 4-Quadrant plot of all measurements in all trimesters excluding measurements with <80% signal quality, using a* ***central exclusion zone of 15% and excluding large variations in CO of >150%****, for the CHD cohort. The concordance rate is 100.0% (54/54). CO – cardiac output; ECHO – echocardiogram; PF – PhysioFlow®. Time points: T1 – rest; T2 – immediately after exercise; T3 – after recovery.*

Table 3.15 *– Concordance analysis of the trending ability of PhysioFlow® tested against transthoracic echocardiogram with 4-Q plots using concordance rates as well as angular bias and radial limits of agreement from transformation of cartesian data into polar data. Data for different central exclusion zones as well as exclusion of >150% variations in cardiac output in the CHD cohort. 4-Q – 4-Quadrant plots.*

4-Q Plot Analysis	Concordance rate	Angular Bias (Mean of ϑ)	Standard Deviation of ϑ	Radial limits of Agreement*
10% Central Exclusion Zone	100%	4.1º	±6.5º	[-8.9, 17.1]
15 % Central Exclusion Zone	100%	4.4º	±6.5º	[-8.6, 17.5]
10% Central Exclusion Zone + **Exclusion >150%**	100%	3.5º	±6.9º	[-10.3, 17.3]
15% Central Exclusion Zone + **Exclusion >150%**	100%	3.9º	±6.8º	[-9,7, 17.5]

*Extrapolated from the 95% CI of the angular bias.

3.5. Discussion

3.5.1. *Validation of impedance cardiography (PhysioFlow®) against transthoracic echocardiography for trending in cardiac output, in low-risk pregnancy*

Women in the low-risk cohort had a median age of 33 years and were mainly of white race, had a normal BMI (22.5kg/m^2), were in their first pregnancy (G1 = 52.1%) and had never smoked, which is in keeping with the majority demographics of women having their babies at CW. Most women delivered after 39 weeks of gestation babies of normal weight (BW centile[186] = 65.0), and had a spontaneous vaginal delivery, which is to be expected in a low-risk pregnant population and also in keeping with the delivery data of CW.

The reproducibility and association of echocardiographic measurements between operators were acceptable with a correlation coefficient, *r*, of >0.90 for all measurements, meaning there was a very strong correlation between the two operators.[196] Linear regression, r^2, (Pearson or Spearman, as appropriate) was also high for all measurements (> 0.8). Intraclass correlation, *ICC*, showed excellent correlation (ICC > 0.75 for all measurements)[196]. Bias was low, as demonstrated by p values >0.05 (Paired t-test/Wilcoxon, as appropriate). The measurement which showed least association was the LVOT area (r = 0.90, r^2 = 0.81 and ICC = 0.85) but the correlation between operators was still very strong. For the calculation of the CSA, the LVOT diameter is divided by two (to obtain the radius) which is then squared, so small errors in the LVOT diameter measurement lead to bigger errors in area.[197] However, the association was still acceptable for the purpose of the study and the LVOT measurement and the measurements derived from the LVOT diameter showed very strong correlation by *r* and excellent correlation by *ICC* with no bias (r = 0.90, r^2 = 0.81 and ICC = 0.85).

When using PhysioFlow® for CO measurement, the software provides a signal quality (in percentage) which is based on morphological assessment of the SV curve. Although there was no official manufacturer guidance on a specific cut-off which represents an adequate signal, we contacted the manufacturer of the device, who informed us that the signal quality measurement in this specific device

is based on the analysis of the concordance of the saxophonic-shaped systolic waveform on the dZ/dt signal in relation to the timing of a QRS ECG signal. They advised us to reject measurements with a signal quality <60% but we opted to exclude all measurements with a signal quality <80% as an additional safeguard against error. Therefore, only data with signal quality ≥80% were used.

CO measured by the PhysioFlow® provided, overall, higher readings of CO than that measured by TTE at all time points and in all trimesters, which has also been reported by other authors.[72] Detailed analysis of CO measurement by both devices and trends will be discussed in detail in Chapter 4. The differences of CO measured by both devices were statistically significant, meaning that, effectively, the two devices were not measuring CO in the same way. This fact has been reported previously and it appears that ICG does not show accurate absolute values of CO in pregnancy.[25, 72] However, PhysioFlow® appears suitable for trend analysis of the changes in CO through time, which was the purpose of this study.[25] The correlation between the measurement of CO by TTE with the ICG measurement at T2 in the second trimester and the upright measurement in the third trimester were not statistically significant, which may have been due to chance variation. Percentage increases were calculated to define the average magnitude of CO increase and therefore identify data that could potentially be excluded due to very high variations in CO. CO measured by TTE showed a greater increase from T1 to T2 when compared with ICG. It has been reported that ICG can overestimate CO at rest and that the overestimation is less during exercise[72], which might explain the smaller relative increase from T1 to T2.

Sound statistical analysis is paramount to correctly deem a device as appropriate and accurate for CO monitoring in clinical practice.[94] Bias and precision statistics were used to compare absolute CO values between the two methods using Bland-Altman analysis.[198] The difference between comparative measurements provides the bias (accuracy) and the calculation of the mean difference and correspondent standard deviations allow calculation of the limits of agreement (LOA = mean difference ± 2 * SD), which provide the precision.[198] Data were analysed separately by trimester and time point, as the original Bland-Altman analysis was designed for independent measurements.[198] The mean difference varied between -0.9 to -2.2 L/min, showing, at best, a minimum difference of 0.9 L/min in

CO measurement between the two devices (corresponding to approximately 20% difference of the mean CO), which in clinical practice would not be acceptable.[192] Bland-Altman analysis has become the standard statistical method for evaluation of agreement between two devices and has increasingly been used over the years since its development in 1986.[94, 191, 192, 198]

There are not, however, defined criteria for acceptable mean differences and LOA from the Bland-Altman analysis.[191] 'Acceptable LOA' are defined clinically and depend on the studied population and the range of values of the measurements obtained from the devices being studied. In our study population, CO ranged from 6.0 – 6.2L/min (mean CO: 6.1L/min, in the low-risk group) when measured by PhysioFlow® (please see Chapter 4) and applying a combined percentage error of 28.3% as proposed by Critchley *et al.*[192], the acceptable limits of agreement would be ±1.7L/min. CO measured at T2 in the first trimester (Figure 3.1), T1 in the second trimester (Figure 3.4), T2 in the second trimester (Figure 3.5), T3 in the second trimester (Figure 3.6), T2 in the third trimester (Figure 3.8) and T3 in the third trimester (Figure 3.9) show that the CO is within the calculated acceptable LOA. However, most clinicians would probably not accept a difference of 1.7L/min of CO measured between two devices as such a large difference may have important clinical implications. Classic Bland-Altman analysis also assumes that data are independent and unrelated[191], hence the analysis provided here was done separately for each trimester and time point. Bland and Altman have reviewed their original publication and published further methodologies for repeated measurements (multiple measurements per subject) to avoid smaller standard deviations and, potentially, LOA that could underestimate the variability of readings.[199] Bland and Altman[199] explain that with repeated measurements in the same subject, the 'true' value measured by a specific method will be scattered around the mean of measurements for that particular method, but there will be bias if a particular method provides higher readings than the other and also heterogeneity when the differences between methods vary more than expected between individuals (the so-called subject by method interaction). Averaging repeated measurements for a particular subject only eliminates the variation in that subject and the resulting limits of agreement might be narrower and may lead to falsely assuming that two methods are closely related when in fact they are not.[199]

In the context of trend analysis, Bland-Altman analysis provides an idea of precision but does not address trending.[191] In this particular study, where we measured CO before and after exercise (repeated measurements with variation over time), the precision does not improve as it would when measuring a variable that does not change over time (as an example, the degree of carotid artery stenosis from one day to the other). Another limitation of Bland-Altman analysis for trending analysis is that four readings instead of two (ΔCO) are used per subject, which increases the standard deviation by a factor of √2.[191] Overall, the standard deviation which is an integral part for the calculation of the LOA will be widened by approximately 1.4 due to the use of ΔCO and, at the same time, narrowed due to repeated measurements.[191] Bland-Altman analysis cannot confirm trending ability and alternative methods have been designed to assess this.[191, 200]

Correlation and regression analyses, once frequently used, simply shows the relationship between methods and does not show agreement.[94] In order to maintain the independence of measurements, correlation and regression analysis was performed per trimester and per time point. The highest correlation was observed for time point T3 in the second trimester ($r = 0.53$, $p<0.001$). Data were scattered at all time points and in all trimesters and correlation and regression analysis failed to show good correlation between the two devices. This type of analysis has many flaws, which were the main drivers for the development of the Bland-Altman analysis.[94, 192] Correlation analysis should not be used as a single statistical method when comparing two methods of CO measurement.[94]

Critchley *et al*.[192] proposed the calculation of percentage error (%error) to be able to quantify the acceptable LOA between two techniques, which involved combining the errors of precision of each measurement technique (calculated as 2 * SD of the mean difference divided by the mean of measurements). Once again, these were calculated for each time point and each trimester to maintain the independence of the measurements. All %errors were above 30%, which is the acceptable limit defined in the literature.[192] For the calculation of %errors, the two independent vectors set at right angles from each measurement are defined and, by following the Pythagorean approach, the length of the hypotenuse by adding variances and an error-gram is constructed.[192] As an example, if the two study

methods have an inherent error of ±20%, the combined LOA would be 28.3% which is rounded up to 30% for practical purposes. The measurement of physiological variables such as CO often lacks precision and errors of ±10-20% are common, even with thermodilution, due to cyclical changes. CO measurements also show errors that are proportional to the magnitude of the CO variance and Bland-Altman analysis does not compensate for the magnitude relationship. The use of %error allows for the proportionality effect to be taken into consideration.[192] However, this should only be applied if the reference method and the studied method have inherent errors of ±20%.[94] More recently, the 30% cut-off has been questioned and Peyton *et al*.[201] have proposed a new cut-off of 45%. Their argument is that the safety, convenience, adaptability and cost of a new device should be considered, without only focusing on the absolute accuracy.

Growing interest in a device's ability to track changes over time have led to the development of concordance analysis based on regression and the creation of 4-quadrant (4Q) plots.[202] For this, ΔCO is calculated and plotted on a scatter plot and visual inspection of the plot determines if the data are randomly distributed or falls along the line of identity (X = Y).[191] The upper right and lower left quadrants are the quadrants of agreement (depending on the direction of change: right upper quadrant if both devices denote an increase in CO and lower left quadrant if both denote a decrease in CO) and the concordance rate is simply the number of data points that fall in either of these quadrants divided by the total number of data points.[191, 200] The direction of change in trend analysis is not important.[203] The key to a reliable trend analysis is the definition of a central exclusion zone – data at the centre of the plot tend to be randomly distributed and represent statistical noise.[191, 200] Definitive guidance on suitable sizes for the exclusion zone does not exist, however, a central exclusion zone of 15% was suggested as optimal in a large review.[191] As discussed above, data at the centre of the plot represent small changes in CO and do not reflect trending ability; this only becomes apparent when data move away from the centre.[191] Authors have historically used exclusion zones of 0.5-1.0L/min based on Perrino *et al*.[202] (who used <1.0L/min), whose original description was based on ROC curve analysis and on the mean CO in their cohort, which was approximately 5L/min. More recently, a percentage change has been more commonly used: 10-15% CO change.[191] However, the exclusion zone should

take into consideration the range of CO in the study population.[200] In this study, there was a large range of CO as we were investigating changes with exercise, therefore an exclusion zone of 15% was used as per more recent guidance.[200, 203] Very large variations in CO may also affect trending analysis but guidance on exclusion bands for very large variations is lacking.[191] The maximum average increase in CO in this study was 123% so we decided to exclude individual measurements with variations larger than 150%. There is a current debate on whether exclusion of large variations should be performed for trending analysis and the optimal approach for high CO changes is not yet defined.[94, 200, 203]

Until recently, guidance on acceptable concordance rates was lacking and Critchley *et al*. have suggested that concordance rates >95% are associated with good trending ability, concordance rates of 90-95% associated with marginal trending ability and concordance rates <90% associated with poor trending ability. When applying the recommended 15% exclusion zone, the PhysioFlow® showed good trending ability in the low-risk group. Concordance analysis has its limitations and, overall, there is a data trend when the ΔCO increases from 50% disagreement to total agreement. Some authors believe that a critical band should be defined to reduce sampling bias and reliably detect trending, however, this is associated with exclusion of a large proportion of data.[191]

To overcome the hurdles of concordance analysis, Critchley *et al*.[203] have developed the polar plot methodology. The advantages of this technology are that there are specific cut-off values for angular bias and radial limits of agreement to define good trending ability.[200] However, the polar plot is a complex method which requires a high level of skill to construct and to interpret the data.[200] It involves transformation of X-Y Cartesian data into polar data: the length of the X-Y vector in the 4-Q plot is calculated as the hypotenuse of a right angle triangle formed by the X and Y of the ΔCO ($\sqrt{(X^2+Y^2)}$); the angle at which data are no longer concordant is easier to define as opposed to using the 45^o from the line of identity (X = Y) in the 4-Q plot.[203] Further refinement of the plot involves rotation of the polar plot by 180^o which makes reading the data more difficult.[200] The advantage of the polar plot is that it is able to exclude the most discordant measurements.[203] The polar plot technology is not recommended as a superior statistical approach to the 4-Q plot.[200] More recently, a method has been

developed[200] to calculate the angular bias and radial limits of agreement from the 4-Q plot which has been explained in the methods section. The 4-Q plot is an intuitive graph that is easy to read and shows the magnitude and direction of change of both study devices.[200] Using the polar plot methodology, we were able to calculate the angular bias and radial limits of agreement which were within the acceptable limits when a central exclusion zone of 15% and exclusion of large variations (>150%) were used. Without excluding large variations in CO and using solely a 15% central exclusion zone, the concordance rate was still good (95.3%), the radial limits of agreement were within the acceptable limits (-8.3 – 18.5), however, the angular bias was higher than the recommended 5º (angular bias = 5.1º). Although, this would still be acceptable.

There has been growing interest in the development and validation of non-invasive devices to monitor CO changes.[25, 203] CO is one of the most important haemodynamic parameters in obstetrics and several reports have shown changes in this important parameter in a multitude of obstetric conditions, such as FGR, PE, PPH and sepsis.[25] Early data on ICG show that the agreement of ICG with the pulmonary artery thermodilution method was poor but, more recently, the mathematical equations have been improved, software have been redeveloped and ICG is showing more promising results.[94] The pulmonary artery thermodilution method is frequently used as a reference method in clinical studies comparing technologies measuring CO, as it is considered the gold standard. PhysioFlow® has been validated in the non-pregnant population at rest and during submaximal exercise using this methodology.[99] Authors report mean difference of as little as 0.07L/min at rest and 0.26L/min during exercise, making the PhysioFlow® a clinically acceptable method of CO monitoring in subjects with normal cardiovascular function.[99] PhysioFlow® has also been validated during incremental exercise against the Fick method and, once again, was shown to be an accurate and clinically acceptable method to monitor CO during exercise.[100] In the context of research, using invasive methods in low-risk pregnancy for validation studies is unacceptable.[25] The criterion standard for the validation of new CO measurement technologies is yet to be established.[94] The use of TTE has been validated for use in pregnancy with bias as low as 0.21L/min and a percentage error of 19.1%, which defines this as an acceptable and accurate method for use in pregnancy.[86] We used a non-invasive, reliable and 'ideal'

technology as our reference, considered by some as the reference technology to use in pregnancy.[25] The PhysioFlow® has the added advantage that it is easier to use, provides continuous data, is operator independent, does not require specialised skills, and is easily reproducible, as it is independent of electrode placement.[98, 204]

3.5.2. *Validation of impedance cardiography (PhysioFlow®) against transthoracic echocardiography for trending in cardiac output, in pregnant women with CHD*

In the CHD cohort, women had a median age of 33 years of age, most were of white race, had a normal booking weight and BMI (23.5kg/m^2) and were in their first pregnancy (G1 = 48%). Most women delivered at term normal sized babies (BW centile = 30) and most delivered by Caesarean section, which is not uncommon in patients with CHD.[32, 57] Women had a variety of cardiac lesions, the most common being repaired ToF (n = 7) followed by repaired CoA (n = 5), lesions which should not interfere significantly with aortic flow and therefore should not affect the ability of the ICG in measuring SV. Most women were not on any medication but 21% were taking beta-blockers (two participants on 2.5mg of Bisoprolol once daily, one participant on Bisoprolol 5mg once daily and one participant on Labetalol 10mg once daily). Beta-blockers can interfere with fetal growth[55, 57] and with the ability of participants increasing their HR with exercise to the level required for the study protocol (further details in Chapter 4). We did not compare the maternal characteristics and obstetric outcomes between the two studied populations, as the main purpose of this study was the validation of the PhysioFlow® against TTE for trending analysis of CO in the two different populations. These data are, therefore, simply descriptive of each of the populations. Differences in maternal characteristics and obstetric and delivery data between the two groups are discussed in Chapters 4-7.

I excluded the first trimester data from the analysis due to the small number of participants in this trimester (n = 4). Participants with CHD were recruited from the Joint Cardiac Obstetric Medicine Clinic and, by the time they attended their first appointment, most were outside the study window for

the first study visit (10-14 weeks of gestation), or could not participate on the day of recruitment and a suitable date could not be found prior to 14 weeks of gestation.

Once again, ICG provided higher CO readings than TTE at each trimester and at all timepoints, except for T2 in the second trimester which might have happened by chance. However, this specific time point also showed a statistically significant correlation between the two devices ($r = -0.63$, $p = 0.009$). At this time point, the Bland-Altman analysis showed a small (0.7L/min) mean difference which could be clinically acceptable. In the CHD group, CO ranged from 5.1 – 6.5L/min (mean CO: 5.8L/min) when measured by PhysioFlow® (please see Chapter 4) and applying a combined percentage error of 28.3% as proposed by Critchley *et al.*[192], the acceptable limits of agreement would be ±1.6L/min. CO measured at any of the time points (T1-T3) in the second and third trimester were within the acceptable calculated LOA. However, as for the low-risk group, most clinicians would probably not accept a difference of 1.6L/min of CO measured between two devices. All the remaining time points showed poor correlation when absolute values were used, as discussed above for the low-risk cohort.

The percentage error in the CHD cohort was higher than the recommended 30%[191, 200] for absolute values at all time points. Percentage errors were much higher in the CHD cohort compared with the low-risk cohort (lowest %error: 46.2% *vs* 38.1% and highest %error 95.2% *vs* 70.8%). As discussed above, the use of %error allows for the proportionality effect to be taken into consideration. Although I did not specifically compare the %errors as this was beyond the scope of this study, it seems that the CO measured by PhysioFlow® and TTE compared worse in the CHD cohort with higher %errors.

The most surprising fact in this study was that in the CHD cohort we found a concordance rate of 100%, irrespective of using 10% or 15% exclusion zone and irrespective of excluding measurements with a high CO variation of >150%. Angular bias and radial limits of agreement were also within the recommended acceptable limits[191, 200, 203] irrespective of which exclusion zone was used. Most of the participants in the CHD had their lesions repaired and, in those with native lesions, these were minor and with minimal haemodynamic implications. There is a critical band of ΔCO where the ability to

reliably detect CO changes is best.[191] There is usually a trend in the data when the ΔCO increases from 50% disagreement to total agreement and this 'optimal band' seems to lie in between 20-50% of ΔCO.[191] A few participants in the CHD cohort did not achieve the target heart rate or did not maintain their heart rate above the target for >1min (as per protocol) either due to the use of beta-blockers or due to fatigue. Importantly, all of those who did not achieve the target heart rate reported fatigue as the reason. This might explain why the concordance rate and associated angular bias and radial limits of agreement are better for the CHD cohort. The ΔCO in the low-risk cohort was broad (please see Chapter 4 for details), which leads to inhomogeneous and skewed data, a well-recognised phenomena in studies of trending analysis.[200] However, restricting the data analysis in the low-risk cohort to such a narrow band (20-50%) would lead to loss of a significant amount of data and was deemed not appropriate.

3.6. Conclusion

PhysioFlow® showed good trending ability when compared to TTE in low-risk pregnancy, as demonstrated by a concordance rate of 95.4%, angular bias of 4.5° and radial limits of agreement ≤30° when a central exclusion zone of 15% was used and when measurements with a variation of >150% were excluded.

In low-risk pregnancy, CO measured by PhysioFlow® at any of the trimesters did not correlate with CO measured by TTE at the same time points and, therefore, PhysioFlow® did not provide an accurate measure of absolute CO.

PhysioFlow® showed good trending ability when compared to TTE in pregnant women with CHD, with a concordance rate of 100%, angular bias of 4.1° and radial limits of agreement of ≤30° when a central exclusion zone of 10% was used.

In pregnancy complicated by CHD, CO measured by PhysioFlow® during the second and third trimesters did not correlate with CO measured by TTE at the same time points and therefore, PhysioFlow® did not provide an accurate measure of absolute CO in these circumstances.

Although PhysioFlow® has been used for monitoring of CO in pregnancy,[103-105] it had not yet been validated for use in pregnancy. Our study shows that PhysioFlow® can safely and reliably be used in pregnancy to study trends in CO across trimesters or potentially, during labour or delivery and may potentially help identify cardiovascular deterioration or FGR and PE, at earlier stages.

Chapter 4.

Haemodynamic parameters in pregnancy:

longitudinal assessment of haemodynamic response to exercise in low-risk pregnancy and in pregnancy complicated by congenital heart disease

Chapter 4. Haemodynamic parameters in pregnancy: longitudinal assessment of haemodynamic response to exercise in low-risk pregnancy and in pregnancy complicated by congenital heart disease

4.1. Introduction

4.1.1. *Cardiovascular adaptation in low-risk pregnancy*

Intravascular volume increases in pregnancy from as early as six weeks of gestation.[2] Peripheral vasodilatation is the initial trigger, causing a reduction in the effective arterial blood volume.[16-18] This, in turn, activates the renin-angiotensin-aldosterone system, with the release of arginine vasopressin, leading to sodium and water retention by the kidneys.[16, 18-20] The drop in TVR causes a compensatory increase in CO.[16, 21]

CO increases by at least 30% in normal low-risk pregnancy in response to the increasing demands of the fetus and placenta.[22, 24] Robson *et al.*[26] showed that CO increased as early as five weeks of gestation and by 12 weeks of gestation that increase was 34-39% above non-pregnant values. CO further increased up to 43-48% above non-pregnant values by 24 weeks of gestation and these levels were then maintained until term.

Different values have been reported regarding the magnitude and pattern of increase of CO throughout pregnancy, most likely as a result of different study design and methodologies.[24] Even greater variations are reported regarding CO in the third trimester.[27] A meta-analysis published in 2016 concludes that CO is increased compared to non-pregnant levels as early as the first trimester and that it reaches a peak by early third trimester[24] (Please see Figure 4.1).

The haemodynamic changes of normal low-risk pregnancy also include increases in the HR (15-25% above non-pregnant values) and SV (20-30% above non-pregnant values).[22, 28] SV is mainly responsible

for the increase in CO in the first half of pregnancy and the gradual increase in HR is mainly responsible for the increase in CO thereafter.[29]

TVR decreases until 20-28 weeks of gestation (30% decrease compared to non-pregnant values)[24] and this is followed by a small increase thereafter.[26] The fall in TVR and the increase in CO largely compensate for one other, hence there are only small differences in the MAP during pregnancy.[22] BP, the result of the product of SV and TVR,[25] changes in pregnancy following a 'J curve' pattern with a trough of systolic and diastolic BP around 24-28 weeks of gestation compared to early pregnancy (< 12 weeks of gestation) and an increase in BP from 28 weeks of gestation towards term, with higher BP at 40 weeks of gestation when compared to early pregnancy.[205, 206] The typical 'mid-trimester fall' in BP has been disputed by some authors and Nama *et al.*[207] showed that BP rose progressively in pregnancy without a significant 'mid-trimester drop'. More recently, Green *et al*.[208] who included >1,000 low-risk pregnant women, demonstrated that systolic BP decreased from 12 weeks of gestation to a nadir around 18 weeks of gestation (drop of -1.0mmHg) and, a progressive increase in systolic BP from 19 weeks to a maximum median at 40 weeks of gestation (increase of +7mmHg). Diastolic BP also decreased from 12 weeks of gestation to a nadir around 19 weeks (drop -1mmHg) with progressive increase thereafter to 40 weeks of gestation (increase +9mmHg).[208]

Data on HR and peripheral oxygen saturations (SpO_2) in pregnancy are limited.[208] HR increases progressively up to around 34 weeks of gestation, decreasing thereafter.[208-211] SpO_2 decreases moderately during pregnancy to a median value of 97% at 40 weeks of gestation.[208, 212]

4.1.2. *Cardiovascular adaptation to pregnancy in women with congenital heart disease*

Cardiovascular adaptation to pregnancy in women with CHD has not been extensively studied and most available data are retrospective.[31] In women with CHD, obstetric and offspring complications seem to be related to their cardiovascular function, however the pathophysiology of this relationship is not yet understood.[32]

In women with structural heart disease, cardiovascular adaptation to pregnancy is attenuated.[31, 33] There is an increase in CO and SV as observed in normal low-risk pregnancies, however, the magnitude of the increase is less and, after a steep increase in the first half of pregnancy, it plateaus for the second half of pregnancy.[33] The largest longitudinal study on cardiovascular adaptation to pregnancy in women with CHD (n = 125)[31] showed that women with CHD have worse LV systolic function when compared to controls and the slope of LV ejection fraction is significantly different. However, although the absolute values on LV function and dimensions in women with CHD were different to controls, pregnancy did not generally lead to worsening cardiac function.[33] Another study from 2010 reported no impairment in systolic function and no persistent dilatation of the LV in women with pre-existing heart disease when compared to controls, except for women with ToF where a persistent dilatation of the subpulmonary ventricle was found.[34]

4.2. Hypotheses and Aims

Hypothesis 5: *There is a second trimester drop in BP in normal low-risk pregnancy and this is not demonstrated in pregnant women with CHD.*

Aim: To investigate longitudinally the maternal BP in low-risk pregnant women and compare this to the BP of pregnant women with CHD.

Hypothesis 6: *In pregnancy, there is a progressive increase in the HR with advancing gestation.*

Aim: To investigate longitudinally the maternal HR in low-risk pregnant women and compare this to the HR of pregnant women with CHD.

Hypothesis 7: *In pregnancy, there is a decrease in peripheral oxygen saturations (SpO_2) with advancing gestation.*

Aim: To investigate longitudinally the maternal SpO_2 in low-risk pregnant women and compare this to the SpO_2 of pregnant women with CHD

Hypothesis 8: *In pregnancy, there is a progressive increase in CO until 34 weeks of gestation.*

Aim: To investigate longitudinally the maternal haemodynamics (SV and CO) as assessed by measurement of cardiac output with ICG and TTE of low-risk women.

Hypothesis 9: *In pregnancies complicated by heart disease, there is a suboptimal increase in CO and SV and/or a suboptimal decrease in TVR.*

Aim: To investigate longitudinally the maternal haemodynamics as assessed by measurement of CO and SV with ICG and TTE and investigate differences in BP between pregnant women with CHD and low-risk pregnant women.

4.3. Materials and Methods

4.3.1. *Study design and participant recruitment*

As described in Chapter 2.

4.3.2. *Study procedures*

Demographic data were collected on the first visit and included age, race, booking height, weight and BMI, gravidity, parity, EDD (based on CRL from first trimester ultrasound), GA at the time of the visit, smoking status, type of CHD lesion (if applicable) and use of medication. Data on delivery information, collected at a later date, included GA at delivery, onset of labour (spontaneous or IOL and indications, where applicable), MOD and indications for assisted delivery or Caesarean section where applicable, sex of the newborn, BW, BW centile according to the Aberdeen Maternity and Neonatal Databank nomograms [188] and EBL.

After a 5-minute rest period, six PhysioFlow® electrodes were placed according to the manufacturer's instructions.[204] Women were asked to lie in the left lateral position (15º tilt and 15º head elevation), the right arm circumference was measured and the blood pressure also measured on that same arm (unless

contraindicated, in which case the left arm was used; this only occurred once). A peripheral oxygen saturations probe was placed on the right index finger (unless contraindicated, in which case the left index finger was used) and a measurement of SpO_2 and HR was performed. The PhysioFlow® device was then calibrated according to the manufacturer's instructions and live continuous monitoring initiated. Please see Chapter 2 for detailed information about each of these steps.

PhysioFlow® measurements were continuous, data were analysed offline and, to allow direct comparison between participants, measurements at the following time points were taken (please see Figures 4.1 and 4.2):

- **T1 (rest)**: average measurement after attaching the PhysioFlow® leads and after signal stabilisation, and before transfer to ergometer cycle. Measurement performed in left lateral. After this, an annotation was made when the participants moved from left lateral to sitting upright on the ergometer cycle ('TX BIKE').
- **Upright (*1)**: peak HR, and corresponding CO and SV measurements were taken when participants were sitting upright in the ergometer cycle waiting for the HR to stabilise prior to starting exercise.
- **'Baseline' prior to exercise (*2)**: New 'baseline' HR, and corresponding CO and SV, prior to starting exercise. An annotation was made when the exercise was started ('START EXERCISE'). Participants could exercise at their own pace/intensity and the goal was to achieve the target HR as described in the methodology. An annotation was made once participants achieved the target HR and were asked to maintain HR above the target HR for at least 60 seconds.
- **Peak Exercise (*3)**: peak HR, and corresponding CO and SV measurements were taken at peak HR during exercise. An annotation was made when exercise finished and participants transferred back to the left lateral position ('TX BED')
- **T3 (recovery)**: Average HR, and corresponding SV and CO, in the recovery phase (waited for HR to be ±20% of initial HR).

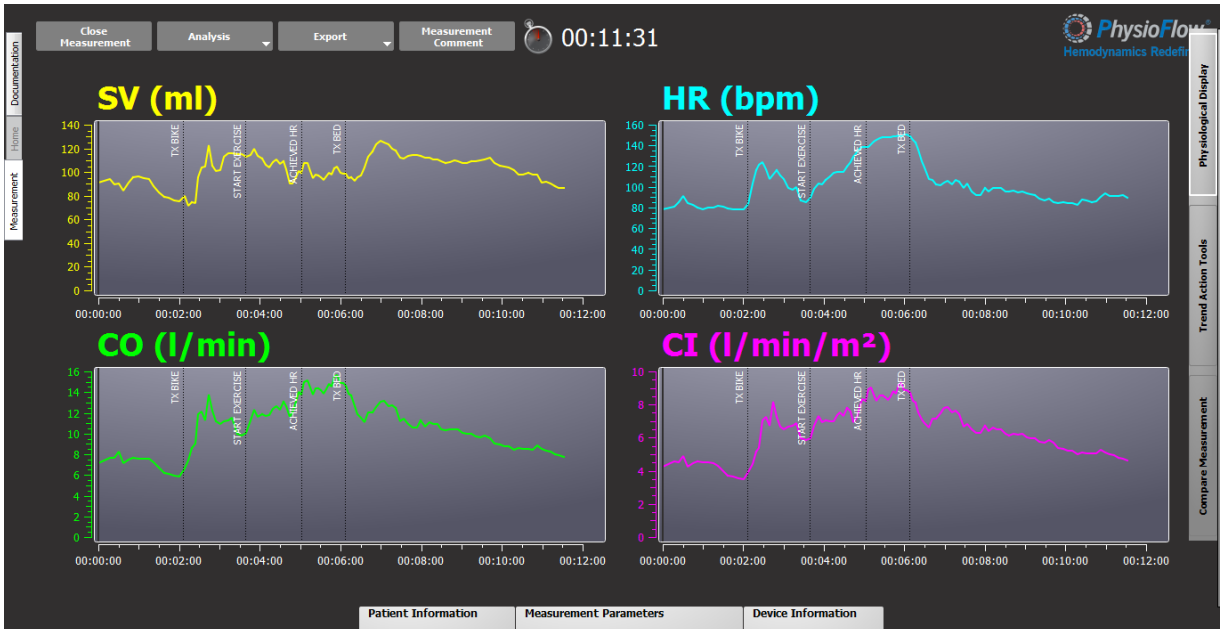


Figure 4.1 *– Representation of the screen on the PhysioFlow® software illustrating four parameters (user's choice) being continuously measured over time: stroke volume (SV), heart rate (HR), cardiac output (CO) and cardiac index (C_i). Please note the annotations made relating to study procedures to allow direct comparison between participants and data analysis.*

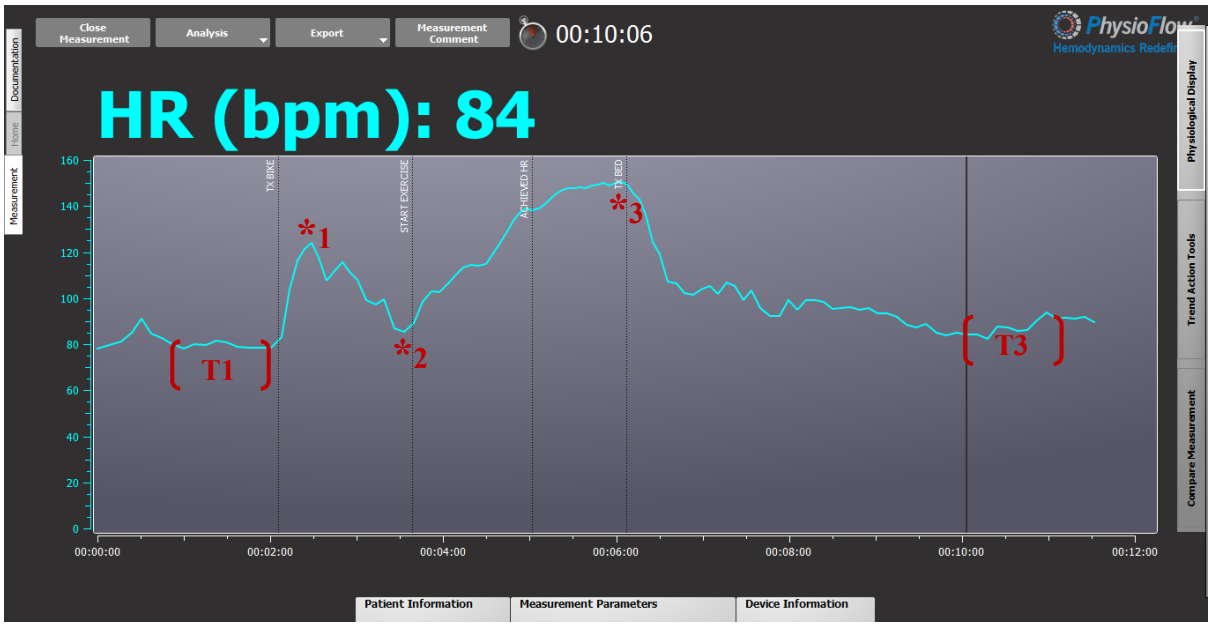


Figure 4.2 *– PhysioFlow® software screen illustrating changes in heart rate (HR) over time and marked with relevant study procedures' labels. T1 – time point one (rest); T3 – time point three (after recovery); *1 – Maximum HR after transfer to cycle; *2 – Pre-exercise HR; *3 – Peak exercise HR.*

For participants having TTE, three additional electrodes were placed (as per standard locations) to provide a timing ECG signal for the TTE machine. The PLAX view was obtained for measurement of the LVOT. Then, the apical A5C view was obtained to acquire the LVOT VTI (at least three consecutive good quality waves for each time point). Measurements were continuous for the ICG and obtained at three time points from the TTE: T1 – rest, T2 – immediately after exercise and T3 – after recovery. For

the purpose of direct comparison, it was marked on the PhysioFlow® software when the acquisition of the Doppler VTI was taking place at each of the time points and an average measurement used for each time point. After acquisition of T1, participants were asked to exercise on an ergometer cycle to achieve at least 70% of the predicted maximum heart rate, and once this was achieved, to continue above this level for at least 60 seconds, after which they immediately transferred to the left lateral position and the second TTE measurement was made (T2, marked on PhysioFlow® software). A second measurement of BP, HR and SpO_2 was also taken at the same time. Women were then allowed to rest and, once their HR was ±20% of the T1 HR, a third TTE measurement was taken (T3, marked on PhysioFlow® software), alongside a third measurement of BP, HR and SpO_2. Please note figures 4.3 and 4.4.

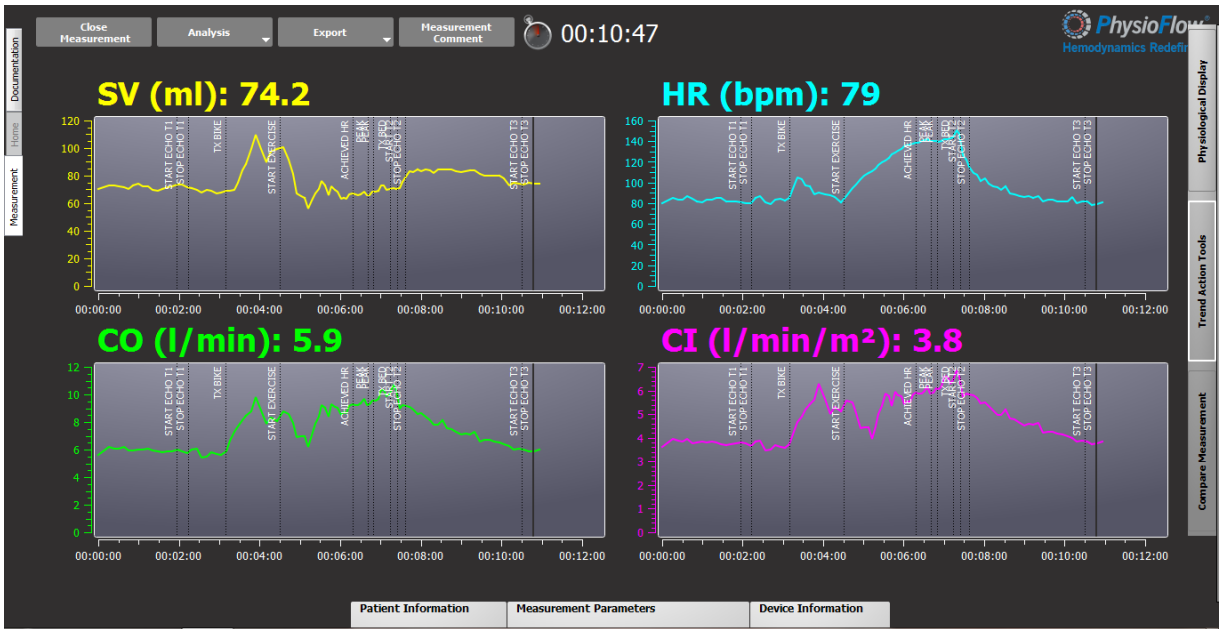


Figure 4.3 *– Representation of the screen on the PhysioFlow® software illustrating four parameters (user's choice) being continuously measured over time: stroke volume (SV), heart rate (HR), cardiac output (CO) and cardiac index (C_i) in participants having transthoracic echocardiogram. Please note the annotations entered for when echocardiogram assessment and other study procedures were taking place – these were to allow direct comparison between PhysioFlow® and echocardiographic measurements, and direct comparison between participants.*

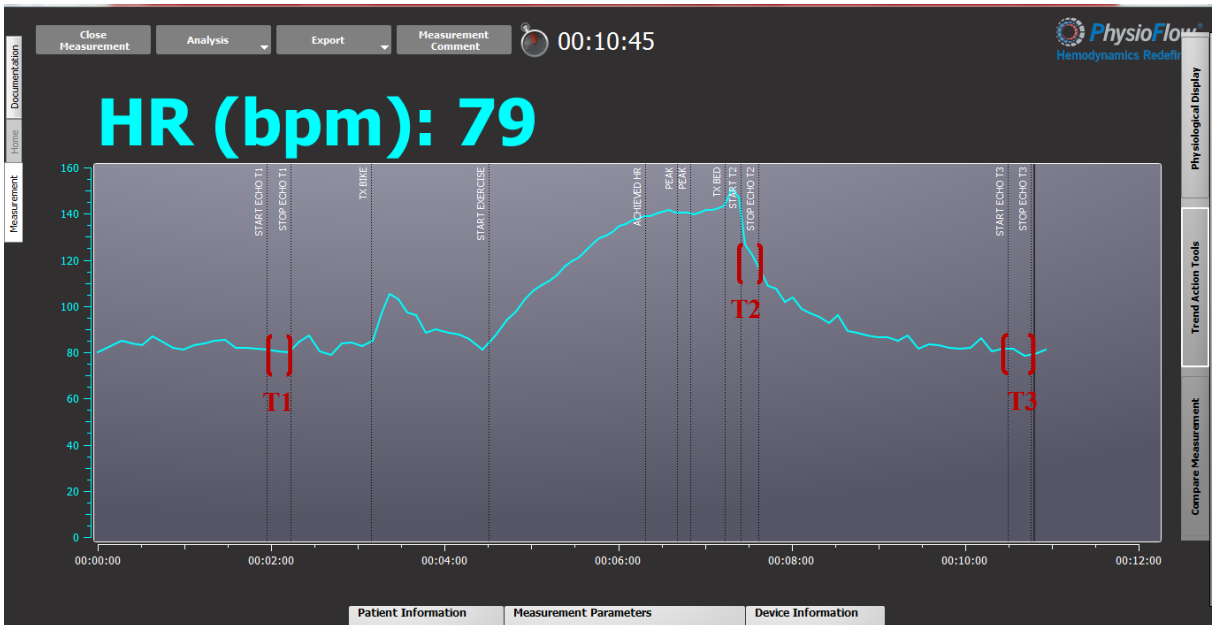


Figure 4.4 *– PhysioFlow® software screen illustrating changes in heart rate (HR) over time and marked with relevant study procedures. T1 – average HR between the start and end of velocity-time integral (VTI) acquisition at rest; T2 – average HR between the start and end of the VTI acquisition immediately after exercise; T3 - average HR between the start and end of the VTI acquisition after recovery.*

To allow comparison between participants having PhysioFlow® and TTE measurements (for the purpose of validation of ICG against TTE [Chapter 3] and for the purpose of direct comparison between participants [Chapter 4]), the following time points were analysed:

- **Rest (T1)**: Average HR, CO and SV measured on PhysioFlow®, between the start and end of the TTE acquisition of VTI at rest. Measurements were obtained in left lateral.
- **Immediately post exercise (T2)**: Average HR, CO and SV measured on PhysioFlow®, between the start and end of the TTE acquisition of VTI immediately post-exercise. Measurements obtained in left lateral.
- **Recovery (T3)**: Average HR, CO and SV measured on PhysioFlow®, between the start and end of the TTE acquisition of VTI in the recovery phase (defined as above).

The cardiac physiologist was blinded for the ICG measurements and the researcher operating the ICG was blinded for the TTE measurements. All data were analysed offline.

4.3.3. *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics, version 26.0, 2019 (*IBM Corp., Armonk, New York, USA*) and Microsoft® Excel for MAC, version 16.30, 2019 (*Microsoft Corp., Redmond, WA, USA*). The Kolmogoroff–Smirnoff and the Shapiro-Wilk tests were used to assess normality of the distribution of the data, as well as visual inspection of the histograms and Q-Q' normality plots. Differences between the two study groups were performed using Mann-Whitney U or Chi-square test, as appropriate. Mean ± SD were calculated at each time point and for each device, if data followed a Gaussian distribution. Median (min-max) were calculated if data did not follow a Gaussian distribution. Trends between trimesters in each of the study groups were assessed using Paired t-test for Gaussian data and Wilcoxon matched-pair signed-rank for non-Gaussian data. Differences between the two study groups were analysed using Mann-Whitney U tests.

4.3.4. *Sample size*

Sample size calculation was performed using the G*Power software (*G*Power for Mac Os X, v. 3.1, February 2020, Heinrich-Heine-Universität, Dusseldorf, Germany*).

To demonstrate a similar effect to Mahendru *et al.*[43] in systolic and diastolic BP and for HR changes across the three trimesters of pregnancy, for an alpha = 0.05 and a power of 95%, we would need 35 study subjects. To obtain a similar result to Masini *et al.*[83] with regard to CO changes in pregnancy, for an alpha = 0.05 and a power of 80%, we would need 42 study subjects. For differences between groups, to detect a similar result to Uebing *et al.*[34], for an alpha = 0.05 and 80% power, we would need 160 subjects in each group.

4.4. Results

4.4.1. *Maternal characteristics and obstetric outcomes*

Table 4.1 describes the maternal characteristics and obstetric outcomes of the participants included in the longitudinal study and Table 4.2 the specific cardiac lesions of the participants with CHD.

Table 4.1 – *Characteristics and obstetric outcomes of participants included in the 'Haemodynamic parameters in pregnancy: longitudinal assessment across trimesters and haemodynamic response to exercise in low-risk pregnancy and in pregnant women with congenital heart disease' study. LR – low-risk pregnant women; CHD – pregnant women with congenital heart disease; P – parity. Significance level: p<0.05.*

	LR N = 45	**CHD** N = 28	**p value**
Age, years, median (min - max)	33 (24-42)	31 (23-39)	0.018*
Race, n (%)			0.51
White	39 (86.7)	26 (92.8)	
Non-white	6 (13.3) [5 Asian, 1 Middle-Eastern]	2 (7.2) [1 Asian, 1 Middle-Eastern]	
Weight, kg, median (min - max)	63.0 (43.0-100.0)	66.5 (49.0-101.0)	0.33
BMI, kg/m^2, median (min-max)	22.5 (18.0-34.0)	24.1 (17.6-34.9)	0.22
Smoking, n (%)			0.41
Never smoked	38 (84.4)	25 (89.2)	
Current/past smoker	7 (15.6)	3 (10.8)	
Parity, n (%)			0.23
P0	33 (73.3)	16 (57.1)	
P ≥1	12 (26.7)	12 (42.9)	
Mode of delivery, n (%)			0.55
Spontaneous Vaginal Delivery	16 (35.6)	9 (32.1)	
Ventouse Vaginal delivery	3 (6.6)	1 (3.7)	
Forceps Vaginal Delivery	5 (11.1)	2 (7.1)	
Emergency Caesarean Section	14 (31.1)	7 (25.0)	
Elective Caesarean Section	7 (15.6)	9 (32.1)	
Mode of delivery, n (%)			0.34
Vaginal (all, including instrumental)	24 (53.3)	12 (42.9)	
Caesarean Section (all)	21 (46.7)	16 (57.1)	
Gestational Age at delivery median (min - max)	39+5 (35+4-42+0)	38+5 (34+3-41+0)	<0.001***
Sex of the baby, n (%)			0.23
Female	16 (35.6)	14 (50.0)	
Male	29 (64.4)	14 (50.0)	
Birthweight, g, median (min - max)	3360 (2580-5220)	2940 (1970-3855)	0.003**
Birthweight Centile, %, median (min-max)	55.0 (3.0-100.0)	30.0 (6.0-97.0)	0.007**
Estimated Blood Loss, mL, median (min - max)	400 (100-2005)	500 (100-1800)	0.15

*p<0.05, **p<0.01, ***p<0.001

Table 4.2 – *Specific cardiac lesions of participants with congenital heart disease (CHD) included in the study. AS – aortic stenosis; ASD – atrial septal defect; BAV – bicuspid aortic valve; CoA – coarctation of the aorta; PDA – patent ductus arteriosus PVR – pulmonary valve regurgitation; r – repaired; SA – subaortic stenosis; ToF – Tetralogy of Fallot; TCPC – total cavopulmonary connection; TGA – transposition of great arteries; TVR – tricuspid valve regurgitation; VSD – ventricular septal defect. N=28. Main lesion in bold and lesion-related features in italic.*

CHD lesion	N
rTOF	**8**
rTOF only	*7*
rTOF + rSA stenosis	*1*
rCoA	**5**
rCoA only	*1*
rCoA + BAV +rVSD	*1*
rCoA + BAV	*3*
rTGA	**4**
rTGA (arterial switch)	*2*
rTGA (arterial switch) + PS	*1*
rTGA (atrial switch)	*1*
VSD (unrepaired)	**3**
AS	**1**
Fontan TCPC	**1**
Ebstein's Anomaly	**1**
Right hemi-truncus	**1**
Pulmonary Atresia + PVR + TVR	**1**
Mixed Lesions	**3**
ASD + rVSD + BAV	*1*
rVSD + rPDA + BAV	*1*
BAV	*1*

In the low-risk group, none of the participants were taking medication. In the CHD group, nine participants (32%) were taking medication: two were on low-molecular-weight heparin (LMWH) and aspirin, one on aspirin, one on thyroxine and metformin, and five on beta-blockers (two on bisoprolol 2.5mg once daily, one on bisoprolol 5mg once daily, one on labetalol 100mg once daily, and one on sotalol 80mg twice daily and sertraline).

4.4.2. *Blood pressure, heart rate and peripheral oxygen saturations' trends across trimesters in low-risk pregnancy and in pregnant women with CHD – differences between groups*

The results for BP, HR and SpO2 trends across trimesters and at each time point are presented in Tables 4.3 and 4.4 for the low-risk group and in Tables 4.5 and 4.6 for the CHD group. With the exception of the first trimester data for the CHD group where BP, HR and SpO_2 did not follow a Gaussian distribution, for both groups and at all trimesters BP and HR data followed a Gaussian distribution and SpO_2 data were non-Gaussian.

Table 4.3 – *Systolic and diastolic blood pressure (BP), heart rate (HR) and peripheral oxygen saturations (SpO_2) at the different study time points (T1 – rest, T2 – immediately post exercise, T3 – recovery) and in each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation) in low-risk pregnancy. N = 45 in each trimester (135 measurements, 3 per participant).*

Trimester	Time point	**Systolic BP** Mean ± SD	**Diastolic BP** Mean ± SD	**HR** Mean ± SD	**SpO_2** Median (min-max)
A	**T1**	99.7 ± 9.5	53.2 ± 9.9	72 ± 10	98 (96-99)
	Post-exercise	124.6 ± 15.2	66.3 ± 12.9	110 ± 15	98 (95-99)
	T3	103.4 ± 10.7	56.2 ± 10.3	77± 9	98 (96-99)
B	**T1**	95.7 ± 9.8	48.8 ± 9.4	73 ± 9	98 (96-99)
	Post-exercise	119.0 ± 13.7	63.2 ± 12.8	114 ± 13	98 (90-99)
	T3	99.4 ± 9.9	54.0 ± 10.0	77 ± 8	98 (96-99)
C	**T1**	98.6 ± 8.8	52.5 ± 8.3	81 ± 12	97 (95-99)
	Post-exercise	121.4 ± 14.0	61.9 ± 11.8	113 ± 12	98 (96-99)
	T3	101.3 ± 10.4	54.9 ± 9.9	83 ± 11	98 (94-99)

Table 4.4 – *Summary of Paired t-test comparing differences in blood pressure (BP) and heart rate (HR) and summary of the Wilcoxon matched-paired signed-rank test for peripheral oxygen saturations (SpO_2) between trimesters (A, B and C) in low-risk pregnancy. Significance level: $p<0.05$.*

Trimesters	Time point	Systolic BP	Diastolic BP	HR	SpO_2
Comparison A/B	T1	**0.009****	**0.003****	0.53	0.11
	Post-exercise	**0.036***	0.17	0.16	0.43
	T3	**0.015***	0.15	0.87	0.55
Comparison B/C	T1	0.09	**0.003****	**<0.001*****	**0.012***
	Post-exercise	0.19	0.58	0.56	0.07
	T3	0.16	0.60	**<0.001*****	0.16
Comparison A/C	T1	0.37	0.65	**<0.001*****	**<0.001*****
	Post-exercise	0.10	0.08	**<0.001*****	0.11
	T3	0.14	0.47	**<0.001*****	0.12

*p<0.05, **p<0.01, ***p<0.001. All significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 4.5 *– Systolic and diastolic blood pressure (BP), heart rate (HR) and peripheral oxygen saturations (SpO_2) at the different study time points (T1 – rest, T2 – immediately post exercise, T3 – recovery) and in each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation) in pregnant women with congenital heart disease (CHD). Data not truly longitudinal.*

Trimester	Time point	Systolic BP Median (min-max)	Diastolic BP Median (min-max)	HR Median (min-max)	SpO_2 Median (min-max)
A, N = 4 (2 participants unable to achieve target HR)[1]	**T1**	106.0 (97-122)	55.5 (47-58)	76 (71-78)	97 (96-99)
	Post-exercise	134.0 (123-138)	67.5 (63-72)	105 (75-127)	98 (93-99)
	T3	110.0 (106-117)	55.0 (50-66)	78 (71-84)	98 (97-99)
B, N = 18 (1 participant unable to achieve target HR)[2]	**T1**	101.0 (80-115)	50.5 ± (34-62)	78 (66-102)	98 (96-99)
	Post-exercise	122.0 (99-140)	63.0 (42-80)	113 (93-128)	98 (92-99)
	T3	99.5 (84-115)	54.5 (37-63)	80 (66-113)	98 (96-99)
C, N = 20 (3 participants unable to achieve target HR)[3]	**T1**	105.5 (88-124)	52.5 (42-78)	84 (65-97)	97 (95-99)
	Post-exercise	126.0 (105-170)	69.0 (50-100)	109 (75-138)	98 (92-99)
	T3	109.0 (92-127)	61.0 (45-81)	83 (68-99)	97 (93-99)

[1] One participant on Sotalol 80mg twice daily. Both participants unable to achieve target HR due to fatigue.
[2] Participant unable to achieve target HR due to fatigue. Not on beta-blockers.
[3] One participant on Sotalol 80mg twice daily. All three participants unable to achieve target HR due to fatigue.

Table 4.6 *– Summary of Wilcoxon matched-paired signed-rank comparing differences in blood pressure (BP), heart rate (HR) and peripheral oxygen saturations (SpO_2) between trimesters in pregnant women with congenital heart disease (CHD). Significance level: $p<0.05$.*

Trimester	Time point	Systolic BP	Diastolic BP	HR	SpO_2
Comparison	T1	0.65	0.18	1.00	1.00
A/B	Post-exercise	0.18	0.18	0.18	0.43
N = 2	T3	0.18	0.32	0.32	0.18
Comparison	T1	0.84	0.35	0.10	0.12
B/C	Post-exercise	0.51	0.53	0.18	0.06
N = 11	T3	0.13	0.33	0.07	0.56
Comparison	T1	1.00	0.11	0.18	0.16
A/C	Post-exercise	0.11	0.59	0.16	0.41
N = 3	T3	0.59	1.00	0.79	0.18

All significance values have been adjusted by the Bonferroni correction for multiple tests

BP, HR and SpO2 in the CHD group did not follow a Gaussian distribution in the first trimester and therefore, for the differences between the two study groups, median (min-max) values are presented below – a summary of the Mann-Whitney U test for differences between the study groups are presented in Table 4.7. Median systolic and diastolic BP, HR and SpO_2 differences between the two study groups and across the three trimesters for T1 (measurements at rest) are presented in Figures 4.5-4.16. Table 4.8 shows an overall summary of the differences (not separated by trimester) in BP, HR and SpO_2 between the two study groups by study time point.

Table 4.7 *– Summary of Mann-Whitney U test for differences between the CHD (congenital heart disease) and the LR (low-risk) cohort at of the study time points (T1 – rest, T2 – immediately post exercise, T3 – after recovery) at each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation). Significance level: p<0.05*

Trimester	**Time point**	**Systolic blood pressure, mmHg** Median (min-max)			**Diastolic blood pressure, mmHg** Median (min-max)			**Heart rate, bpm** Median (min-max)			**Peripheral oxygen saturations, %** Median (min-max)		
		LR	CHD	**p value**	LR	CHD	**p value**	LR	CHD	**p value**	LR	CHD	**p value**
A LR=45 CHD=4	T1	98 (82-122)	106 (97-122)	**0.12**	52 (30-76)	55.5 (47-58)	**0.56**	71 (55-95)	76 (71-78)	**0.49**	98 (96-99)	97 (96-99)	**0.19**
	T2	126 (89-153)	134 (123-138)	**0.29**	65 (45-108)	67.5 (63-72)	**0.61**	108 (80-144)	105 (75-127)	**0.60**	98 (95-99)	98 (93-99)	**0.67**
	T3	101 (83-127)	110 (106-117)	**0.10**	56 (41-88)	55 (50-66)	**0.86**	78 (58-96)	78 (71-84)	**0.91**	98 (96-99)	99 (97-99)	**0.47**
B LR=45 CHD=18	T1	94 (76-114)	101 (80-115)	**0.11**	48 (31-80)	50.5 (34-62)	**0.30**	71 (60-94)	78 (66-102)	**0.005****	98 (96-99)	98 (96-99)	**0.96**
	T2	119 (92-146)	122 (99-140)	**0.50**	61 (41-116)	63 (42-80)	**0.71**	115 (89-146)	113 (93-128)	**0.53**	98 (90-99)	98 (92-99)	**0.72**
	T3	99 (81-131)	99.5 (84-115)	**0.56**	51 (39-81)	54.5 (37-63)	**0.70**	78 (59-97)	80 (66-113)	**0.047***	98 (96-99)	98 (96-99)	**0.91**
C LR=45 CHD=20	T1	98 (81-119)	105.5 (88-124)	**0.007****	52 (39-75)	52.5 (42-78)	**0.19**	78 (60-105)	84 (65-97)	**0.26**	97 (95-99)	97 (95-99)	**0.56**
	T2	120 (91-153)	126 (105-170)	**0.11**	60 (42-96)	69 (50-100)	**0.012***	114 (83-137)	109 (75-138)	**0.038***	98 (96-99)	98 (92-99)	**0.59**
	T3	100 (72-120)	109 (92-127)	**0.009****	54 (31-74)	61 (45-81)	**0.08**	83 (58-103)	83 (68-99)	**0.79**	98 (94-99)	97 (93-99)	**0.24**

*p<0.05, **p<0.01, ***p<0.001.

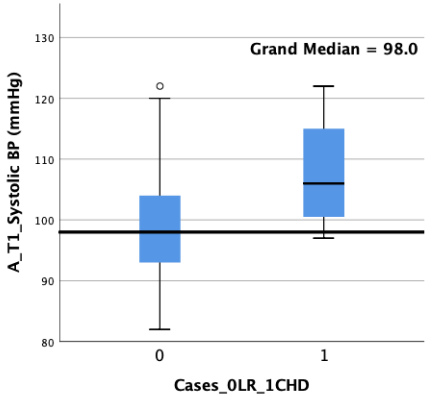


Figure 4.5 *– Systolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 1st trimester (A).*

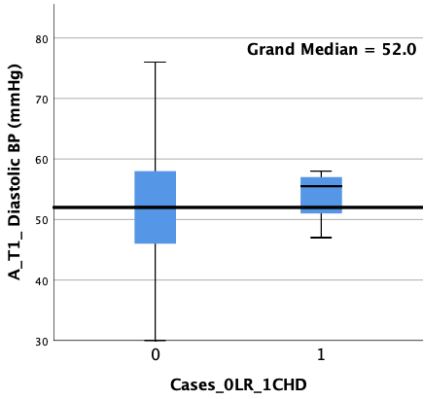


Figure 4.6 *– Diastolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 1st trimester (A).*

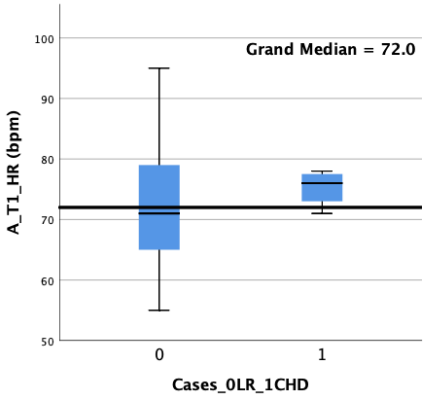


Figure 4.7 *– Heart rate (HR) in the two study groups (0 – LR, 1 – CHD) in the 1st trimester (A).*

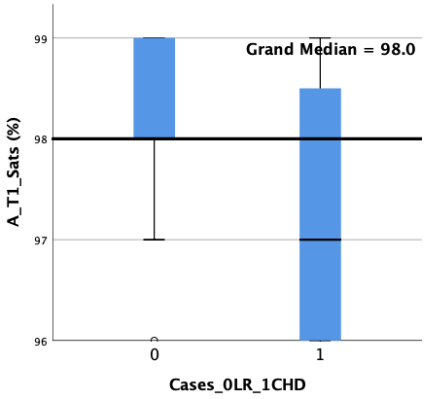


Figure 4.8 *– Peripheral oxygen saturations (SpO_2) in the two study groups (0 – LR, 1 – CHD) in the 1st trimester (A).*

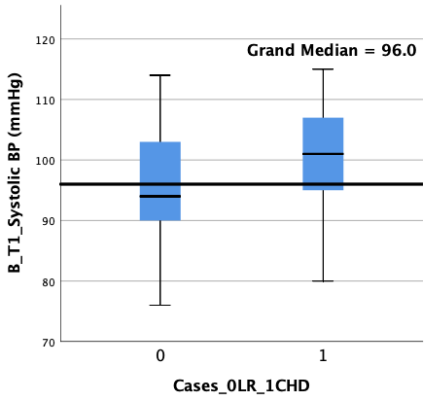


Figure 4.9 *– Systolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 2nd trimester (B).*

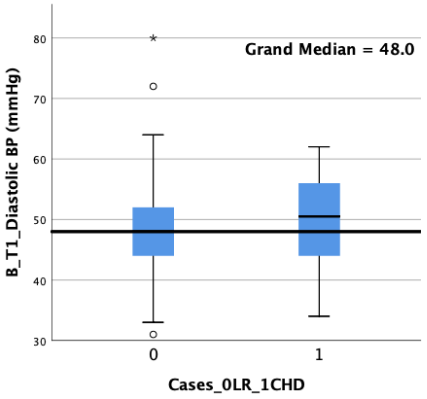


Figure 4.10 *– Diastolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 2nd trimester (B).*

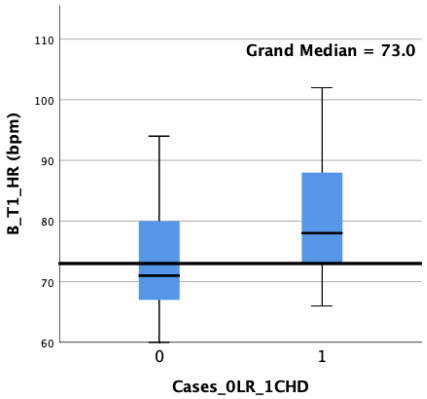


Figure 4.11 *– Heart rate (HR) in the two suay groups (0 – LR, 1 – CHD) in the 2nd trimester (B).*

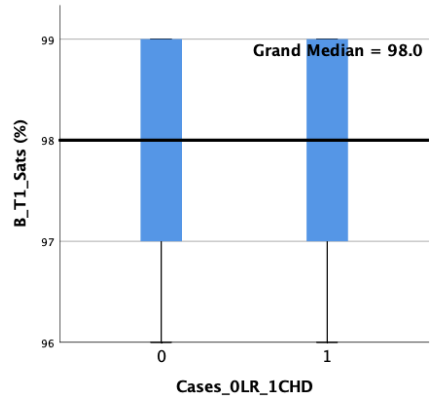


Figure 4.12 *– Peripheral oxygen saturations (SpO_2) in the two study groups (0 – LR, 1 – CHD) in the 2nd trimester (B).*

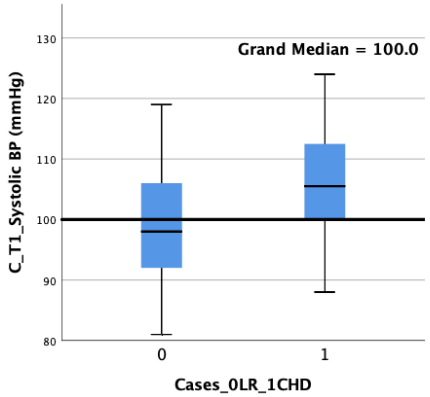


Figure 4.13 *– Systolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 3rd trimester (C).*

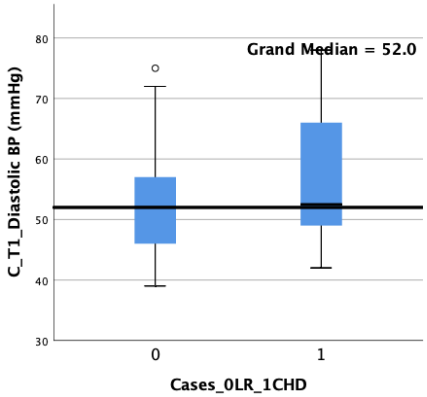


Figure 4.14 *– Diastolic blood pressure (BP) in the two study groups (0 – LR, 1 – CHD) in the 3rd trimester (C).*

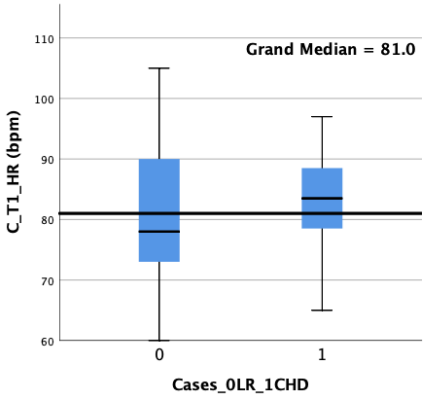


Figure 4.15 *– Heart rate (HR) in the two study groups (0 – LR, 1 – CHD) in the 3rd trimester (C).*

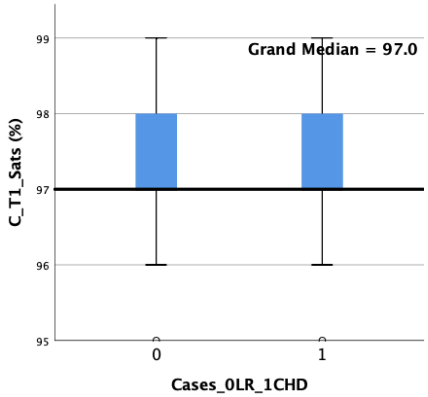


Figure 4.16 *– Peripheral oxygen saturations (SpO_2) in the two study groups (0 – LR, 1 – CHD) in the 3rd trimester (C).*

Table 4.8 *– Summary of Mann-Whitney U test for overall differences (not separated by trimester) in blood pressure (BP), heart rate (HR) and peripheral oxygen saturation (SpO_2) between the two study groups by time point (T1 – rest, T2 – immediately post exercise, T3 – recovery). Significance level: p<0.05*

Time point	Systolic BP	Diastolic BP	HR	SpO_2
T1	**0.001*****	0.14	**0.001*****	0.09
T2	0.18	0.08	0.12	0.58
T3	**0.019***	0.23	**0.031***	0.40

*p<0.05, **p<0.01, ***p<0.001.

4.4.3. *Stroke volume, cardiac output and heart rate trends measured with PhysioFlow® - longitudinal assessment*

SV, CO and HR measured by PhysioFlow® followed a Gaussian distribution in the low-risk group – results presented in Tables 4.9 and 4.10.

Table 4.9 *– Stroke volume (SV), cardiac output (CO) and heart rate (HR) measured by PhysioFlow® at the different study time points (T1 – rest, peak exercise, T3 – recovery) and in each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation) in low-risk pregnancy. N = 45 in each trimester (135 measurements, 3 measurements per participant).*

Trimester	Time point	SV (ml) Mean ± SD	CO (l/min) Mean ± SD	HR (bpm) Mean ± SD
A	T1	85.0 ± 16.2	6.1 ± 1.2	73 ± 10
	Peak Exercise	106.0 ± 22.1	14.2 ± 2.6	135 ± 8
	T3	90.3 ± 18.9	7.1 ± 1.6	79 ± 10
B	T1	82.7 ± 15.2	6.0 ± 1.1	74 ± 10
	Peak Exercise	107.1 ± 26.3	14.3 ± 3.3	135 ± 11
	T3	91.0 ± 18.6	7.0 ± 1.6	78 ± 8
C	T1	78.6 ± 15.0	6.2 ± 1.3	80 ± 12
	Peak Exercise	106.2 ± 25.2	14.0 ± 3.0	135 ± 12
	T3	90.4 ± 19.1	7.4 ± 1.6	83 ± 11

Table 4.10 *– Summary of Paired t-test comparing differences in stroke volume (SV), cardiac output (CO) and heart rate (HR) at the different time points (T1 – rest, peak exercise, T3 – recovery) across trimesters, in low-risk pregnancy. Significance level: p<0.05.*

Trimester	Time point	SV (ml)	CO (l/min)	HR (bpm)
Comparison A/B	T1	0.28	0.54	0.50
	Peak Exercise	0.69	0.76	0.89
	T3	0.77	0.49	0.25
Comparison B/C	T1	0.052	0.38	**<0.001*****
	Peak Exercise	0.83	0.60	0.92
	T3	0.80	**0.042***	**0.001*****
Comparison A/C	T1	**0.018***	0.70	**<0.001*****
	Peak Exercise	0.94	0.68	0.97
	T3	0.98	0.20	0.06

*p<0.05, **p<0.01, ***p<0.001. All significance values have been adjusted by the Bonferroni correction for multiple tests.

4.4.4. *Stroke volume, cardiac output and heart rate response to exercise (exercise above 70% of predicted or maximum heart) assessed by impedance cardiography (PhysioFlow®) in the low-risk group*

Table 4.11 illustrates the haemodynamic response to exercise in the low-risk group. The different time points studied are described in detail in the methods section.

Table 4.11 *– Heart rate (HR), stroke volume (SV) and cardiac output (CO) measured by PhysioFlow® at different time points in each of the trimesters (A – 10-14 weeks of gestation; B – 18-22 weeks of gestation; C – 30-34 weeks of gestation) for the low-risk group. T1 (rest): average measurement at rest; Upright: maximum HR and corresponding SV and CO after changing position from left lateral to sitting upright on the ergometer cycle; Relative increase ([Upright-T1]/Upright): relative increase in HR, SV and CO between upright and T1; 'Baseline' prior to exercise: baseline HR, SV and CO after HR returned to levels similar to those of T1 HR; Relative increase (['Baseline'-T1]/'Baseline): relative increase in HR, SV and CO between new 'baseline' after change of position and rest; Peak Exercise: Peak HR and corresponding SV and CO at peak HR during exercise; Relative increase ([PeakEx-T1]/PeakEx): relative increase in HR and corresponding SV and CO between peak exercise and rest; Relative increase ([PeakEx-'Baseline']/PeakEx): relative increase in HR and corresponding SV and CO between peak exercise and 'baseline' after position change; T3 (recovery):measurement in the recovery phase.*

		T1 (Rest)	**Upright** (supine to upright position change [***1**])	**Relative Increase, %** (Upright-T1/Upright)	**'Baseline' Prior to Ex** (After HR returned to T1 **[*2]**)	**Relative Increase, %** from rest	**Peak Exercise [*3]**	**Relative Increase, %** (PeakEx-T1/PeakEx)	**Relative Increase, %** (PeakEx-'Baseline')/ PeakEx)	**T3** (Recovery)
A	**HR (bpm)** Median (min-max)	74 (53-95)	105 (82-136)	31 (18-42)	83 (64-103)	10 (-6-23)	135 (115-151)	47 (24-63)	39 (18-56)	79 (58-99)
	SV (ml) Median (min-max)	81.7 (51.0-115.2)	87.1 (29.4-133.6)	0.5 (-73-47)	99.2 (8.1-164.5)	16 (-41-57)	100.4 (74.0-168.0)	18 (-14-55)	1.0 (-33-91)	88.6 (40.8-127.0)
	CO (l/min) Median (min-max)	6.3 (3.1-8.9)	9.0 (3.0-13.9)	31 (-10-62)	8.2 (0.7-15.0)	26 (-26-59)	13.7 (10.4-23.3)	57 (34-78)	41 (24-94)	6.9 (2.9-10.6)
B	**HR (bpm)** Median (min-max)	73 (56-92)	104 (81-132)	31 (6-46)	80 (58-113)	9 (-11-35)	137 (102-158)	45 (20-60)	41 (3-59)	77 (59-98)
	SV (ml) Median (min-max)	81.7 (48.0-111.7)	82.8 (46.0-128.2)	3 (-72-5)	103.4 (59.9-157.6)	19 (-10-44)	99.8 (70.2-177.3)	22 (-0.8-52)	1.4 (-33-52)	91.8 (53.3-133.4)
	CO (l/min) Median (min-max)	6.1 (3.9-9.3)	8.8 (4.8-14.3)	32 (-19-63)	8.3 (5.1-13.4)	23 (-2-92)	13.6 (8.0-23.5)	56 (35-75)	41 (8-65)	7.0 (4.1-11.6)
C	**HR (bpm)** Median (min-max)	78 (60-106)	112 (82-140)	27 (16-42)	84 (63-111)	4 (-8-29)	135 (95-155)	41 (19-59)	39 (4-53)	84 (59-105)
	SV (ml) Median (min-max)	77.1 (45.2-113.8)	82.7 (47.5-152.1)	0.4 (-55-39)	105.3 (61.7-144.4)	23 (-12-45)	101.9 (66.1-177.4)	25 (-5-57)	3.3 (-29-40)	89.4 (48.7-135.6)
	CO (l/min) Median (min-max)	6.1 (3.5-9.9)	8.6 (5.5-14.4)	28 (-27-61)	8.3 (5.4-12.5)	28 (-19-50)	13.2 (9.8-20.6)	57 (25-73)	39 (7-64)	7.2 (4.5-12.3)

4.4.5. *Stroke volume, cardiac output and heart rate trends across trimesters using transthoracic echocardiography in the low-risk group*

Longitudinal data was available for 14 low-risk participants (Table 4.12). SV, CO, HR and VTI measured by TTE followed a Gaussian distribution. LVOT on TTE followed a non-Gaussian distribution. Table 4.13 summarises the comparison across trimesters.

Table 4.12 *– Left ventricular outflow tract (LVOT) diameter, velocity-time integral (VTI), stroke volume (SV), cardiac output (CO) and heart rate (HR) in the low-risk group, measured by transthoracic echocardiography. (N=14, 42 measurements, 3 measurements per participant).*

Trimester	**Time point**	**LVOT (cm)** Median (min-max)	**VTI (cm^2)** Mean ± SD	**SV (ml)** Mean ± SD	**CO (l/min)** Mean ± SD	**HR (bpm)** Mean ± SD
A	**T1**	2.0 (1.8-2.2)	20.4 ± 4.3	62.5 ± 15.0	4.1 ± 1.1	66 ± 10
	T2		24.3 ± 2.5	75.2 ± 13.8	9.4 ± 2.1	126 ± 17
	T3		22.3 ± 3.5	71.1 ± 10.0	5.2 ± 1.2	76 ± 13
B	**T1**	2.0 (1.8-2.1)	24.2 ± 12.3	58.2 ± 16.2	4.3 ± 0.7	69 ± 9
	T2		27.7 ± 15.8	70.2 ± 19.4	9.2 ± 1.9	124 ± 10
	T3		25.9 ± 17.5	64.4 ± 16.4	5.0 ± 0.6	74 ± 9
C	**T1**	2.0 (1.7-2.3)	17.2 ± 3.7	53.0 ± 12.9	4.0 ± 0.9	77 ± 14
	T2		21.6 ± 4.1	66.2 ± 14.1	7.9 ± 1.9	120 ± 11
	T3		18.9 ± 4.5	57.3 ± 14.5	4.5 ± 1.1	79 ± 11

Table 4.13 *– Summary of Paired t-test comparing differences in velocity-time integral (VTI), stroke volume (SV), cardiac output (CO) and heart rate (HR) and summary of the Wilcoxon matched-paired signed-rank test for differences in left ventricular outflow tract (LVOT), across trimesters in low-risk pregnancy in those participants with longitudinal echocardiographic data. Significance level: p<0.05.*

Trimester	**Time point**	**LVOT (cm)** Median (min-max)	**VTI (cm^2)** Mean ± SD	**SV (ml)** Mean ± SD	**CO (l/min)** Mean ± SD	**HR (bpm)** Mean ± SD
Comparison A/B	T1	0.64	0.31	0.39	0.55	0.31
	T2		0.44	0.28	0.48	0.63
	T3		0.44	0.26	0.59	0.44
Comparison B/C	T1	0.73	0.053	0.18	0.07	0.08
	T2		0.19	0.46	0.051	0.18
	T3		0.14	0.25	0.13	0.09
Comparison A/C	T1	0.71	0.14	0.09	0.71	**0.030***
	T2		0.11	0.057	**0.021***	0.25
	T3		**0.015***	**0.003****	0.10	0.28

*p<0.05, **p<0.01. All significance values have been adjusted by the Bonferroni correction for multiple tests.

4.4.6. *Stroke volume, cardiac output and heart rate trends measured with impedance cardiography (PhysioFlow®) across trimesters in pregnant women with CHD*

SV, CO and HR measured by PhysioFlow® followed a Gaussian distribution in the CHD group – results presented in Tables 4.14 and 4.15.

Table 4.14 *– Stroke volume (SV), cardiac output (CO) and heart rate (HR) at the different study time points (T1 – rest, peak exercise, T3 – recovery) and in each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation) in pregnant women with congenital heart disease (CHD).*

Trimester	Time point	SV (ml) Mean ± SD	CO (l/min) Mean ± SD	HR (bpm) Mean ± SD
A N=4	T1	68.7 ± 12.9	5.1 ± 0.8	74 ± 4
	Peak Exercise	94.3 ± 8.2	10.6 ± 0.9	114 ± 8
	T3	72.7 ± 19.7	5.6 ± 1.7	76 ± 5
B N=18	T1	80.8 ± 15.0	6.2 ± 1.1	78 ± 10
	Peak Exercise	108.8 ± 18.1	13.6 ± 1.8	127 ± 17
	T3	87.6 ± 15.8	7.2 ± 1.3	83 ± 10
C N=20	T1	80.0 ± 12.4	6.5 ± 1.0	82 ± 9
	Peak Exercise	111.0 ± 20.5	14.1 ± 2.8	127 ± 13
	T3	91.1 ± 12.2	7.7 ± 1.2	85 ± 8

Table 4.15 *– Summary of Paired t-test for differences in stroke volume (SV), cardiac output (CO) and heart rate (HR) across trimesters (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation) at the different time points (T1 – rest, peak exercise, T3 – recovery in the congenital heart disease (CHD) group. Significance level: p<0.05*

Trimester	Time point	SV (ml)	CO (l/min)	HR (bpm)
Comparison A/B (2 pairs)	T1	0.45	0.64	0.50
	Peak Exercise	0.08	0.34	0.52
	T3	0.32	0.32	0.39
Comparison B/C (11 pairs)	T1	0.26	0.41	0.06
	Peak Exercise	0.90	0.82	0.77
	T3	0.52	0.23	0.06
Comparison A/C (3 pairs)	T1	0.75	0.28	0.19
	Peak Exercise	0.87	0.83	0.69
	T3	0.54	0.22	0.21

All significance values have been adjusted by the Bonferroni correction for multiple tests.

4.4.7. *Heart rate, stroke volume and cardiac output response to exercise (above 70% of predicted or maximum heart rate) assessed by impedance cardiography (PhysioFlow®) in the CHD group*

Table 4.16 illustrates the haemodynamic response to exercise in the CHD group.

Table 4.16 *– Heart rate (HR), stroke volume (SV) and cardiac output (CO) measured by PhysioFlow® at different time points in each of the trimesters (A – 10-14 weeks of gestation; B – 18-22 weeks of gestation; C – 30-34 weeks of gestation) for the congenital heart disease (CHD) group. T1 (rest): measurement at rest; Upright: maximum HR and corresponding SV and CO after changing position from left lateral to sitting upright on the ergometer cycle; Relative increase ([Upright-T1]/Upright): relative increase in HR, SV and CO between upright and T1; 'Baseline' prior to exercise: baseline HR, SV and CO after HR returned to similar T1 HR levels; Relative increase (['Baseline'-T1]/'Baseline): relative increase in HR, SV and CO between new 'baseline' after change of position and rest; Peak Exercise: Peak HR and corresponding SV and CO at peak HR during exercise; Relative increase ([PeakEx-T1]/PeakEx): relative increase in HR and corresponding SV and CO between peak exercise and rest; Relative increase ([PeakEx-'Baseline']/PeakEx): relative increase in HR and corresponding SV and CO between peak exercise and 'baseline' after position change; T3 (recovery):measurement in the recovery phase.*

		T1 (Rest)	**Upright** (supine to upright position change**[*1]**)	**Relative Increase, %** (Upright-T1/Upright)	**'Baseline' Prior to Ex** (After HR returned to T1**[*2]**)	**Relative Increase, %** from rest	**Peak Exercise [*3]**	**Relative Increase, %** (PeakEx-T1/PeakEx)	**Relative Increase, %** (PeakEx-'Baseline')/ PeakEx)	**T3** (Recovery)
A	**HR (bpm)** Median (min-max)	73 (70-79)	106 (77-112)	27 (9-37)	83 (70-92)	9 (0-18)	115 (104-120)	35 (33-38)	28 (23-32)	77 (70-82)
	SV (ml) Median (min-max)	63.6 (59.7-87.7)	83.3 (76.6-90.6)	20 (3-28)	93.3 (80.9-103.6)	25 (15-38)	92.7 (87.2-104.6)	34 (-0.6-38)	-0.5 (-19-22)	64.8 (59.4-101.9)
	CO (l/min) Median (min-max)	4.8 (4.5-6.2)	8.5 (6.4-10.1)	39 (28-49)	8.1 (5.7-8.6)	34 (19-43)	10.7 (9.3-11.5)	57 (33-58)	26 (11-48)	5.1 (4.2-7.9)
B	**HR (bpm)** Median (min-max)	76 (64-103)	108 (75-142)	28 (0-43)	84 (67-109)	5 (-4-29)	133 (93-146)	37 (10-55)	37 (5-53)	79 (71-112)
	SV (ml) Median (min-max)	75.7 (58.3-111.9)	80.9 (56.8-114.2)	3 (-37-21)	94.1 (55.3-139.1)	16 (-25-32)	111.1 (80.5-141.0)	26 (0-52)	14 (-23-35)	89.3 (60.3-118.4)
	CO (l/min) Median (min-max)	6.1 (4.7-8.5)	8.5 (5.5-13.3)	30 (-1-53)	7.8 (4.4-11.5)	22 (-22-37)	13.6 (10.3-17.0)	54 (37-66)	40 (20-61)	7.5 (5.2-9.4)
C	**HR (bpm)** Median (min-max)	84 (68-99)	110 (75-133)	26 (0-35)	83 (66-95)	1.0 (-10-15)	127 (106-145)	36 (19-50)	36 (18-48)	86 (71-99)
	SV (ml) Median (min-max)	82.8 (55.4-101.1)	85.0 (34.2-104.5)	6 (-118-26)	106.1 (31.0-133.0)	22 (-140-47)	112.7 (74.1-142.7)	28 (3-47)	5 (-22-78)	93.4 (58.3-115.5)
	CO (l/min) Median (min-max)	6.6 (4.4-8.1)	8.6 (4.6-11.6)	25 (-61-45)	8.3 (2.7-10.8)	24 (-174-44)	14.2 (8.9-18.0)	56 (41-66)	40 (13-85)	7.9 (5.1-9.2)

4.4.8. *Stroke volume, cardiac output and heart rate trends across trimesters using transthoracic echocardiography in the CHD group*

The mean ± SD of the SV, CO, HR and VTI measurements by TTE for all trimesters, and the median of the LVOT measurement by TTE, for the CHD group, are presented in table 4.17. Table 4.18 summarises the comparison across trimesters.

Table 4.17 *– Left ventricular outflow tract (LVOT) diameter, velocity-time integral (VTI), stroke volume (SV), cardiac output (CO) and heart rate (HR) in the CHD (congenital heart disease) group, measured by echocardiography, at the different study time points (T1 – rest, peak exercise, T3 – recovery) and in each trimester (A – 1st trimester, 10-14 weeks of gestation; B – 2nd trimester, 18-22 weeks of gestation; C – 3rd trimester, 30-34 weeks of gestation).*

Trimester	**Time point**	**LVOT (cm)** Median (min-max)	**VTI (cm^2)** Mean ± SD	**SV (ml)** Mean ± SD	**CO (l/min)** Mean ± SD	**HR (bpm)** Mean ± SD
A N=3	T1	2.0 (2.0-2.3)	17.3 ± 3.9	59.2 ± 7.4	4.3 ± 0.6	73 ± 2
	T2		16.9 ± 3.5	58.0 ± 8.6	5.3 ± 1.2	92 ± 17
	T3		14.1 ± 1.2	48.8 ± 3.9	3.6 ± 0.4	73 ± 3
B N=16	T1	2.1 (1.6-2.9)	18.5 ± 4.3	67.9 ± 26.5	5.5 ± 2.7	79 ± 11
	T2		23.8 ± 10.6	83.6 ± 32.6	9.9 ± 3.8	118 ± 13
	T3		18.5 ± 4.7	67.6 ± 26.8	5.7 ± 2.7	83 ± 10
C N=17	T1	2.2 (1.9-2.4)	18.4 ± 5.1	65.4 ± 19.9	5.3 ± 1.7	81 ± 10
	T2		23.0 ± 4.4	82.6 ± 22.6	9.6 ± 2.8	116 ± 15
	T3		19.9 ± 4.7	71.5 ± 23.3	6.0 ± 1.8	82 ± 8

Table 4.18 *– Summary of Paired t-test comparing differences in velocity-time integral (VTI), stroke volume (SV), cardiac output (CO) and heart rate (HR) and summary of the Wilcoxon matched-paired signed-rank test for left ventricular outflow tract (LVOT) across trimesters in pregnant women with congenital heart disease (CHD) in those participants with echocardiographic data. Significance level: p<0.05.*

Trimester	**Time point**	**LVOT (cm)**	**VTI (cm^2)**	**SV (ml)**	**CO (l/min)**	**HR (bpm)**
Comparison A/B	T1	0.32	-	-	-	-
	T2		-	-	-	-
	T3		-	-	-	-
Comparison B/C	T1	1.00	0.27	0.35	0.67	0.88
	T2		0.63	0.69	0.39	0.06
	T3		0.16	0.06	**0.024***	0.46
Comparison A/C	T1	1.00	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
	T2		**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
	T3		**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****

*p<0.05, **p<0.01, ***p<0.001. All significance values have been adjusted by the Bonferroni correction for multiple tests.

4.4.9. *Differences between groups: differences in heart rate, stroke volume and cardiac output measured by impedance cardiography and by transthoracic echocardiography in both study groups*

The differences in HR, SV and CO measured by ICG in both study groups are presented in Table 4.19 and by TTE in Table 4.20.

Table 4.19 *– Differences in heart rate (HR), stroke volume (SV) and cardiac output (CO) measured by ICG* ***(PhysioFlow®)*** *between the two study groups at different time points. Significance level: p<0.05.*

		T1 (Rest)			**Upright** (supine to upright position change)			**'Baseline' Prior to Ex** (After HR returned to T1)			**Peak Exercise**			**T3** (Recovery)		
		LR	CHD	p	LR	CHD	p	LR	CHD	p	LR	CHD	p	LR	CHD	p
A	**HR (bpm)** Median	74	73	0.87	105	106	0.79	83	83	0.93	135	115	**0.001*****	79	77	0.53
	SV (ml) Median	81.7	63.6	0.09	87.1	83.3	0.82	99.2	93.3	0.42	100.4	92.7	0.36	88.6	64.8	0.058
	CO (l/min) Median	6.3	4.8	**0.044***	9.0	8.5	0.56	8.2	8.1	0.54	13.7	10.7	**0.003****	6.9	5.1	0.06
B	**HR (bpm)** Median	73	76	0.13	104	108	0.76	80	84	0.39	137	133	0.18	77	79	0.09
	SV (ml) Median	81.7	75.7	0.52	82.8	80.9	0.84	103.4	94.1	0.18	99.8	111.1	0.59	91.8	89.3	0.49
	CO (l/min) Median	6.1	6.1	0.50	8.8	8.5	0.90	8.3	7.8	0.75	13.6	13.6	0.68	7.0	7.5	0.43
C	**HR (bpm)** Median	78	84	0.29	112	110	0.50	84	83	0.55	135	127	**0.017***	84	86	0.56
	SV (ml) Median	77.1	82.8	0.75	82.7	85.0	0.73	105.3	106.1	0.97	101.9	112.7	0.37	89.4	93.4	0.76
	CO (l/min) Median	6.1	6.6	0.13	8.6	8.6	0.72	8.3	8.3	0.99	13.2	14.2	0.83	7.2	7.9	0.14

*p<0.05, **p<0.01, ***p<0.001.

Table 4.20 *– Differences in heart rate (HR), stroke volume (SV) and cardiac output (CO) measured by* ***transthoracic echocardiography (TTE)*** *between the two study groups at different time points. Significance level: p<0.05.*

		T1 (Rest)			**T2** (Immediate post Exercise)			**T3** (Recovery)		
		LR	CHD	p	LR	CHD	p	LR	CHD	p
A LR 28 CHD 3	**HR (bpm)** Median	71	73	0.59	131	92	**0.007****	75	73	0.76
	SV (ml) Median	61.0	59.2	0.79	74.3	58.0	**0.019***	60.7	48.8	**0.005****
	CO (l/min) Median	4.1	4.3	0.61	9.9	5.3	**0.007****	5.2	3.6	**0.019***
	VTI	20.2	17.3	0.28	24.8	16.9	**0.009****	21.4	14.1	**0.005****
B LR 32 CHD 16	**HR (bpm)** Median	71	79	**0.015***	130	118	**0.013***	75	83	**0.028***
	SV (ml) Median	59.8	67.9	0.47	74.2	83.6	0.47	62.8	67.6	0.90
	CO (l/min) Median	4.3	5.5	0.21	9.5	9.9	0.84	4.8	5.7	0.65
	VTI	19.7	18.5	0.30	24.8	23.8	0.15	20.3	18.5	0.06
C LR 34 CHD 17	**HR (bpm)** Median	76	81	0.15	124	116	0.11	81	82	0.25
	SV (ml) Median	55.2	65.4	0.15	70.3	82.6	**0.049***	59.9	71.5	**0.048***
	CO (l/min) Median	4.1	5.3	**0.012***	8.4	9.6	0.44	4.4	6.0	**0.006****
	VTI	17.3	18.4	0.98	22.5	23.0	0.78	19.0	19.9	0.51

*p<0.05, **p<0.01, ***p<0.001.

4.5. Discussion

4.5.1. *Demographic and obstetric data*

Although women in the CHD group were heavier by 3.5kg and had a BMI which was higher by 1.6 kg/m^2, there were no statistically significant differences between the two studied groups with regard to weight or BMI. The same was also observed for race, smoking status, parity, mode of delivery and estimated blood loss. There were more nulliparous women in the low-risk group (73% *vs* 57%) but despite this, more women in the CHD group were delivered via Caesarean section (57% *vs* 47%). This reflects the generally increased rate of caesarean section in women with CHD described in the literature.[213, 214] Participants were younger by two years in the CHD group ($p = 0.018$) and the gestational age at delivery was significantly shorter by one week ($p < 0.001$), which is again consistent with what has been described in the literature[215]. The birthweight and birthweight centiles were statistically different between the two groups ($p = 0.003$ and $p = 0.007$, respectively) with babies of mothers with CHD weighing, on average, 420g less than babies from mothers in the low-risk group. Poor cardiac function, associated with poor placental function and the use of beta-blockers, are associated with lower birthweight in the CHD population.[55, 57] Higher rates of PPH are reported in the CHD population,[216] and although the CHD participants had a higher blood loss at delivery (by 100mL), the difference was not statistically significant in this cohort.

4.5.2. *Blood pressure, heart rate and peripheral oxygen saturation trends across trimesters in low-risk pregnancy*

Systolic BP decreased from the first to the second trimester (99.7mmHg to 95.7mmHg), a decrease that was statistically significant ($p = 0.009$). Systolic BP levels rose in the third trimester to levels similar to those in the first trimester. In a large recent longitudinal study conducted in the UK[208], systolic blood pressure also decreased from 114mmHg (50^{th} centile) at 12 weeks of gestation to 113mmHg (its nadir) at 19 weeks and then, progressively increased to 121mmHg by 40 weeks of gestation.

Diastolic BP also decreased from the first to the second trimester (53.2mmHg to 48.8mmHg, p = 0.003) and then increased in the third trimester to levels similar to those in the first trimester (52.5mmHg, p = 0.003). Green *et al.*[208] reported a similar trend in diastolic BP throughout pregnancy with diastolic BP decreasing from 70mmHg at 12 weeks to 69mmHg at 19 weeks and then a progressive increase to 78mmHg at 40 weeks of gestation. Longitudinal studies of BP in normal pregnancy show conflicting results.[217] Previous longitudinal studies on BP in pregnancy have shown a 'mid-pregnancy fall' in BP of as much as 5mmHg for systolic and 10-15mmHg in diastolic BP.[205, 217-219] Other studies show a smaller decrease in the second trimester.[220, 221] Nama *et al.*[207] studied a homogeneous population (primigravid, white, normotensive at booking and with normal outcomes) and showed that systolic BP increased progressively (no mid-trimester fall noted) after 11 weeks of gestation and diastolic BP also increased progressively after a non-significant dip at 22 weeks of gestation. A recent systematic review[219], which included 39 studies in their meta-analysis (more than 124,000 BP readings), showed an increase in systolic BP from 10-40 weeks of gestation with a slight drop (1mmHg) in the second trimester. They also reported that diastolic BP was lowest at 21 weeks of gestation and increased thereafter until 40 weeks of gestation.[208] There was no difference in the BP of nulliparous and parous women.[219] We showed a small drop in systolic BP in the second trimester (4mmHg) and a small drop in diastolic BP (4.4mmHg), however we had small numbers (n = 45) and the study population was heterogeneous. Mahendru[210] *et al.* reported a BP profile throughout pregnancy (54 women of which, 33 nulliparous and 21 multiparous) similar to ours, with a reduction in systolic BP from 104mmHg to 103mmHg in the second trimester and a drop in diastolic BP from 65mmHg to 63mmHg.

HR increased throughout pregnancy, from 72bpm in the first trimester to 73bpm in the second and to 81bpm in the third trimester (Table 4.3). The increase was statistically significant from the second to the third trimester (p <0.001). Several authors reported similar increases in HR consistent with our findings. Stein *et al.*[222] reported an increase of 7bpm from early to late pregnancy and also reported a decline in HR variability during pregnancy. Green *et al.*[208] reported an increase of 9bpm from the first to the third trimester and Loerup *et al.*[219] also showed an increase of HR throughout pregnancy (7.6bpm

on average). Mahendru *et al.*[210] showed an increase in HR from 71bpm in early pregnancy to 80bpm in the third trimester.

There was a small statistically significant drop in SpO_2 throughout pregnancy, from 98% in the first trimester to 97% in the third trimester ($p < 0.001$) and from 98% in the second trimester to 97% in the third trimester ($p = 0.012$). This was also consistent with the findings of Green *et al.*[208] who reported a drop in SpO_2 in low-risk pregnancy to as low as 94% in the third trimester.

4.5.3. *Blood pressure, heart rate and peripheral oxygen saturation trends across trimesters in pregnant women with CHD*

There was no statistically significant difference in the BP of pregnant women with CHD between trimesters. However, this was not a true longitudinal sample and the number of participants in each of the trimesters was different (four in the first trimester and 18 in the second). As far as I am aware, there are no studies of longitudinal blood pressure profiling during pregnancy in women with CHD.

There was also no statistically significant difference in the HR of pregnant women with CHD between trimesters. There were only data available for four participants in the first trimester so there was low power to detect a difference. There were two participants on beta-blocker therapy in the CHD group which could have affected the results. Studies of haemodynamic performance during pregnancy in women with CHD are limited.[33] Cornette *et al.*[33] included 29 pregnant women with CHD and showed an average increase in HR from 75bpm in the first trimester to 76bpm in the second and third.

There was minimal change in SpO_2 in the CHD cohort and no statistically significant differences were found between trimesters. The small number of participants in the first trimester meant there was low power to detect a difference.

4.5.4. *Blood pressure, heart rate and peripheral oxygen saturation differences between participants with low-risk pregnancy and pregnant women with CHD*

There was a statistically significant difference in systolic BP between groups only in the third trimester ($p = 0.007$) with participants with CHD having higher systolic BP than healthy controls (105.5mmHg vs 98mmHg) at rest (T1). There was no significant difference in diastolic BP at rest.

HR was significantly higher in the second trimester in the CHD cohort (78bpm vs 71bpm, $p = 0.005$) but no significant differences were noted in the first or third trimesters. Reduced HR variability is particularly pronounced after ToF repair and these patients have a lower HR compared to controls in pregnancy.[223] Twenty-nine percent (eight out of 28 participants) of our CHD cohort had repaired ToF. Of those, two were taking beta-blockers (both on 2.5mg of Bisoprolol once daily). However, Niwa *et al.*[223] showed that in a CHD population with a mixture of conditions (similar to our cohort), HR was not different to controls. None of the rToF participants in this study were seen in the first trimester. Five were seen in the second trimester, and six in the third trimester.

A high proportion (>50%) of patients with CHD have chronotropic incompetence.[170] In the third trimester, HR immediately post-exercise was lower in the CHD group (109bpm) than in the low-risk group (114bpm, $p = 0.038$). Diastolic BP was significantly higher in the CHD group at this particular time point (69mmHg *vs* 60mmHg, $p = 0.012$), suggesting an impaired response to exercise in the third trimester. A total of four participants with CHD were unable to achieve the target HR as defined by the study protocol during exercise (two in the first trimester: Fontan TCPC and BAV + ASD + rVSD; one in the second trimester: Fontan TCPC [same participant as in the first trimester]; and three in third trimester: Fontan TCPC [same participant as in the first trimester], rToF and TGA – arterial switch). Of these, only one was taking beta-blockers (Sotalol 80mg twice daily) and on all occasions, participants stopped exercise due to reported fatigue. As a comparison, in the low-risk group, all participants achieved the target HR as per study protocol.

There was no significant difference in SpO_2 in any of the trimesters. All women in the CHD cohort had either repaired CHD or small/simple defects with minimal haemodynamic disruption which may explain the minimal differences between the groups.

4.5.5. *Longitudinal assessment of stroke volume, cardiac output and heart rate with impedance cardiography (PhysioFlow®) and transthoracic echocardiography in low-risk pregnancy*

SV decreased during pregnancy from 85.0mL in the first trimester to 78.6mL in the third trimester, when measured by PhysioFlow®. The differences were only statistically significant between the first and third trimesters (p = 0.018). SV followed a similar trend when measured by TTE, decreasing from 62.5mL in the first trimester to 53.0mL in the third trimester. Differences were also statistically significant between the first and second trimesters (p = 0.003). A large systematic review which included nearly 1,500 measurements of CO and related parameters measured via TTE and ICG also showed a reduction in SV from first to the third trimester after a small increase in the early second trimester (80, 84 and 72mL for first, early second trimester and third trimester, respectively).[24] Although we did not exactly replicate SV trends as per the mentioned systematic review, SV can be measured in a number of ways on TTE (Doppler techniques, ventricular dimensions such as Simpson, Teichholz), giving a wide variation in reported SV values which do not allow direct comparison.[22] Other studies using ICG show a similar trend to the one observed in our study, with a gradual decline in SV in pregnancy when measured in the supine position.[224-226]

There were no statistically significant differences in CO between the trimesters when this was measured by PhysioFlow® or TTE. When measured with PhysioFlow®, CO decreased slightly from 6.1L/min in the first trimester to 6.0L/min in the second trimester and then rose again slightly to 6.2L/min in the third trimester. When measured by TTE, CO increased slightly from 4.1L/min in the first trimester to 4.3L/min in the second and then decreased to 4.0L/min in the third trimester. None of these differences were statistically significant for either device. CO is the product of SV and HR and therefore, its

measurement will be affected by the SV measurement.[22] The consensus from the literature is that CO increases in the first two trimesters but there is considerable disagreement regarding trends in the third trimester, with some authors reporting an increase and others a decrease or plateau.[22, 24, 27] Increases in CO in the first trimester (compared to the non-pregnant state) seem to be largely a result of reduced afterload secondary to a reduction in systemic vascular resistance with subsequent stimulation of the sympathetic system and an increase in HR.[24] SV seems to play a minor role in the CO increase.[24] CO trends from the first to the second trimester depend on the method used: measurements of CO with ICG usually show a decrease in CO with gestation, those with indicator dilution show a plateau, and those with TTE show an increase.[27] Meah *et al.*[24] provided new insights into the increase of CO in normal pregnancy and showed that CO increased until the early third trimester, but by the late third trimester CO values decreased again to levels below those of the second trimester (early and late) until term. Blood flow in the uteroplacental circulation reaches its peak in the late third trimester[227] and this, combined with a possible reduction in venous return due to compression of the inferior vena cava by the growing uterus, could be responsible for a decrease in CO in the third trimester.[27] A recent longitudinal study using an inert gas rebreathing technique in over 400 pregnant women, showed a peak CO at 15.2 weeks of gestation and a subsequent decrease until term, which is similar to our results.[83] van Oppen *et al.*[228] showed an increase in CO from first to the second trimester and a subsequent decrease in the third trimester (7.26L/min, 7.60L/min and 6.37L/min for first, second and third trimesters, respectively). Our findings from TTE are consistent with this. Conflicting results from longitudinal studies of CO in pregnancy may be due to inter-individual variation.[228] Our findings from PhysioFlow® are consistent with Moertl *et al.*[225], Atkins *et al.*[229] and San-Frutos *et al.*[230], who showed decrease in CO with advancing gestation. The often referenced study by Robson *et al.*[26], which showed an increase in CO up to 24 weeks of gestation and a plateau thereafter, only reported on 13 women.

HR increased throughout pregnancy when measured both by PhysioFlow® and TTE, and the differences were statistically significant ($p < 0.001$ for PhysioFlow® and $p = 0.030$ for TTE), as described in section 4.5.1. Because the measurement of HR is simple, it would have been surprising

and unexpected if there had been any difference between the two techniques. The HR increase in the second half of pregnancy is the main contributor to CO change.[29]

4.5.6. *Longitudinal assessment of stroke volume, cardiac output and heart rate with impedance cardiography (PhysioFlow®) and transthoracic echocardiography in pregnant women with CHD*

SV and CO increased during pregnancy in the CHD cohort with PhysioFlow®, and with TTE these parameters increased from the first to the second trimester with a subsequent drop in the third trimester. However, this sample was not a true longitudinal nor a true cross-sectional sample and all data available per trimester were analysed together, therefore the data should be interpreted with caution.

Longitudinal data on pregnancy in women with CHD are limited.[33] Cornette *et al.*[33] included, over a three year period, 29 women with structural heart disease who had echocardiographic assessment from preconception to the postpartum period. They describe a steep increase in CO and SV in the first half of pregnancy with a 'plateau-like' phase in the second half of pregnancy.[33] The magnitude of the increase described by the authors was less than previously described for normal pregnancy: approximately 5.7L/min at 10 weeks of gestation to 5.8L/min at 20 weeks of gestation and to 5.9L/min at 30 weeks of gestation, suggesting an attenuated cardiac adaptation to pregnancy.[33] We do not have preconception data but our study also shows an increase with advancing gestation when using the PhysioFlow®. However, with TTE, as described above, we found an increase from the first to the second trimester and then a decrease in the third trimester. The pattern was similar to that found in the the low-risk participants. None of the changes reached statistical significance when measured with PhysioFlow® but changes from the first to the third trimester measured by TTE were all highly statistically significant ($p < 0.001$). However, due to the small number of participants studied in the first trimester ($n = 4$) compared to the third trimester ($n = 20$), and because there were only two truly longitudinal samples in the CHD group, these results should not be considered definitive.

Looking at changes from the second to the third trimester only, SV changes were consistent with those found in the low-risk group, with a very small decrease from 80.8mL to 80.0mL. However, CO increased in the third trimester, from 6.2L/min in the second to 6.5L/min in the third trimester.

HR changes were consistent with those described previously in section 4.5.3 for the CHD cohort and those described in section 4.5.1 for the low-risk population with a progressive increase with advancing gestation. However, the changes did not reach statistical significance.

4.5.7. *Response to postural change and exercise in low-risk pregnancy, measured by impedance cardiography (PhysioFlow®)*

Changes to haemodynamic parameters in response to postural change (left lateral to sitting position) were almost immediate and characterised by an increase in HR and CO. Although HR returned to values similar to rest after a few seconds, CO remained raised even after the return of HR to baseline. This is consistent with the findings by Del Bene *et al.*[231] and D'Silva *et al.*[224] There was minimal change in SV with postural change (0.4-3%). D'Silva *et al.*[224] report a decrease in SV with a change from supine to sitting position (due to a reduction in preload). We observed a similar effect but recorded the SV when the HR reached its peak after position change and, by this time, some women had already increased their SV, which probably accounts for the minimal change in SV we observed.

Maternal cardiovascular function is maintained during submaximal exercise and responses are similar to those in non-pregnant individuals.[175] Systolic BP increased by approximately 25mmHg with exercise and diastolic BP increased by approximately 10-15mmHg, which is consistent with the literature.[232] BP returned to pre-exercise values once HR was, as per study protocol, ±20% of baseline.

CO increased 56-57% with exercise in all of the trimesters, mainly due to an increase in HR (increase of 41-47%) and a smaller increase in SV (18-25%). During exercise, there is an increase in SV to support the increasing CO.[233]

4.5.8. *Response to postural change and exercise in pregnant women with CHD, measured by impedance cardiography (PhysioFlow®)*

Haemodynamic response to postural change in the second and third trimesters was similar to that in the low-risk group. In the first trimester there was a higher increase in SV and lower increase in HR but, as mentioned previously, there were only four participants at this time point and therefore no definitive conclusion can be drawn.

Exercise can unmask abnormalities in cardiac function that are non-apparent at rest.[175] Interestingly, when compared to low-risk participants, the increase in CO in response to exercise and in comparison with the resting state, was very similar (54-57%) but, likely due to chronotropic incompetence, the SV seemed to play a more important role in maintaining this CO, as it increased more than in the low-risk group (26-34% in CHD *vs* 18-25% in LR). As discussed above, four participants with CHD did not achieve the required target HR with exercise on six occasions. Of these, one was taking beta-blockers (Sotalol 80mg twice daily). Correspondingly, HR increased less than in the low-risk participants (35-37% *vs* 41-47%). To date, I am not aware of any published studies evaluating haemodynamic response to exercise in pregnant women with CHD.

4.5.9. *Response to exercise and differences between low-risk pregnancy and pregnancy in CHD*

4.5.9.1. Differences of measurements with impedance cardiography (PhysioFlow®)

In the first trimester, there was a marginally statistically significant difference between the two studied populations ($p = 0.044$) in CO at rest (LR: CO 6.3L/min; CHD: CO 4.8L/min). However, as mentioned above, these results need to be interpreted with caution due to the small number of CHD participants ($n = 4$) and the individual characteristics of these participants. This cohort included a patient with Fontan circulation (TCPC), two participants with TGA (arterial switch) and a participant with BAV + ASD + rVSD. Exercise capacity in patients after TGA repair is reduced, even in asymptomatic patients[234] and patients with Fontan circulation are known to have worse cardiac function when compared to other

forms of CHD.[14] This is further demonstrated by the fact that at peak exercise, these participants were unable to increase their HR as much as the LR group (115bpm *vs* 135bpm, p = 0.001) and were also unable to increase their CO as much (maximal CO 10.7l/min *vs* 13.7l/min, p = 0.003). No statistically significant differences were found in SV changes and no statistically significant differences were found between those taking beta-blockers and those not taking beta-blockers. In the second trimester, there were no differences between groups in any of the parameters and CO was similar at rest and at peak exercise between the groups. In the third trimester, the only statistically significant difference was HR at peak exercise, with participants in the CHD group having a lower HR (127bpm) than the LR group (135bpm), p = 0.017. Of note, three participants were unable to achieve their target heart rate during exercise and it has been reported that a significant proportion of patients with CHD have chronotropic incompetence[170], as described above, which might have had an impact on these results. There were no statistically significant differences in any of the parameters when comparing participants on beta-blockers with those not on beta-blockers. At this particular time point, only one of the three participants was on beta-blockers (Sotalol 80mg twice daily). Unexpectedly, there were no major differences between the two studied populations at rest or during exercise, suggesting an adequate cardiovascular adaptation to pregnancy in the CHD group. Uebing *et al.*[34] studied the ventricular function of 53 women with heart disease (mainly CHD) prior to and 1.5years post pregnancy, matched to a population of women with similar heart conditions who did not have a pregnancy and found that pregnancy was not associated with impairment of ventricular function of the systemic ventricle, nor was it associated with systemic ventricular dilatation. This was a novel finding at the time and was suggested as a reassuring feature when counselling women with CHD planning a pregnancy.[34] Although our assessment of cardiac function with PhysioFlow® was somewhat limited due to the restricted exercise capacity, our data seem to suggest minimal differences between CHD and the healthy group. Our CHD participants were mostly fit and well and in class 1-2 of the New York Heart Association (NYHA) Functional Classification. In a large cohort of pregnant women with heart disease, 19.4% suffered a primary cardiac event but those with impaired subpulmonary ventricular systolic function and/or severe pulmonary regurgitation were at highest risk.[54] Khairy *et al*. reported in 2006 the results of a large cohort study (90 pregnancies in 53 women with CHD) which found that women with left heart obstruction, poor

functional class and cyanosis had worse outcomes of pregnancy.[53] Our relatively stable population of CHD women might explain the minimal differences in response to exercise in the two groups.

4.5.9.2. Differences between groups in measurements with transthoracic echocardiography

In the first trimester, there were no differences at rest or in the recovery period but statistically significant differences in all studied parameters immediately post exercise. HR was significantly lower in the CHD group (92bpm *vs* 131bpm, p = 0.007), SV was significantly lower (58.0mL *vs* 74.3mL, p = 0.019) and CO was also significantly lower (5.3L/min *vs* 9.9L/min, p = 0.009). Of note, only three of the CHD participants had TTE data in the first trimester (Fontan, TGA, BAV + ASD + VSD) which limits the interpretation of the results. In the second trimester, there was a statistically significant difference in HR at rest (79bpm for CHD compared to 71bpm for LR, p = 0.015). CO was also significantly different at rest in the third trimester, with women with CHD having a higher CO (5.3L/min vs 4.1L/min, p = 0.012) which, although consistent with our longitudinal assessment of haemodynamic changes throughout pregnancy, is an unexpected and isolated finding.

4.6. Conclusion

Systolic and diastolic BP decreased significantly in the second trimester and then in the third trimester returned to levels similar to those in the first trimester. HR increased and SpO_2 decreased with advancing GA. BP throughout pregnancy was similar between the two studied groups, apart from systolic BP in the third trimester which was significantly higher in the CHD group.

In the low-risk group, SV decreased with advancing gestation and CO remained stable throughout pregnancy when measured by ICG. When measured by TTE, SV decreased with advancing gestational age and CO increased in the second trimester with a subsequent decrease in the third trimester. In the CHD group, SV remained stable in the second and third trimesters and CO increased when measured by ICG. When TTE was used, SV increased throughout pregnancy and CO followed a similar pattern to that of women in the low-risk group. There were no major differences in CO, SV and HR between

the two studied groups, suggesting similar cardiac function in low-risk women and in women with repaired/simple CHD at rest, which is a novel finding. There were, however, differences in measurements taken immediately after exercise, with women with CHD showing evidence of chronotropic incompetence, lower SV and CO in the first trimester.

The most important novel finding was that the CO increase in response to exercise was similar in low-risk pregnancy and in pregnant women with CHD, however (probably due to chronotropic incompetence) the increase in CO in the CHD group was mainly due to an increase in SV rather than in HR.

Chapter 5.

Maternal haemodynamics and fetal Doppler indices:

association of maternal haemodynamics in the third trimester with fetal Doppler indices, estimated fetal weight and birthweight

Chapter 5. Maternal haemodynamics and fetal Doppler indices: association of maternal haemodynamics in the third trimester with fetal Doppler indices, estimated fetal weight and birthweight

5.1. Introduction

Placentation directly and/or indirectly influences cardiovascular adaptation to pregnancy and there is an association between impaired placentation and impaired cardiovascular adaptation to pregnancy.[37, 38] The maternal vascular response to placentation is compromised in FGR[39] and it has also been demonstrated that defective placentation can precede FGR.[40]

Following implantation, trophectodermal cells arising from the blastocyst epithelium form the primitive syncytium which invades the maternal endometrium.[41] Following the formation of the lacunae system, the cytotrophoblasts give origin to primary villi which differentiate into secondary and tertiary villi and which then invade the blood vessels.[41] Utero-placental malperfusion secondary to incomplete spiral artery conversion can lead to FGR.[40] After 12 weeks of gestation, uterine artery remodelling transforms the spiral arteries into large diameter vessels with reduced contractility, which allows oxygen delivery at low vascular pressure.[41]

BW is linked to neonatal, infant and childhood morbidity and mortality.[43] FGR is usually defined as a pathological condition in which the fetus fails to achieve its full biological growth potential.[44] However, there are inconsistencies in the literature and there is no universally agreed definition.[44-46] The commonest inconsistency is defining FGR as being SGA. The RCOG define SGA as a fetus with an EFW or an AC below the 10th percentile and severe SGA as a fetus with EFW or AC below the 3rd percentile.[46] However, many babies < 10th percentile BW are constitutionally small normal babies and many babies who are FGR, weigh > 10th percentile at birth, as discussed in Chapter 1.[47, 48] The RCOG state that FGR "*implies a pathological restriction of the genetic growth potential*" and that, therefore, these fetuses can show signs of fetal compromise such as abnormal Doppler studies or reduced liquor volume.[46] The ACOG says that "*the most widely used definition of fetal growth restriction in the United*

States is an estimated fetal weight that is less than the 10th percentile" but points out that this definition "*does not take into account the individualized growth potential of each fetus, and its use may fail to identify larger fetuses that have not achieved their growth potential and may be at risk of adverse outcomes. Conversely, this definition will result in the misdiagnosis of fetal growth restriction for some constitutionally small fetuses SGA for newborns whose BW is below the 10th percentile*".[45] It has been suggested that to make a more accurate diagnosis of true FGR more than one antenatal measurement must be performed, with those measurements showing a growth rate below that expected.[44, 46] A recent consensus from 2018[44] distinguishes between early and late FGR and proposes the following definition (Figure 5.1):

Consensus-based definitions for early and late FGR.

Early FGR: gestational age <32 weeks, in the absence of congenital anomalies
AC/EFW <p3 or AEDF in the umbilical artery
Or
1. AC/EFW <p10 **combined with**
2. PI in the uterine artery >p95 **and/or**
3. PI in the umbilical artery >p95

Late FGR: gestational age ≥32 weeks, in the absence of congenital anomalies
AC/EFW <p3
Or
At least 2 out of 3 of the following
1. AC/EFW <p10
2. Crossing centiles of more than 2 quartiles on growth centiles[a]
3. CPR <p5

[a] Growth centiles are noncustomized centiles.

Figure 5.1 *– Consensus-based definitions for early and late fetal growth restriction (FGR). pX – percentile; AEDF – absent end-diastolic flow; PI – pulsatility index; CPR – cerebroplacental ratio.* Image reproduced with permission of the rights holders, Elsevier © 2019 Elsevier

FGR is associated with a threefold increase in mortality and an increased incidence of necrotising enterocolitis and respiratory distress syndrome.[49]

In the general population, both FGR and PE are associated with lower CO and high TVR, and in women with CHD it is postulated that their impaired haemodynamic adaptation can lead to inadequate placentation (rather than the reverse) and therefore a higher incidence of FGR and PE.[11] There is a reported positive correlation between CO and BW.[51]

FGR has been reported in up to 38% of pregnancies of women with CHD, depending on the type of cardiovascular abnormality.[53-55] In normotensive pregnancies, TVR is inversely correlated with centile birthweight (in normotensive pregnancies) and can be used as a surrogate marker for cardiovascular function during pregnancy.[50] In a study comparing pregnant women (normotensive, non-smoker and not on any medications) who had intra-uterine growth restricted (IUGR) babies to women who had non-IUGR babies, CO and SV were significantly lower in the IUGR group by 23% and 19%, respectively, and TVR was higher by 33%.[56] There was also a significant correlation between fetal weight and maternal cardiac output.[56] Another study reporting on 20 FGR pregnancies showed lower CO and higher TVR in this group compared to controls.[35]

Gelson *et al*.[55] reported perinatal complications of 34% in a cohort of 331 women with heart disease (as opposed to 15% in the control group) and the most common complication was SGA, accounting for 61% of cases. Median BW percentile was 31 in the heart disease group compared with 48 in the control group, and women in the cyanotic or low CO group had the smallest babies.[55] A more recent review of 1321 pregnancies in women with heart disease reported that their babies were smaller compared to the control group and that the difference was more pronounced in those with Fontan circulation, pulmonary hypertension, prosthetic heart valves, systemic right ventricle, Marfan syndrome, repaired ToF and cardiomyopathy.[57]

Correlation of haemodynamic parameters assessed longitudinally throughout pregnancy, but particularly during the first trimester, and their association with BW percentile can provide an insight into how haemodynamic (mal)adaptation influences growth.

Doppler studies in Obstetrics have for long been used to study the fetal and placental circulations in order to diagnose and monitor conditions such as FGR.[58] Commonly used Doppler studies in clinical practice include uterine artery Doppler (UtAD), umbilical artery Doppler (UAD), middle cerebral artery (MCA) Doppler and ductus venosus (DV).[58] Doppler ultrasound is a non-invasive and reliable way of assessing uteroplacental circulation.[59]

The use of UtAD has been mainly to screen for PE, typically between 20-24 weeks of gestation and a raised pulsatility index (PI), raised resistance index (RI) or presence of notching is considered to be abnormal.[58] It also allows the distinction between placental and other causes of FGR.[58] Cnossen *et al.*[60] performed a large systematic review and concluded that UtAD predicts PE better than FGR and that an increased PI with notching in the second trimester best predicted PE in both low-risk and high-risk women. An increased PI with or without notching best predicted severe FGR in a low-risk population and in high-risk subjects the best predictor was an increased RI.[60] Only women with risk factors should be referred for screening by means of UtAD, as there is limited predictive value in low-risk women.[58] Screening can be started as early as the first trimester and the detection rate for early PE can be increased to nearly 90% when UtAD is used.[61] In women with a low-risk pregnancy affected by FGR or hypertensive disorders of pregnancy, placentation is often found to be impaired and this translates into abnormal Doppler studies of the uterine and umbilical arteries.[32]

Melchiorre *et al*.[62] studied the relationship between raised UtAD PI/notching and the presence of cardiac defects. The authors report nine cases of undiagnosed functionally significant maternal structural cardiac anomalies in 205 women with raised UtAD PI (4.4%), with only one of such cases in 286 women with normal resistance (0.35%, RR = 12.6, 95% CI [1.60-98.34]).[62] The authors conclude that high resistance UtAD PI may indicate suboptimal haemodynamic adaptation rather than defective placentation.[62] A systematic review of uteroplacental blood flow and pregnancy outcomes concluded that there is a place for routine UtAD in women with known cardiovascular dysfunction, as this can reveal cardiovascular maladaptation and guide antenatal management.[63] RCOG guidelines[46] recommend that women with an abnormal UtAD at 20-24 weeks of gestation (PI >95th centile and/or presence of notching) should have serial growth scans and UAD from 26-28 weeks of gestation.

UAD is a surrogate marker of placenta vascular resistance – as gestation progresses, there is reduced vascular resistance and indices such as UAD PI decrease.[64] Abnormal UAD wave patterns (increased PI or absent/reversed EDF) precede decelerations on antenatal cardiotocography and can, therefore, be used as predictors of fetal compromise.[65]

The role of MCA Doppler in FGR is less clear than that of UAD.[58] A 'brain-sparing effect' was reported by Wladimiroff *et al.*[66] in 1986 and was described as an "*increased peripheral vascular resistance in the fetal body and placenta and a compensatory reduction in peripheral vascular resistance in the fetal cerebrum*". MCA is not a useful predictor for adverse perinatal outcomes preterm and MCA Doppler should only be used to plan time of delivery if it is abnormal (even with a normal UAD) at term.[46] More recently, the cerebroplacental ratio (CPR), the ratio of the MCA PI divided by the UAD PI, correlates better than birth weight as a marker for placental insufficiency in the third trimester.[67]

Pieper *et al.*[32] performed UtAD and UAD studies at 20 and 32 weeks of gestation in over 200 women with CHD and compared the results to 70 low-risk women (controls). UtAD and UAD PI and RI were on average higher throughout pregnancy in the CHD group compared to the low-risk group, and PI/RI in both arteries decreased throughout pregnancy in both groups.[32] Abnormalities in Doppler flow was associated with more adverse obstetric and offspring events in women with CHD – higher rates of hypertensive disorders/PE (fourfold), preterm delivery and SGA (fourfold).[32] Another study by Goya *et al.*[68] including 127 women with CHD showed that their UtAD PI at 20 weeks was statistically significantly higher than in a healthy pregnancy comparison group, and that UAD PI at 32 weeks of gestation was also statistically significantly higher in the heart disease group.

Lesion-specific studies such as the study by Kampman *et al.*[69] concluded that women with ToF have a higher incidence of abnormal UtAD and UAD which are indicative of abnormal placentation and that RV function was correlated to the Doppler flow parameters. In women with repaired CoA, RV function was also impaired and correlated with Doppler flow parameters.[70]

5.2. Hypotheses and Aims

Hypothesis 10: *Fetal growth is related to maternal CO and impaired cardiovascular adaptation to pregnancy can lead to defective placentation and, therefore, FGR.*

Aim: To obtain a measure of EFW (as defined by the study protocol), BW and BW centiles, and correlate these with maternal haemodynamics: (a) CO at rest measured by ICG and TTE; (b) cardiac

function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 11: *Increased UtA PI in the third trimester is associated with lower EFW and, consequently, with lower BWs and associated BW centiles.*

Aim: To obtain a measure of UtAD PI in the third trimester (between 30-34 weeks of gestation) and correlate this with EFW in the third trimester (between 30-34 weeks of gestation), BW and BW centiles.

Hypothesis 12: *Defective placentation can be secondary to cardiovascular maladaptation in pregnancy and there is an association between UtAD PI, CO and MAP.*

Aim: To correlate UtAD indices with: (a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 13: *Increased UAD PI is associated with lower EFW, BW and BW centiles.*

Aim: To obtain a measure of UAD in the third trimester (between 30-34 weeks of gestation) and correlate these with EFW, BW and BW centiles, as well as with the maternal haemodynamics: (a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

Hypothesis 14: *CPR in the third trimester is a surrogate marker for placental insufficiency and a low CPR is associated with high UtAD PI and lower EFW, BW and BW centiles.*

Aim: To correlate CPR with EFW, BW and BW centiles and with the maternal haemodynamics: a) CO at rest measured by ICG and TTE; (b) cardiac function measured as the percentage increase in cardiac output (ΔCO) with maximal exercise to 70% of maximum capacity, measured by ICG and TTE; (c) MAP.

5.3. Materials and Methods

5.3.1. *Recruitment*

Recruitment, inclusion and exclusion criteria were described in Chapter 2. For the low-risk group, a visit was planned between 30-34 weeks of gestation (trimester C). Ultrasound and Doppler studies were all performed prior to the exercise testing. For the low-risk group, this was an extra visit (outside standard care) and measurements and Doppler studies were obtained as per study protocol. For the CHD group, only those having an ultrasound as part of standard care were included, and measurements and Doppler studies were performed as per CW protocol. Therefore, UtAD and MCA Doppler measurements were not performed in all participants.

5.3.2. *Obstetric and haemodynamic data*

Demographic data were collected at the first visit and included age, race, height, weight and BMI at booking, gravidity, parity, EDD (based on CRL from 1st trimester scan), GA at the time of the visit, smoking status, type of CHD lesion and use of medication. Delivery data collected included GA at delivery, onset of labour (spontaneous or IOL, and indication), MOD and indications for assisted delivery or Caesarean section where applicable, BW, sex of the newborn, BW centile according to the Aberdeen Maternity and Neonatal Databank nomograms[188] and EBL.

CO at rest measured by ICG and TTE was obtained as described in Chapter 2. The percentage increase in CO, ΔCO, was calculated as: ΔCO_PF = [(CO at peak exercise – CO at rest)/CO at peak exercise] for measurements obtained from ICG and as ΔCO_TTE = [(CO_{T2} – CO_{T1})/CO_{T2}], as also described in Chapter 4. MAP was calculated as: MAP[50] = [(Diastolic BP + (Systolic BP – Diastolic BP))/3)].

5.3.3. *Ultrasound measurements*

The GE Voluson™ S10 Expert (*General Electric Company, USA*) equipped with a transabdominal C4-8-D probe (frequency 8-2MHz) was used to perform all the scans. In the low-risk group, the scans were performed by two senior obstetric sonographers who were part of the research team: one performed 62.1% of the scans and the other one performed 37.9% of the scans. For the CHD group, as the scans were performed as part of routine care, measurements were performed by a variety of NHS staff.

GA at the scan was confirmed based on the agreed EDD, which was established following the first trimester ultrasound. Measurements of the HC, AC and FL were obtained to calculate the EFW using the Hadlock formula.

For the HC, a measurements of the BPD and OFD were first obtained as per guidelines.[182, 183] A cross-sectional view of the fetal head was obtained at the level of the thalami, ensuring there was a symmetrical view of both ventricles, a continuous midline echo representing the falx cerebri broken in half by the cavum septi pellucidi and that no cerebellum was visualised. The callipers were positioned to measure from an "outer-to-outer" manner at the widest part of the skull and using an angle which was perpendicular to the falx cerebri to obtain the BPD. For the OFD, callipers were positioned in the middle of the bone echo and a measurement performed from the occipital to the frontal bone. The ultrasound machine automatically calculated the HC through the formula: HC = 1.62 (BPD + OFD). For the AC, a transverse section of the fetal abdomen was obtained ensuring this was as circular as possible, that the umbilical vein was at the level of the portal sinus, a stomach bubble was visible and no kidneys were visualised. The AC was measured directly with ellipse callipers from the outer skin line. For the FL, the femur was identified and both ends of the ossified metaphysis visualised. Callipers were positioned at the ends of the ossified diaphysis and the length of the diaphysis measured. The EFW was automatically measured by the ultrasound machine using the Hadlock formula.

Following fetal biometry, the Doppler studies were performed according to the ISUOG guidelines.[184] For UtAD, using a transabdominal approach, the probe's ultrasound beam was directed into the lower quadrant of the abdomen angled medially and then moved laterally, until the uterine artery was seen crossing the external iliac artery. The Doppler gate was placed 1cm downstream from the crossover point and the Doppler waveform obtained, trying to achieve complete alignment with the blood flow. The same procedure was performed for the contralateral side. For the UAD, a free loop of cord was identified, the umbilical artery identified using colour Doppler and the sample volume place over this area. For the middle cerebral artery MCA Doppler, an axial section of the brain which included the thalami and the sphenoid bone wings was obtained and magnified. Colour flow mapping was used to identify the circle of Willis and the proximal MCA. The pulsed-wave Doppler gate was placed in the proximal third of the MCA and the angle between the ultrasound beam and direction of blood flow kept to as close to 0^{o} as possible.

5.3.4. *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics, version 26.0, 2019 (*IBM Corp., Armonk, New York, USA*) and Microsoft® Excel for MAC, version 16.30, 2019 (*Microsoft Corp., Redmond, WA, USA*). The Kolmogoroff–Smirnoff and the Shapiro-Wilk tests were used to assess normality of the distribution of the data as well as visual inspection of the histograms and Q-Q' normality plots. Maternal characteristics were compared using Chi-square or Mann-Whitney U, as appropriate. Spearman-rank correlation (r_s) was used to evaluate correlation between EFW, AC/EFW, BW and BW centile with CO, ΔCO and MAP. Doppler indices were presented as median (min-max) and multiples of the median were used. Spearman-rank correlation (r_s) was also used to evaluate correlations between EFW, AC/EFW ratio, BW and BW centile, CO, ΔCO and MAP with all Doppler indices. Doppler indices were adjusted for gestational age using a linear mixed model.

5.3.5. *Sample size*

Sample size calculation was performed using the G*Power software (*G*Power for Mac Os X, v. 3.1, February 2020, Heinrich-Heine-Universität, Dusseldorf, Germany*).

For the association between Doppler indices and birthweight, to achieve a similar result to that of Bamfo *et al.*[35] with an alpha = 0.05 and a 95% power, we would need 58 low-risk subjects and 17 subjects in the CHD group.

5.4. Results

5.4.1. *Maternal characteristics, obstetric and fetal outcomes*

Table 5.1 describes the characteristics and obstetric outcomes of the participants included in the study of maternal haemodynamics and association with birthweight and fetal Doppler indices. Table 5.2 details the specific cardiac lesions of the participants with CHD.

Table 5.1 *– Maternal characteristics and obstetric outcomes of low-risk (LR) pregnant women and pregnant women with congenital heart disease (CHD) included in the study of maternal haemodynamics and association with birthweight and fetal Doppler indices. Significance level: p<0.05.*

	LR N = 62	**CHD** N = 16	**p value**
Age, years, median (min - max)	33 (24-42)	30 (25-39)	0.009**
Race, n (%)			0.64
White	51 (82.3)	14 (87.5)	
Non-white	11 (17.7) [2 Black, 5 Asian, 2 Middle-Eastern, 2 Mixed]	2 (12.5) [1 Asian, 1 Middle-Eastern]	
Weight, kg, median (min - max)	62.0 (43.0-100.0)	69.0 (53.0-101.0)	0.010**
BMI, kg/m^2, median (min-max)	22.5 (18.0-34.0)	26.5 (17.6-34.9)	0.038*
Smoking, n (%)			0.96
Never smoked	55 (88.7)	14 (87.5)	
Current/past smoker	7 (11.3)	2 (12.5)	
Parity, n (%)			0.98
P0	44 (71.0)	11 (68.8)	
P ≥1	18 (29.0)	5 (31.2)	
Mode of delivery, n (%)			0.23
Spontaneous Vaginal Delivery	30 (48.4)	6 (37.5)	
Ventouse Vaginal delivery	2 (3.2)	0 (0.0)	
Forceps Vaginal Delivery	6 (9.7)	1 (6.3)	
Emergency Caesarean Section	16 (25.8)	3 (31.3)	
Elective Caesarean Section	8 (12.9)	4 (25.0)	
Mode of delivery, n (%)			0.21
Vaginal (all, including instrumental)	38 (61.3)	7 (43.8)	
Caesarean Section (all)	24 (38.7)	9 (56.2)	
Gestational Age at delivery median (min - max)	39+6 (35+6-42+0)	38+5 (34+3-41+0)	0.001***
Sex of the baby, n (%)			0.89
Female	26 (41.9)	7 (43.8)	
Male	36 (58.1)	9 (56.2)	
Birthweight, g, median (min - max)	3435 (2360-5220)	2915 (1970-3855)	0.009**
Birthweight Centile, %, median (min-max)	60.0 (3.0-100.0)	31.0 (7.0-92.0)	0.050*
Estimated Blood Loss, mL, median (min - max)	400 (100-2005)	480 (300-800)	0.52

*p<0.05, **p<0.01, ***p<0.001

Table 5.2 *– Specific cardiac lesions of participants with congenital heart disease included in the study. AS – aortic stenosis; BAV – bicuspid aortic valve; CoA – coarctation of the aorta; LDA – low-dose aspirin; LMWH – low-molecular-weight heparin; PDA – patent ductus arteriosus; r – repaired; SA – subaortic stenosis; ToF – Tetralogy of Fallot; TCPC – total cavopulmonary connection; TGA – transposition of great arteries; VSD – ventricular septal defect. N=16*

CHD lesion	N	Use of medication
rTOF	**5**	One participant on LDA, two participants on beta-blockers
rTOF only	*4*	
rTOF + rSA stenosis	*1*	
rCoA	**2**	
rCoA only	*1*	
rCoA + BAV	*1*	
rTGA	**3**	One participant on beta-blockers, one participant on LMWH
rTGA (arterial switch)	*2*	
rTGA (atrial switch)	*1*	
Fontan TCPC	**1**	On LMWH
Ebstein's Anomaly	**1**	
Right hemi-truncus	**1**	
Mixed Lesions	**3**	
AS + BAV	*1*	
rVSD + rPDA + BAV	*1*	
BAV	*1*	

5.4.2. *Association of estimated fetal weight, birthweight and birthweight centiles with cardiac output at rest, percentage increase in cardiac output with exercise (increase in heart rate to 70% of predicted for age) and with mean arterial blood pressure in low-risk pregnancy and in pregnant women with congenital heart disease*

The association between haemodynamic parameters measured by PhysioFlow® (CO, percentage increase in cardiac output (ΔCO) and MAP) with fetal ultrasound measurements (EFW, AC/EFW ratio) in the third trimester and with BW and BW centile, in low-risk pregnancy, are presented in Table 5.3. Table 5.4 describes the association between these parameters when measured by TTE. BW, BW centile,

UtAD RI, UtAD PI, UtAD PI and RI in multiples of the median (MoM), CPR and CPR MoM did not follow a Gaussian distribution. UAD PI, UAD RI, UAD PI and RI MoM, MCA PI, MCA RI, MCA PI and RI MoM, AC/EFW ratio followed a Gaussian distribution.

Table 5.3 *– Spearman's correlation (r_s) between haemodynamic parameters measured by* ***PhysioFlow®*** *(cardiac output (CO), percentage increase in cardiac output (ΔCO) and mean arterial blood pressure (MAP)) with fetal ultrasound measurements (estimated fetal weight (EFW) and abdominal circumference (AC)/EFW ratio) in the third trimester (Trimester C) and with birthweight (BW) and BW centile in low-risk pregnancy. N = 62. Significance level: p<0.05.*

		EFW	AC/EFW	BW	BW centile
CO, L/min (rest)	r_s	0.200	-0.218	0.092	0.084
	p value	0.12	0.09	0.48	0.52
ΔCO, % (CO peak exercise - CO rest/CO peak exercise)	r_s	0.036	-0.031	-0.056	0.009
	p value	0.78	0.81	0.67	0.95
MAP, mmHg ([systolic BP+2*diastolic BP]/3)	r_s	0.114	-0.110	-0.199	-0.170
	p value	0.38	0.39	0.12	0.19

Table 5.4 *– Spearman's correlation (r_s) between haemodynamic parameters measured by* ***transthoracic echocardiogram*** *(cardiac output (CO) and percentage increase in cardiac output (ΔCO)) with fetal ultrasound measurements (estimated fetal weight (EFW) and abdominal circumference (AC)/EFW ratio) in the third trimester (Trimester C) and with birthweight (BW) and BW centile in low-risk pregnancy. N = 47. Significance level: p<0.05.*

		EFW	AC/EFW	BW	BW centile
CO, l/min (rest)	r_s	0.044	-0.052	0.036	0.087
	p value	0.77	0.73	0.81	0.56
ΔCO, % (T2-T1/T2)	r_s	**-0.298**	**0.304**	-0.177	-0.153
	p value	**0.042***	**0.038***	0.24	0.30

*p<0.05

The scatter plots for EFW and the AC/EFW ratio against ΔCO are presented in Figures 5.2 and 5.3, as these showed a statistically significant correlation with each other as per table 5.4.

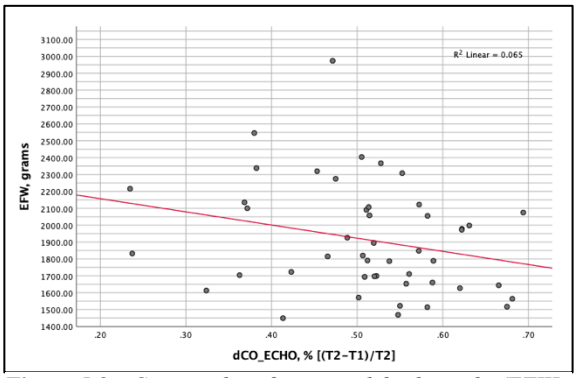


Figure 5.2 *– Scatter plot of estimated fetal weight (EFW), in grams and percentage cardiac output increase (dCO) with exercise, measured by transthoracic echocardiography in low-risk pregnancy with fit line at total.*

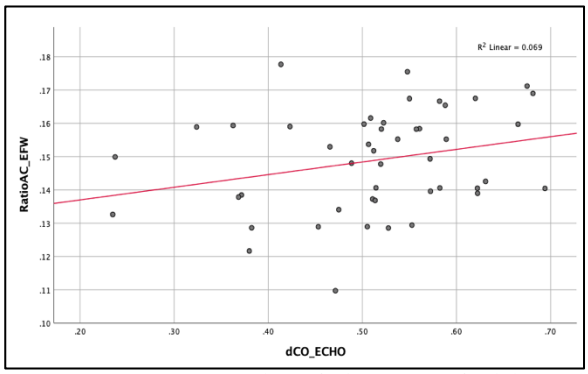


Figure 5.3 *– Scatter plot of the ratio of abdominal circumference (AC) to estimated fetal weight (EFW) and percentage increase in cardiac output (dCO) with exercise, measured by transthoracic echocardiography in low-risk pregnancy with fit line at total.*

The association between the same haemodynamic parameters measured by PhysioFlow® (CO, ΔCO and MAP) with fetal ultrasound measurements (EFW, AC/EFW ratio) in the third trimester and with BW and BW centile, in the CHD group, are presented in Table 5.5. Table 5.6 describes the association between these parameters when measured by TTE.

Table 5.5 *– Spearman's correlation (r_s) between haemodynamic parameters measured by **PhysioFlow®** (cardiac output (CO), percentage increase in cardiac output (ΔCO) and mean arterial blood pressure (MAP)) with fetal ultrasound measurements (estimated fetal weight (EFW) and abdominal circumference (AC)/EFW ratio) in the third trimester (Trimester C) and with birthweight (BW) and BW centile in pregnant women with congenital heart disease (CHD). N = 16. Significance level: p<0.05.*

		EFW	AC/EFW	BW	BW centile
CO, l/min (rest)	r_s	0.256	-0.218	0.478	0.358
	p value	0.34	0.42	0.06	0.17
ΔCO, % (CO peak exercise -CO rest/CO peak exercise)	r_s	0.232	-0.253	**0.559**	0.294
	p value	0.39	0.35	**0.024***	0.27
MAP, mmHg ([systolic BP +2*diastolic BP/3)	r_s	0.000	0.018	0.297	0.142
	p value	1.00	0.95	0.26	0.6

*p<0.05

Table 5.6 *– Spearman's correlation (r_s) between haemodynamic parameters measured by* ***transthoracic echocardiogram*** *(cardiac output (CO) and percentage increase in cardiac output (ΔCO)) with fetal ultrasound measurements (estimated fetal weight (EFW) and abdominal circumference (AC)/EFW ratio) in the third trimester (Trimester C) and with birthweight (BW) and BW centile in pregnant women with congenital heart disease (CHD). N = 14. Significance level: p<0.05.*

		EFW	AC/EFW	BW	BW centile
CO, l/min (rest)	r_s	0.231	-0.235	-0.108	0.174
	p value	0.43	0.42	0.71	0.55
ΔCO, % (T2-T1/T2)	r_s	-0.284	0.319	0.130	-0.020
	p value	0.33	0.27	0.66	0.95

5.4.3. *UtA, UA and MCA Doppler indices and correlation with EFW, BW, BW centile, CO at rest, ΔCO and MAP in low-risk pregnancy, in the third trimester (between 30 and 34 weeks of gestation)*

Table 5.7 describes the GA and Doppler indices performed in the third trimester in the low-risk pregnancy group.

Table 5.7 *– Gestational age and Doppler indices of ultrasound performed in the third trimester in low-risk pregnancy. Data presented as median and min-max. GA – gestational age; MCA – middle cerebral artery; PI – pulsatility index; RI – resistance index; UAD – umbilical artery Doppler; UtA – uterine artery Doppler. N = 62.*

GA Median (Min-Max)	**UtAD PI** Median (Min-Max)	**UtAD RI** Median (Min-Max)	**UAD PI** Median (Min-Max)	**UAD RI** Median (Min-Max)	**MCA PI** Median (Min-Max)	**MCA RI** Median (Min-Max)
31+6	0.698	0.470	0.970	0.630	1.970	0.840
(30+0-34+0)	0.3-1.7	0.3-0.7	0.7-1.4	0.5-0.8	1.3-2.9	0.7-1.0

Correlation between UtAD studies and EFW, BW, BW centile and CO at rest, ΔCO and MAP are presented in figures 5.4 – 5.9. Correlation between UAD studies and EFW, BW, BW centile and CO at rest, ΔCO and MAP are presented in figures 5.10 – 5.15. Correlation between CPR and EFW, BW, BW centile and CO at rest, ΔCO and MAP are presented in figures 5.16 – 5.21. The correlation between UtAD PI MoM and CPR MoM in the low-risk group is illustrated in figure 5.22.

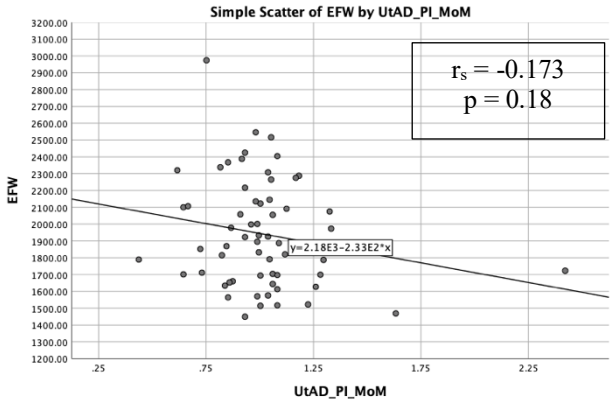


Figure 5.4 *– Scatter plot of estimated fetal weight (EFW) and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

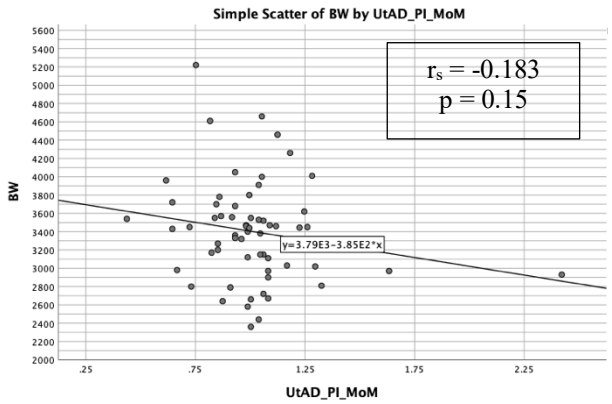


Figure 5.5 *– Scatter plot of birthweight (BW) and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

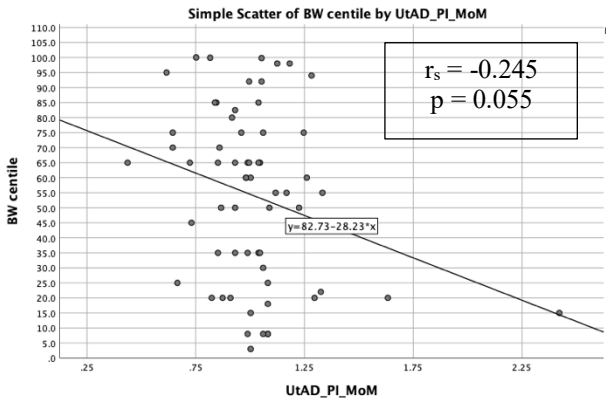


Figure 5.6 *– Scatter plot of birthweight (BW) centile and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

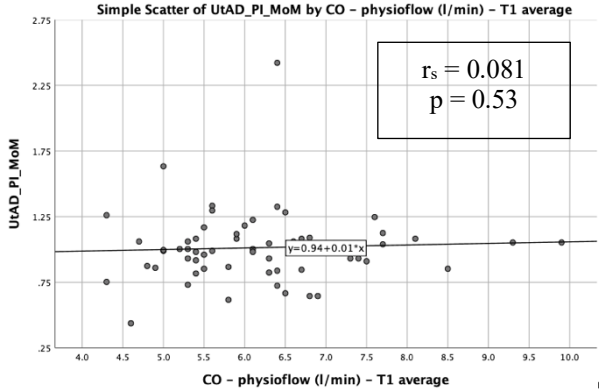


Figure 5.7 *– Scatter plot of cardiac output (CO) at rest, measured by PhysioFlow®, and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

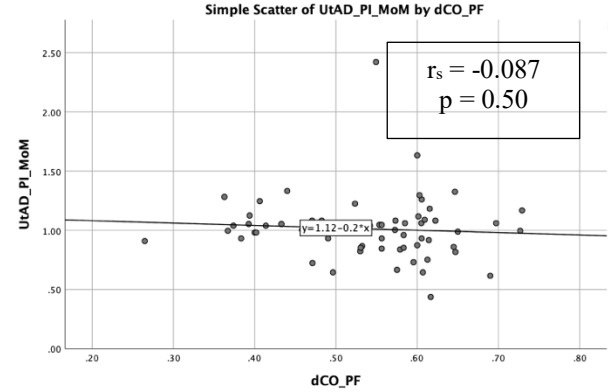


Figure 5.8 *– Scatter plot of percentage increase in cardiac output (ΔCO), measured by PhysioFlow®, and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

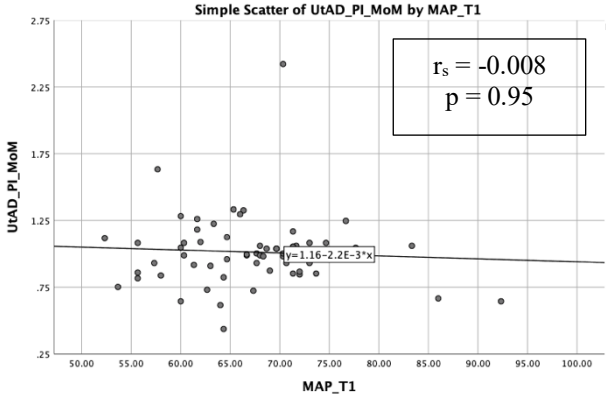


Figure 5.9 *– Scatter plot mean arterial blood pressure (MAP) and uterine artery Doppler (UtAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

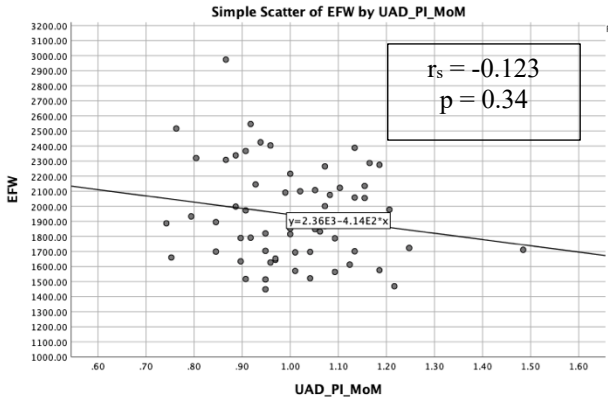


Figure 5.10 *– Scatter plot of estimated fetal weight (EFW) and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

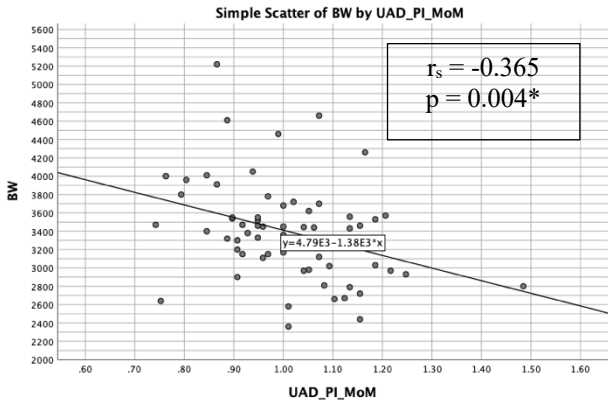


Figure 5.11 *– Scatter plot of birthweight (BW) and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

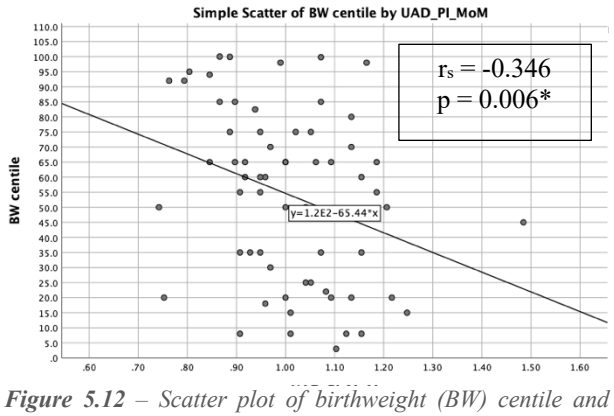


Figure 5.12 *– Scatter plot of birthweight (BW) centile and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

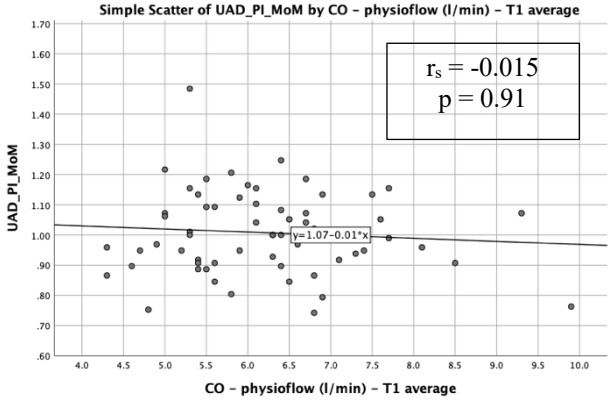


Figure 5.13 *– Scatter plot of cardiac output (CO) at rest, measured by PhysioFlow®, and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

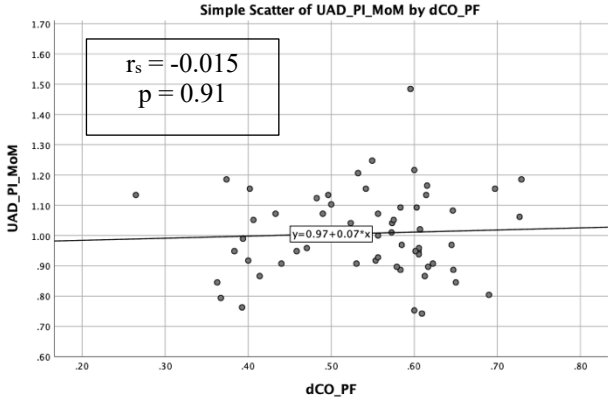


Figure 5.14 *– Scatter plot of percentage increase in cardiac output (CO), measured by PhysioFlow®, and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

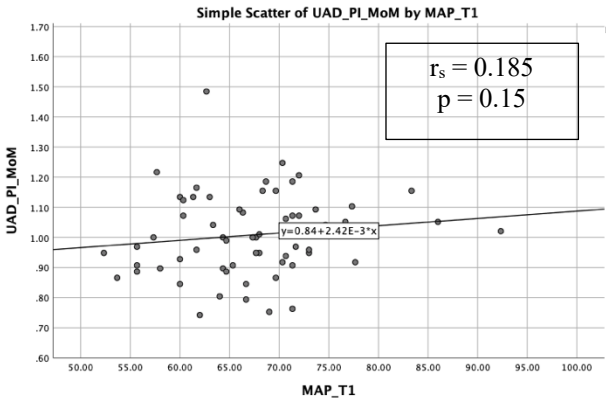


Figure 5.15 *– Scatter plot mean arterial blood pressure (MAP) and umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) in the low-risk group. r_s – Spearman's rho; p – p value*

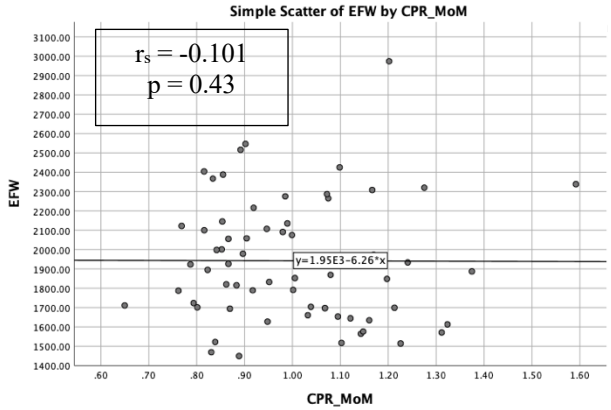


Figure 5.16 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and estimated fetal weight (EFW) in the low-risk group. r_s – Spearman's rho; p – p value*

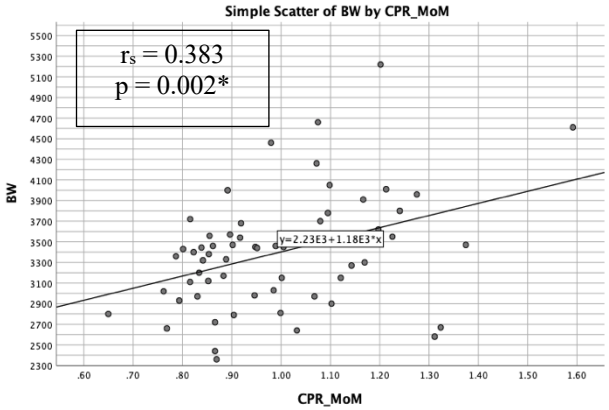


Figure 5.17 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and birthweight (BW) in the low-risk group. r_s – Spearman's rho; p – p value*

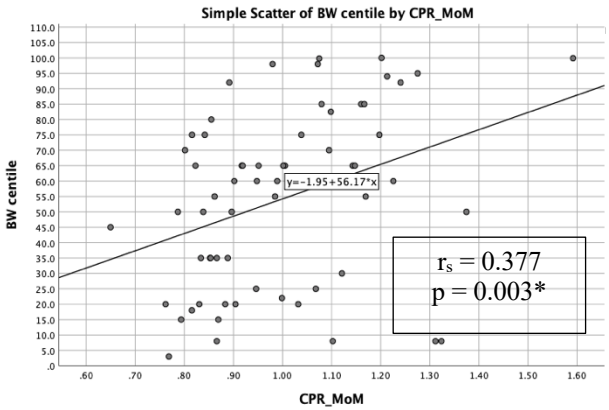


Figure 5.18 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and birthweight (BW) centile in the low-risk group. r_s – Spearman's rho; p – p value*

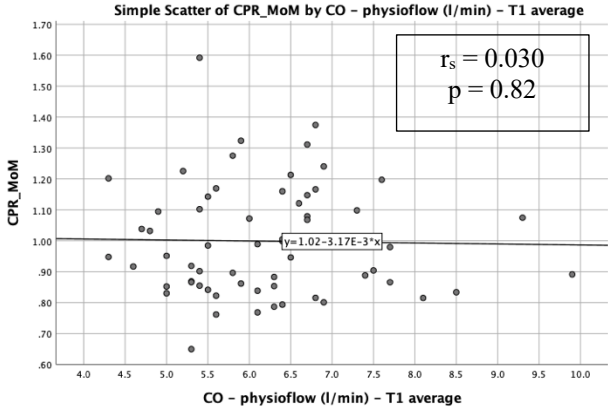


Figure 5.19 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and cardiac output (CO) at rest, measured by PhysioFlow®, in the low-risk group. r_s – Spearman's rho; p – p value*

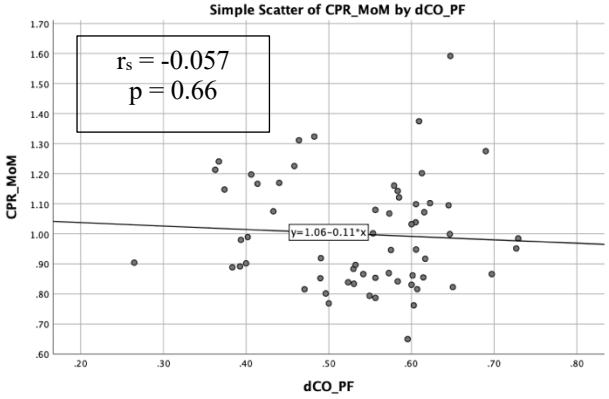


Figure 5.20 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and percentage increase in cardiac output (CO) with exercise, measured by PhysioFlow®, in the low-risk group. r_s – Spearman's rho; p – p value*

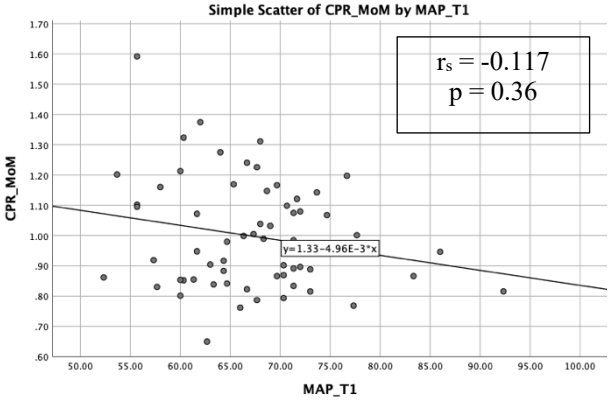


Figure 5.21 *– Scatter plot of cerebroplacental ratio (CPR) in multiples of the median (MoM) and mean arterial blood pressure (MAP) in the low-risk group. r_s – Spearman's rho; p – p value*

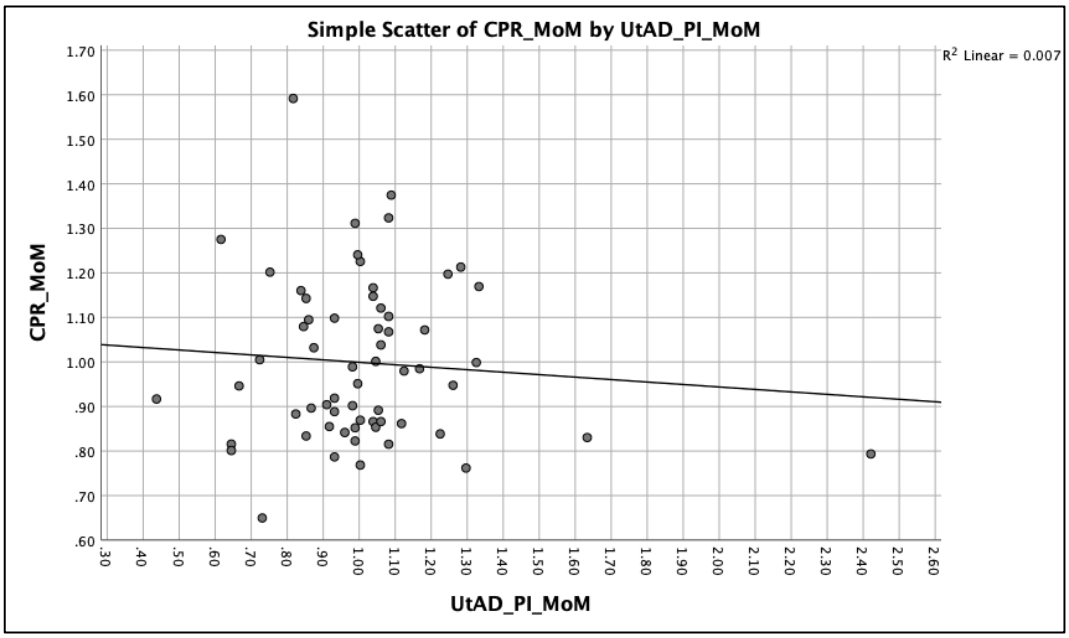


Figure 5.22 – *Scatter plot of uterine artery Doppler (UtAD) pulastility index (PI) in multiples of the median (MoM) and the cerebroplacental ratio (CPR) in MoM in the low-risk group.Spearman's correlation, r: 0.028; p value: 0.82.*

5.4.4. *UtA, UA and MCA Doppler indices and correlation with EFW, BW, BW centile, CO at rest, ΔCO and MAP in pregnant women with CHD, in the third trimester (between 30 and 34 weeks of gestation)*

Table 5.8 describes the GA and Doppler indices of ultrasound performed in the third trimester in the CHD group. Correlation between UAD studies and EFW, BW, BW centile and CO at rest, ΔCO and MAP are presented in figures 5.23 – 5.28. Correlation between UtADs and CPRs and EFW, BW, BW centile and CO at rest, ΔCO and MAP are presented in Table 5.9 due to small numbers.

Table 5.8 *– Gestational age and Doppler indices of ultrasound performed in the third trimester in pregnant women with congenital heart disease (CHD). Data presented as median and min-max. GA – gestational age; MCA – middle cerebral artery; PI – pulsatility index; RI – resistance index; UAD – umbilical artery Doppler; UtA – uterine artery Doppler. Data presented as medians and minimum-maximum. N = number of participants.*

GA Median (Min-Max)	**UtAD PI** Median (Min-Max)	**UtAD RI** Median (Min-Max)	**UAD PI** Median (Min-Max)	**UAD RI** Median (Min-Max)	**MCA PI** Median (Min-Max)	**MCA RI** Median (Min-Max)
	N = 3		N = 16		N = 7	
32+1	0.880	0.570	0.885	0.595	1.810	0.830
(31+6-34+0)	0.6-0.9	0.5-0.6	0.7-1.1	0.5-0.7	1.5-2.4	0.8-0.9

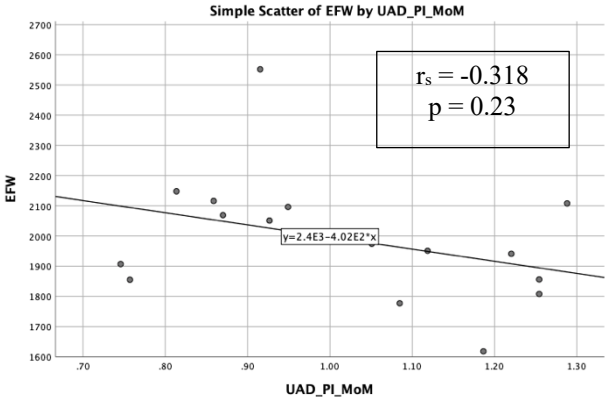


Figure 5.23 *– Scatter plot of estimated fetal weight (EFW) and umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD). r_s – Spearman's rho; p – p value*

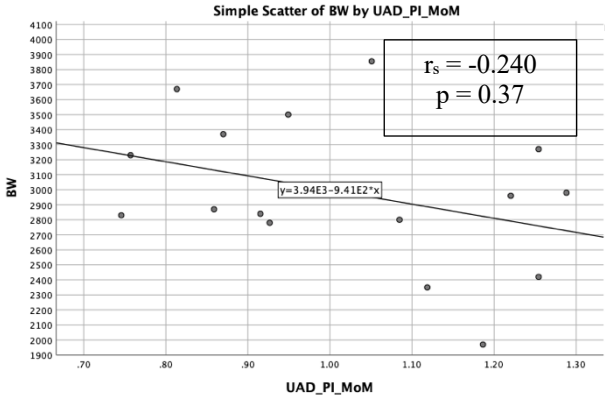


Figure 5.24 *– Scatter plot of birth weight (BW) and umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD). r_s – Spearman's rho; p – p value*

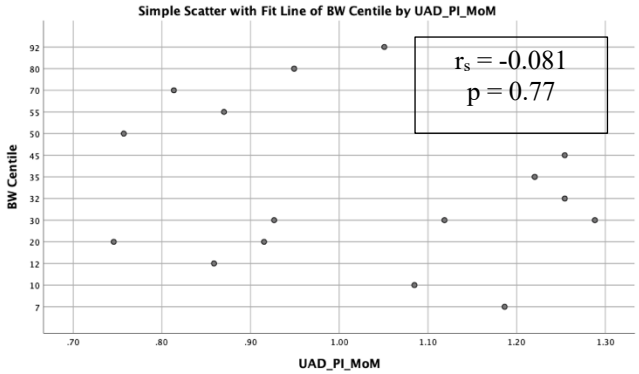


Figure 5.25 *– Scatter plot of birth weight (BW) centile and umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD). r_s – Spearman's rho; p – p value*

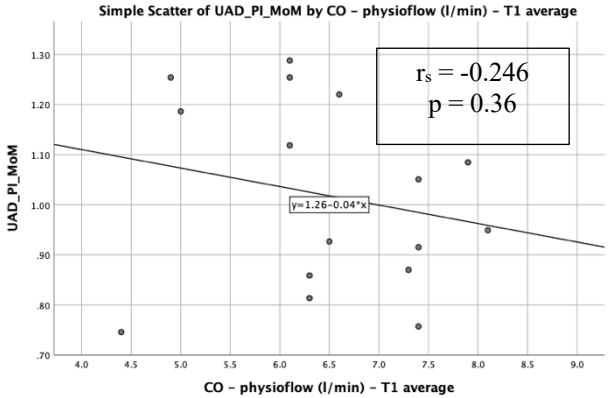


Figure 5.26 *– Scatter plot of umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD) and cardiac output (CO) at rest. r_s – Spearman's rho; p – p value*

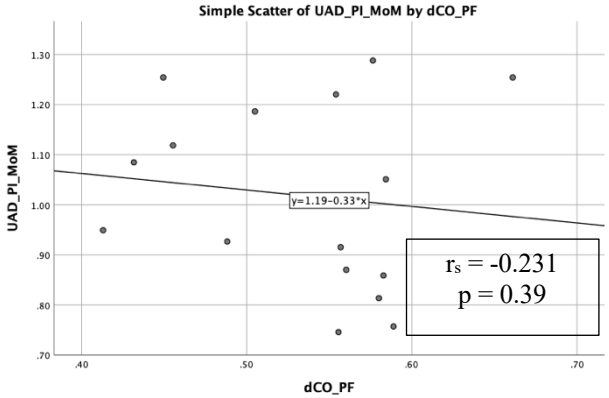


Figure 5.27 *– Scatter plot of umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD) and percentage cardiac output increase (dCO). r_s – Spearman's rho; p – p value*

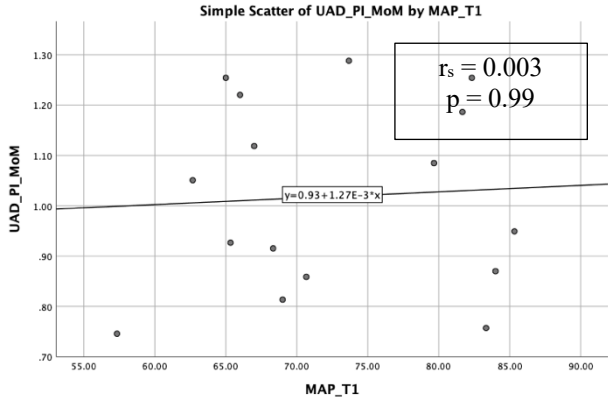


Figure 5.28 *– Scatter plot of umbilical artery Doppler (UAD) studies in multiples of the median (MoM) in the third trimester, in pregnant women with congenital heart disease (CHD) and mean arterial blood pressure (MAP). r_s – Spearman's rho; p – p value*

Table 5.9 *– Spearman's correlation (r_s, and associated p values) for umbilical artery Doppler (UAD) pulsatility index (PI) in multiples of the median (MoM) and cerebroplacental ratio (CPR) in multiples of the median (MoM) and the studied variables: estimated fetal weight (EFW), birthweight (BW), BW Centiles, cardiac output (CO) at rest and ΔCO in pregnant women with congenital heart disease (CHD).*

		EFW	BW	BWc	CO	dCO	MAP
UAD PI MoM N=16	rs	-0.318	-0.240	-0.081	-0.095	-0.231	0.003
	p	0.23	0.37	0.77	0.75	0.39	0.99
CPR MoM N=7	rs	0.071	0.143	0.357	0.143	-0.071	0.071
	p	0.88	0.76	0.43	0.79	0.88	0.88

5.4.5. *Differences in third trimester (between 30 and 34 weeks of gestation) of UtA, UA and MCA Doppler indices between low-risk pregnant women and pregnant women with CHD*

Table 5.10 summarises the differences in Doppler indices between the two studied groups.

Table 5.10 *– Summary of Mann-Whitney U test for differences in Doppler indices between the two studied groups: LR – low-risk pregnancy; CHD – pregnant women with congenital heart disease (CHD). CPR – cerebroplacental ratio; GA – gestational age; MCA – middle cerebral artery; PI – pulsatility index; RI – resistance index; UAD – umbilical artery Doppler; UtA – uterine artery Doppler.*

	LR	CHD	p value
GA Median (Min-Max)	31+6 (30+0 – 34+0) N=62	32+1 (31+6-34+0) N=16	**0.040***
UtAD PI Median (Min-Max)	0.698 (0.3-1.7) N=62	0.880 (0.6-0.9) N=3	0.23
UtAD RI Median (Min-Max)	0.470 (0.3-0.7) N=62	0.570 (0.5-0.6) N=3	0.14
UAD PI Median (Min-Max)	0.965 (0.7-1.4) N=62	0.885 (0.7-1.1) N=16	0.11
UAD RI Median (Min-Max)	0.630 (0.5-0.8) N=62	0.595 (0.5-0.7) N=16	0.07
MCA PI Median (Min-Max)	1.970 (1.3-2.9) N=62	1.810 (1.5-2.4) N=7	0.64
MCA RI Median (Min-Max)	0.840 (0.7-1.0) N=62	0.830 (0.8-0.9) N=7	0.83
CPR Median (Min-Max)	1.961 (1.3-3.2) N=62	2.057 (1.29-2.35) N=7	0.91

Using a linear mixed model, all Doppler indices (UtAD PI, UtAD RI, UAD PI, UAD RI, MCA PI and MCA RI) were corrected for gestational age and no statistically significant difference was found between the groups.

5.5. Discussion

5.5.1. *Maternal characteristics, obstetric and fetal outcomes*

There were no statistically significant differences between the two studied populations with regard to race, smoking status, parity, MOD, sex of the newborn and EBL. Women in the CHD group were delivered by Caesarean section more often than the low-risk cohort (56.2% *vs* 38.7%), despite the CHD group having a slightly higher proportion of multiparous women. Although the results did not reach statistical significance, it is well established from previous studies that women with CHD are more often delivered by Caesarean section[213-215]. Although, as discussed in Chapter 4, pregnant women with CHD tend to have higher rates of PPH[216], we did not find any significant differences in this particular cohort. This might be partly explained by the small number of CHD participants compared to the low-risk participants (n = 16 *vs* n = 62), as we have found a statistically significant difference when the number of participants in each of the groups is similar (please see Chapters 6 and 7), which is also consistent with the literature.[216]

Participants in the CHD group were younger by three years (p = 0.009), heavier by seven kilograms (p = 0.010) and, consequently, their BMI was higher by 4kg/m^2 (p = 0.038). We were unable to perform a subgroup analysis stratifying by age, weight and BMI as this would result in small numbers in each of the categories. GA at delivery was significantly different (p = 0.001), with women in the CHD group delivering, on average, eight days earlier than the low-risk group. This is consistent with our findings in similar cohorts in this thesis (please see Chapters 3, 4, 6 and 7), and with the literature.[55, 215] BW was also statistically different (p = 0.009), with women in the CHD group delivering babies who were, on average, 500g lighter than the low-risk group. The BW centiles showed a trend for BW centiles to be lower in the CHD group (mean centile 31 *vs* 60), although this was not statistically significantly

different in this cohort (p = 0.050), probably due to small numbers (please see Chapters 4, 6 and 7 where we did find a statistically significant difference between the two studied groups). Both poor cardiac function in the CHD group and the use of beta-blockers seem to be associated with lower birthweights.[55, 57] Three of our CHD participants were on beta-blockers (two on Bisoprolol 2.5mg once daily and one on Labetalol 100mg once daily). Although, because of small numbers, we did not perform a statistical test to investigate if there were differences between those taking beta-blockers and those not on any medication, it is unlikely that there would be a difference.

5.5.2. *Association of EFW, BW and BW centiles with CO at rest, ΔCO and MAP in low-risk pregnancy and in pregnant women with CHD*

In the low-risk group, we did not find any statistically significant association between CO at rest, CO percentage increase (ΔCO, measured by ICG) or MAP and EFW, the AC/EFW ratio, BW and BW centiles. When CO was measured by TTE, in the low-risk group, we found a significant association between ΔCO and EFW (p = 0.042) and with the AC/EFW ratio (p = 0.038). No statistically significant association was found between CO and BW or BW centiles. As discussed in Chapter 4, although ICG is useful for trending analysis, the absolute level of CO measured by ICG and by TTE in the same individuals is different and not correlated, which can explain why a difference was detected using one device and not the other. The association between ΔCO and EFW was negative, meaning that in those with larger variations of CO with exercise, EFW was lower. This could potentially be explained by the fact that women with larger percentage increases in CO with exercise have lower baseline COs and therefore "worse" cardiac function, as they would have not increased their CO as much compared to pre-pregnancy values.

In the CHD group, we did not find any statistically significant association between CO at rest (measured by ICG) or MAP and EFW, the AC/EFW ratio or BW centile, but we did find a correlation (p = 0.024) between ΔCO (measured by ICG) and BW. The relationship was, however, positive – those with higher

relative increases in CO had higher BW, which contradicts the conclusions made above for the low-risk population. However, as discussed in Chapter 4, ICG measures, on average, higher CO at rest when compared to TTE and the number of participants in the CHD cohort was small (n = 16), which suggests this might have been a spurious result. When CO was measured with TTE, no statistically significant differences were found between CO at rest, ΔCO and EFW, AC/EFW ratio, BW or BW centiles.

Stott *el at.*[235] showed no significant association between CO (measured non-invasively by bioreactance, NICOM device) and BW or MAP in a low-risk population. However, the authors found a significant association between CO, MAP and BW in the high-risk group with PE.[235] The high-risk group showed higher MAP compared to the low-risk group and those who subsequently developed PE and FGR also had significantly lower CO.[235] Other studies have shown similar results. Bamfo *et al.*[35] showed that in pregnancies complicated by FGR, CO was lower and TVR was higher than in normal pregnancies. In a different study, Bamfo *et al.*[56] described similar findings and suggested that a logistic regression equation incorporating maternal echocardiographic parameters could be used to diagnose FGR. Vasapollo *et al.*[50] also showed that women with FGR had lower CO and higher MAP and TVR compared to women with SGA babies. The authors suggested that maternal cardiovascular function is a contributor to the development of FGR and report that the cardiovascular changes precede the development of FGR/SGA and that TVR might be considered an indirect marker of cardiovascular function in pregnancy.[50] Melchiorre *et al.*[236] showed that women who developed preterm PE showed significantly higher TVR and lower CO mid-gestation (between 20-23 weeks of gestation) than those who developed PE at term, which is also consistent with the findings of Valensise *et al.*[237] Our median BW centile, for the low-risk population, was 60.0 and only four out the 62 participants had BW centiles below the 10th centile. None of our women had notching of UtAD and only one had UtAD PI >1.45. None developed PE and only one participant developed gestational hypertension at term. This makes our population essentially low-risk which might explain the lack of association between CO and EFW, BW, BW centiles and Doppler indices.

5.5.3. *UtA, UA and MCA Doppler indices and correlation with EFW, BW, BW centile, CO at rest, ΔCO and MAP in low-risk pregnancy, in the third trimester (between 30 and 34 weeks of gestation)*

The values of the Doppler indices for the low-risk population were within the normal ranges for the gestation studied (30+0-34+0). UtAD RI median was within normal range and on the 50th percentile and UtAD PI median was within normal range and slightly above the 50th percentile.[238] UAD PI and RI medians were within normal ranges and the median was in the 50th percentile.[238] MCA PI and RI medians were within normal ranges and on the 50th percentile.[238] Cnossen *et al.*[60] performed a systematic review evaluating 15 Doppler indices and showed that UtAD PI was the best predictor for PE in low and high-risk populations and that UtAD PI best predicted severe growth restriction in low-risk populations, whereas UtAD RI was the best predictor for high-risk populations. As we only obtained UtAD in three of the CHD participants (high-risk), we decided to perform our analysis using UtAD PI as the majority of our cohort was in the low-risk category.

We found no significant correlation between UtAD PI in MoM and EFW, BW or BW centile, although higher values of UtAD PI were associated with lower EFW, lower BW and lower BW centiles. However, for BW centile, there was a trend with a p value of 0.055 and perhaps with a higher number of participants, significance would have been achieved. UtAD is a non-invasive tool for the assessment of the placental circulation in the first and second trimesters and correlates with BW.[239] Prefumo *et al.*[240] demonstrated that the proportion of decidual vessels in placentae of women with low resistance UtAD indices was higher than in those with high-resistance circulation.

We found no association between UtAD PI and CO at rest, ΔCO or MAP. We report on UtAD PI but also performed analysis of UtAD RI and still found no correlation. We also report on both CO measured by ICG and CO measured by TTE and still found no statistically significant correlation. Velensise *et al.*[241] showed a negative association between UtAD RI and CO. The authors used TTE and CO was measured from SV derived from the Teicohholz formula. We cannot assess whether differences in

methodology could explain the differences in our results as the populations studied are too small to compare the results. In a different study, Valensise *et al.*[242] showed that in women with low-risk pregnancies, who were non-smokers, not on any medication and with no history of cardiac disease, but who nonetheless had a high UtAD RI and notching and who subsequently developed a pregnancy complication, CO was significantly lower than in those who did not have an adverse pregnancy outcome. The authors also showed higher TVR in these patients, suggesting poor cardiovascular adaptation to pregnancy and inadequate volume expansion.[242] The placental bed might be responsible for about 20% reduction in TVR and a decrease in UtAD RI by a factor of one, causing a reduction in TVR by a factor of 0.26.[241]. Prefumo *et al.*[239] also reported worse diastolic function in women with increased resistance in the UtAD indices. Novelli *et al.*[243], who performed TTE at 24 weeks of gestation in normotensive women with bilateral notching of the uterine arteries, demonstrated lower CO and higher MAP in those who developed pregnancy complications. Pieper *et al.*[32] also demonstrated that UtAD and UAD parameters were associated with ventricular and valvular function and that, in women with CHD, abnormal values were associated with worse cardiac function.

CPR is a strong and independent predictor of stillbirth and perinatal death (more so than UtAD PI)[67], hence we studied the correlation of CPR in MoM and fetal and maternal parameters. There was a positive and statistically significant association between CPR and BW and BW centile in the low-risk group. There was an association between UtAD PI and CPR. Women with higher UtAD PI had lower CPR, suggesting that increased UtAD PI, a marker of placental perfusion, is associated with fetal ischemic response. This association did not, however, reach statistical significance ($p = 0.82$), probably due to the small number of participants. Even in fetuses deemed appropriate for gestational age, low CPR is associated with abnormal fetal growth and lower BW[244], and our findings are consistent with this.

5.5.4. *Differences between groups*

Participants in the CHD cohort had higher UtAD indices (both PI and RI) but statistical significance was not reached, probably due to the small number of participants in the CHD cohort (n = 3). UAD indices were surprisingly higher in the low-risk group although, once again, differences did not reach statistical significance. One possible explanation is that women in the CHD group had their scans and Doppler studies later in the pregnancy (GA median CHD: 32+1 *vs* GA median LR: 31+6, p = 0.040) and the UAD PI decreases with gestation.[238] The number of CHD participants with data on UAD was also small compared to the low-risk group (n = 16 *vs* n = 62), which could explain these findings. The CHD lesions were multiple (11 different lesions in 16 participants), which could cause scatter of the data as different cardiac lesions will have different haemodynamic implications. After correcting for gestational age, no difference was found between the groups. MCA PI and RI were slightly lower in the CHD group but not statistically significant.

Pieper *et al.*[32] who performed Doppler studies in about 150 pregnant women with CHD (compared with 70 low-risk pregnancies) showed that both UtAD and UAD PIs and RIs were significantly higher in the CHD group at 20 and 32 weeks of gestation, and that a high UAD PI at 32 weeks was associated with "obstetric events" in the CHD group. Goya *et al.*[68] also showed increased UtAD PI and increased UAD PI in 170 pregnant women with heart disease (of whom 70% had CHD; compared with 75 low-risk pregnancies) and a raised UAD PI at 32 weeks of gestation was associated with "obstetric events". Obstetric events in these studies were a combination of outcomes such as SGA/FGR and PE. Both these studies included participants with a broad mixture of CHD lesions and in different functional classes. Kapman *et al.*[69] report on 62 pregnant women with ToF (69 controls) and showed that UAD PI was significantly higher at 32 weeks of gestation in the ToF group but no differences were found between the two groups with regards to UtAD indices. Siegmund *et al.*[70], however, did not show a significant difference between UtAD and UAD RIs and PIs at 20 or 32 weeks of gestation in 49 women with repaired CoA (69 controls), which the authors explain could be due to small sample size.

Existing evidence from Doppler studies in pregnancy in women with CHD and their association with cardiac function is scarce.[63] Kampman *et al.*[63] report in their systematic review that women with heart disease have a higher incidence of abnormalities in uteroplacental blood flow indices and poor obstetric outcome and, at the same time, women with abnormal uteroplacental flow are also more likely to have cardiac dysfunction. The placentation process causes a low-resistance system in the utero-placental interface and blood flow depends mainly on maternal cardiovascular function due to the absence of autoregulation in the uteroplacental interface.[63]

Our results regarding UtAD differences in the two groups are in line with the literature.[32, 68, 69] Still, we cannot draw any conclusions as we only had results for three of the participants with CHD. Our results regarding UAD indices are somewhat contradictory with those in the literature, as UAD indices were lower in the CHD group. As discussed above, perhaps the fact that we had only a few women with CHD and UAD measurements, and the fact that they had their Doppler studies done later in gestation, explains these results. MCA Doppler RI/PI will reduce between 30-34 weeks of gestation in the general population and in our cohort they were lower in the CHD group, as expected based on the results from the UAD indices. We could not find any data regarding MCA Dopplers in a population with CHD and therefore cannot comment if this difference is expected or not.

5.6. Conclusion

There was no association between CO at rest, percentage increase in CO with exercise measured by ICG in the third trimester or MAP, and EFW, BW or BW centile, in a low-risk pregnant population. There was a statistically significant association between percentage increase in CO with exercise (a measure of cardiac function) measured by TTE in the third trimester and EFW, which can be explained by the fact that those with larger percentage increases in CO with exercise have lower baseline CO and, low CO is associated with lower BW.

There was a statistically significant correlation between percentage increase in CO with exercise measured by ICG in the third trimester in the CHD cohort and BW. These results are difficult to interpret

due to the small sample size but suggest that those with the ability to increase their CO with exercise by a greater degree have better cardiac function and therefore larger BW. No association was found in this cohort between CO at rest or percentage increase in CO with exercise measured by TTE in the third trimester or MAP, and EFW, BW or BW centile.

No association was found between UtAD PI and EFW, BW, BW centiles, CO, ΔCO and MAP in either population. In the low-risk group, UAD was negatively associated with BW and BW centile suggesting lower PI, and therefore less resistance in the uteroplacental circulation, is associated with higher BW. This association was further reinforced by the correlation found between CPR and BW and BW centile in the low-risk group. No correlations were found between UAD indices and the studied parameters in the CHD group.

UtAD indices were higher in CHD population, as expected, although we only had data for three CHD participants. The most surprising finding in this study was that women with CHD had lower UAD indices when compared to the low-risk group, which contradicts existing literature. However, CHD had Doppler studies at a later gestation (resistance decreases with advancing gestation) and also numbers were small and therefore results need to be interpreted with caution. After correcting for gestational age, using a linear mixed model, there were no differences between the groups.

Chapter 6.

Expression of B-type natriuretic peptide in low-risk pregnancy and in pregnancy complicated by congenital heart disease:

association with cardiac function measured by Impedance Cardiography (Physioflow®)

Chapter 6. Expression of B-type natriuretic peptide in low-risk pregnancy and in pregnancy complicated by congenital heart disease: association with cardiac function measured by Impedance Cardiography (Physioflow®)

6.1. Introduction

BNP is secreted by cardiac myocytes in response to changes in LV loading.[115] Myocyte stretch induced by pressure overload, volume expansion or increased myocardial wall stress, stimulates the secretion of the BNP pro-peptide, which is then transformed into two main molecules: the NT pro-BNP (inactive) and BNP (bioactive).[115-117] The physiological haemodynamic changes characteristic of pregnancy would, in the non-pregnant state, induce the release of BNP.[118] BNP has natriuretic, vasodilator and diuretic effects.[117] Concentration of BNP is higher in women, increase with age and declining renal function and decrease with increasing BMI.[115]

Outside pregnancy, BNP can reliably discriminate between HF and other causes of dyspnoea, and predict the risk of sudden death in patients with established HF, but it correlates poorly with levels of LV loading.[119, 120] The upper limits of normal in the non-acute setting are <35pg/mL for BNP and, <125pg/mL for NT pro-BNP. In the acute setting, higher values are used and the cut-offs are <100pg/mL for BNP and, <300pg/mL for NT pro-BNP; patients with BNP concentrations below these levels, are highly unlikely to have HF.[121] Diagnosing heart disease in pregnancy can be difficult as the normal symptoms of pregnancy can mimic those of heart decompensation or failure.[118] It is hypothesised that the volume load of pregnancy is responsible for some of the cardiovascular events in pregnancy and that BNP could play a role in predicting them.[120]

Studies on BNP in pregnancy have shown conflicting results[122-124] and reference values for pregnancy are yet to be defined.[125] In many studies, BNP was measured to assess the strain on the LV secondary to HTN or PE.[127, 128] The largest study assessing BNP levels in pregnancy, by Mayama *et al.*[125], included 773 women with low-risk pregnancy and showed mean concentrations of 17.9 pg/mL (95% CI [0-44.7]) between 35-37 weeks of gestation, levels which were higher compared to non-pregnant individuals

(mean concentration of 11.8 pg/mL (95% CI [0-27.5]). Burlingame *et al.*[129] performed longitudinal measurements of BNP at six time points during pregnancy/postpartum (the only study profiling longitudinal BNP measurements during pregnancy/postpartum) in 116 women with low-risk pregnancy and showed stable levels (median concentrations of around 25pg/mL) during pregnancy, with an increase within 48 hours postpartum but a return to baseline by 6-12 weeks postpartum. The study by Yurteri-Kaplan *et al.*[128] included 104 women and reported that BNP concentrations in pregnancy at term are similar to those of non-pregnant individuals (median concentration 20 pg/mL [IQR 5-70]). Hameed *et al.*[132] showed no significant change of BNP throughout pregnancy but median concentrations in pregnancy were double those of non-pregnant individuals (n = 29, median [IQR] 19 pg/mL [0-143] *vs* 10 pg/mL [10-37]). Resnik *et al.*[134] and Okuno *et al.*[136] reported on stable BNP levels throughout pregnancy at levels <20 pg/mL. More recently, Malhamé *et al.*[126] performed a retrospective review to assess the sensitivity and specificity of BNP in diagnosing HF in pregnancy and found that a cut-off of 111pg/mL had excellent sensitivity and excellent negative likelihood ratio for suspected HF.[126] The authors also report that BNP levels >467pg/mL in pregnant women with preeclampsia-related HF had a good specificity and good positive likelihood ratio for the identification of women with concurrent cardiac conditions.[126] All the remaining publications on BNP in pregnancy had small sample size (15-40 subjects) and focused mainly on BNP concentration in women with low-risk pregnancy and in those with PE/gestational HTN – the latter showing higher BNP levels.[122, 124, 127, 131, 133-135, 137]

In asymptomatic non-pregnant patients with simple CHD (unrepaired ASDs and VSDs), BNP and NT pro-BNP levels are slightly raised when compared to controls. These elevated levels may be related to shunt severity and pulmonary artery pressures (data from systematic review by Eindhoven *et al.*).[117] In complex CHD, BNP levels are also increased when compared to healthy controls, even in asymptomatic patients, and correlated with end-diastolic RV dimensions and pulmonary regurgitation in patients with ToF and with RV function in patients with a systemic RV (data from systematic review from Eindhoven *et al.*).[116]

An early study looking into BNP levels in pregnancy in women with cardiac disease[118] included 78 pregnant women, of whom 66 had cardiac disease (74% of these had CHD) and 12 were controls. They reported that in the healthy controls BNP was low and did not increase during pregnancy (peak median: 35pg/mL [IQR 21-43pg/mL], whereas in women with cardiac disease levels were higher (peak median: 79pg/mL [IQR 51-152pg/ml]) and highest in women with subaortic ventricular dysfunction (peak median: 137pg/mL, IQR 55-261pg/mL). No other differences in BNP levels were found in the remainder of the cardiac disease groups.[118] The same study reported cardiac adverse events in 13% of women with cardiac disease and these women had high BNP levels (peak median: 354pg/mL [IQR 299-805pg/mL]); fetal or neonatal adverse events were not associated with elevated BNP levels.[118]

Kampman *et al.*[120] studied a subsection of the ZAHARA II trial and reported adverse cardiovascular events in 10.3% of pregnancies. NT pro-BNP levels >128pg/mL at 20 weeks of gestation were predictive of events in later pregnancy with an OR 10.6 (95% CI 1.13-98.7, p = 0.039; NPV = 100%). This is the only study suggesting that BNP has a predictive value for cardiac adverse events.

Further research with longitudinal assessment of BNP levels throughout pregnancy in low-risk women, as well as in women with CHD, is therefore needed, specifically how BNP levels change throughout pregnancy and also how BNP levels in pregnant women with CHD compare to low-risk pregnant women.

6.2. Hypotheses and Aims

Hypothesis 15: *Pregnancy is associated with fluid overload which stimulates the release of BNP. However, the levels of BNP do not change significantly across the three trimesters of pregnancy.*

Aim: To obtain a measure of BNP concentration at each of the three trimesters of pregnancy (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation) and establish if there is a difference between the different trimesters of pregnancy in low-risk pregnancy and in pregnant women with CHD.

Hypothesis 16: *Pregnancy is associated with fluid overload which causes release of BNP and suboptimal increase in CO/SV and/or suboptimal decrease of TVR in CHD is associated with higher levels of BNP.*

Aim: To obtain a measure of BNP concentration in low-risk pregnancy and in pregnant women with CHD and establish if there is a difference between the two groups in each of the studied trimesters. To correlate the haemodynamic parameters with levels of maternal BNP, measured longitudinally, in low-risk women and in women with CHD.

6.3. Methodology

6.3.1. *Human BNP ELISA assay: ab193694 – BNP Human ELISA kit*

Initially, the ab193694 – BNP ELISA Kit (*Abcam plc*, UK) was used for the quantitative assessment of Human BNP in the plasma. The detection range for this assay is 14-1000 pg/mL and the sensitivity is 14pg/mL. The inter-assay coefficient of variability is less than 12% and the intra-assay coefficient of variability is less than 10%. Well effects have not been documented for this assay.

6.3.1.1. Reagent preparation

Reagents and standards were equilibrated at room temperature as per manufacturer's instructions. The 5X Diluent Assay B was diluted 5-fold, the wash buffer solution, the Detection Antibody BNP and the 1X HRP-Streptavidin (Glycerol) Solution were prepared.

6.3.1.2. Standard preparation

The vial containing the Human BNP Standard was centrifuged and 400µL of Assay Diluent C (5-Bromo-5-nitro-1,3-dioxane) added to the vial to obtain a 50ng/mL Stock Standard. The following standards were prepared using the following dilutions (Table 6.1):

Table 6.1 *– Standard dilution preparation for Human B-type natriuretic peptide (BNP) enzyme-linked immunosorbent assay (ELISA), ab193694 – BNP ELISA Kit (Abcam plc, UK).*

Standard Number	**Sample diluted**	**Volume diluted** (µL)	**Volume of diluent used** (µL)	**Starting concentration** (pg/mL)	**Final concentration** (pg/mL)
1	Stock Standard	20	980	50,000	1,000
2	Standard 1	300	300	1,000	500
3	Standard 2	300	300	500	250
4	Standard 3	300	300	250	125
5	Standard 4	300	300	125	62.5
6	Standard 5	300	300	62.5	31.25
7	Standard 6	300	300	31.25	15.63
8	None	-	300	0	0

6.3.1.3. ELISA Assay

Samples were removed from the freezer and thawed overnight at 4º Celsius. Samples were then brought to room temperature on the day of the assay and were not diluted. Low-risk participants with longitudinal CO data from the Physioflow® (CO data from PhysioFlow® at all three visits) and longitudinal samples (plasma samples from all three visits) were selected (n = 39), and an extra four participants with longitudinal samples but without longitudinal CO data were added: a total of 43 participants with longitudinal plasma samples.

The standards, positive control and samples were added to each plate in duplicate. Longitudinal samples were analysed on the same plate. An example of how standards, samples and controls were distributed on Plate 1 can be found below (Table 6.2).

For women with CHD, there were only two participants with complete longitudinal samples. The rest of the participants either had samples at one or two trimesters. In total, there were 5 samples for trimester A, 19 for trimester B and 21 for trimester C. The plate layout for all plates was planned ahead of the experiment.

Table 6.2 *– Plate layout showing distribution of standards, control and samples on B-type natriuretic peptide (BNP) plate number 1. All other plates followed a similar distribution. ST – Standard; +C – positive control; Pt – participants; a, b or c – trimester samples collected (a:10-14 weeks of gestation, b: 18-22 weeks of gestation, c: 30-34 weeks of gestation); A and B – samples and its duplicate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST 1A	ST 1B	Pt 1aA	Pt 1aB	Pt 1bA	Pt 1bB	Pt 1cA	Pt 1cB	Pt 9aA	Pt 9aB	Pt 9bA	Pt 9bB
B	ST 2A	ST 2B	Pt 2aA	Pt 2aB	Pt 2bA	Pt 2bB	Pt 2cA	Pt 2cB	Pt 9cA	Pt 9cB	Pt 10aA	Pt 10aB
C	ST 3A	ST 3A	Pt 3aA	Pt 3aB	Pt 3bA	Pt 3bB	Pt 3cA	Pt 3cB	Pt 10bA	Pt 10bB	Pt 10cA	Pt 10cB
D	ST 4A	ST 4B	Pt 4aA	Pt 4aB	Pt 4bA	Pt 4bB	Pt 4cA	Pt 4cB	Pt 11aA	Pt 11aB	Pt 11bA	Pt 11bB
E	ST 5A	ST 5B	Pt 5aA	Pt 5aB	Pt 5bA	Pt 5bB	Pt 5cA	Pt 5cB	Pt 11cA	Pt 11cB	Pt 12aA	Pt 12aB
F	ST 6A	ST 6B	Pt 6aA	Pt 6aB	Pt 6bA	Pt 6bB	Pt 6cA	Pt 6cB	Pt 12bA	Pt 12bB	Pt 12cA	Pt 12cB
G	ST 7A	ST 7B	Pt 7aA	Pt 7aB	Pt 7bA	Pt 7bB	Pt 7cA	Pt 7cB	Pt 13aA	Pt 13aB	Pt 13bA	Pt 13bB
H	BLANK A	BLANK B	Pt 8aA	Pt 8aB	Pt 8bA	Pt 8bB	Pt 8cA	Pt 8cB	Pt 13cA	Pt 13cB	+C A	+C B

One hundred microlitres of standard (x8), sample and a positive control were added to the wells of a 96-well plate. For this experiment, I used a total of 6 plates and left them to incubate overnight at 4º C with gentle shaking. The following morning, the solutions were discarded and the plate washed four times. Following this step, 100µL of Detection Antibody BNP solution was added to each well and incubated for one hour at room temperature with gentle shaking. This solution was then discarded and washed. One hundred microlitres of the HRP-Streptavidin solution was then added to each well followed by a 45-minute incubation at room temperature with gentle shaking. This solution was then discarded and washed. The TMB One-Step solution was then added to each well and allowed to incubate for 30 minutes, in the dark, at room temperature with gentle shaking. Finally, 50µL of the Stop Solution (Sulphuric acid) was added to each well to allow change of colour. Plates were immediately read on a 450nm plate reader.

Results were analysed using the ELISA analysis website (https://elisaanalysis.com/app, ElisaKit.com©, Pty Ltd, 2012, Melbourne, Australia).

6.3.2. *Repeat Human BNP ELISA assay with different dilutions*

Due to unexpectedly high levels of BNP in both the low-risk and CHD groups and after discussing with the manufacturer of the *ab193694 – BNP Human ELISA kit* who advised that the high readings could be secondary to a matrix effect, the above assay was repeated using the same methods described above, but testing for different dilutions in an attempt to identify the ideal dilution that would minimise the matrix effect and yield BNP levels within the expected range. For the dilutional pilot assay test, I used two samples from low-risk participants and one sample from a CHD participant. Samples were selected at random and were tested in duplicate. A total of six strips (each with eight wells) were used. Dilutions tested were: neat (undiluted sample), 1:2, 1:4, 1:5 and 1:10). The plate layout for dilutional testing was as per Table 6.3.

Table 6.3 *–Plate layout showing distribution of standards and samples for the pilot dilution test for BNP ELISA assay. ST – Standard; Pt – participants: LR – low-risk pregnancy and CHD – pregnant women with congenital heart disease; A and B – samples and its duplicate.*

	1	2	3	4	5	6
A	ST 1A	ST 1B	Pt 1 – LR NEAT	Pt 1 – LR NEAT	Pt 2 – LR 1:5	Pt 2 – LR 1:5
B	ST 2A	ST 2B	Pt 1 – LR 1:2	Pt 1 – LR 1:2	Pt 2 – LR 1:10	Pt 2 – LR 1:10
C	ST 3A	ST 3A	Pt 1 – LR 1:4	Pt 1 – LR 1:4	Pt 1 – CHD NEAT	Pt 1 – CHD NEAT
D	ST 4A	ST 4B	Pt 1 – LR 1:5	Pt 1 – LR 1:5	Pt 1 – CHD 1:2	Pt 1 – CHD 1:2
E	ST 5A	ST 5B	Pt 1 – LR 1:10	Pt 1 – LR 1:10	Pt 1 – CHD 1:4	Pt 1 – CHD 1:4
F	ST 6A	ST 6B	Pt 2 – LR NEAT	Pt 2 – LR NEAT	Pt 1 – CHD 1:5	Pt 1 – CHD 1:5
G	ST 7A	ST 7B	Pt 2 – LR 1:2	Pt 2 – LR 1:2	Pt 1 – CHD 1:10	Pt 1 – CHD 1:10
H	BLANK A	BLANK B	Pt 2 – LR 1:4	Pt 2 – LR 1:4	NOT USED	NOT USED

Results were analysed using the ELISA analysis website (https://elisaanalysis.com/app, ElisaKit.com©, Pty Ltd, 2012, Melbourne, Australia).

6.3.3. *ARCHITECT Ci8200 BNP*

Following the pilot assay with different dilutions, BNP levels obtained were still unexpectedly high in both the low-risk and the CHD groups. A second assay was later used to retest the same samples due to issues with the stability of the protein used for the creation of the standards in the first assay. The ARCHITECT Ci8200 BNP (*Abbott, USA*), a chemiluminescent microparticle immunoassay, was used and read on the ARCHITECT iSystem (*Abbott, USA*). The measurement range for this assay is 10-5000pg/mL.

Samples were thawed and brought to room temperature on the day of testing, briefly centrifuged and any cryoprecipitate from protein denaturation was removed with a standard pipette. The samples were then transferred to compatible tubes (200µL) and these were labelled with appropriate barcodes so that the concentration could be read on the ARCHITECT iSystem (*Abbott, USA*). The samples were read in batches (40 samples at a time). For a more extensive and detailed description of the methodology used, please refer to Chapter 2 – Materials and Methods.

6.3.4.*Measurements and Statistical Analysis*

The demographic and delivery data collected included age, race, weight, height and body mass BMI at booking, smoking status, type of CHD lesion (for the CHD group), parity, MOD, GA at delivery, sex of the newborn, BW, BW centile according to the Aberdeen Maternity and Neonatal Databank nomograms[186] and EBL.

Data were analysed using IBM SPSS Statistics, version 26.0, 2019 (IBM Corp., Armonk, New York, USA) and Microsoft® Excel for MAC, version 16.30, 2019 (Microsoft Corp., Redmond, WA, USA). The Kolmogoroff–Smirnoff, the Shapiro-Wilk tests and visual inspection of the histograms and Q-Q plots were used to assess normality of the distribution of the data. Mann-Whitney or chi-square tests, as appropriate, were performed to examine the differences in the demographic characteristics between

the examined populations. Non-parametric tests (Mann-Whitney, Spearman-rank, Wilcoxon-rank) were used to assess differences across trimesters and across populations. Samples were categorised in three different groups: 1 – BNP level < 20.0pg/mL; 2 – BNP level 20.0-39.9 pg/mL and 3 – BNP level ≥40 pg/mL. The lower limit of detection of this assay is 10pg/mL and therefore any values <10pg/mL were recorded as <10pg/mL. Because the majority of measurements were of values <10pg/mL, logarithmic transformation did not allow parametric analysis because the distribution was not Gaussian. Therefore non-parametric analysis of the results by categories was used instead. Where appropriate (in the case of correlation analysis using Spearman-rank), BNP levels <10pg/mL, were given a value of 5pg/mL (as Spearman-rank is independent of the actual BNP value) to allow statistical analysis. Cardiac function was indirectly measured by PhysioFlow® and calculated as the relative increase in CO and SV between the resting and exercise measurements (([T2-T1]/T2) * 100%), please see Chapter 2 – Materials and Methods).

6.3.5.*Sample size*

Sample size calculation was performed using the G*Power software (*G*Power for Mac Os X, v. 3.1, February 2020, Heinrich-Heine-Universität, Dusseldorf, Germany*).

Two studies assessed the change in BNP levels across the three trimesters of low-risk pregnancy.[132, 134] The authors of both studies did not report a statistically significant difference between the levels of BNP across trimesters. For the longitudinal assessment, to obtain and effect size of 0.48 between trimesters (similar to the two studies mentioned above) with an alpha = 0.05 and a power of 80%, we would need 30 samples for each of the groups. One study[118] compared BNP levels in pregnancy in women with heart disease (not specifically CHD) and in low-risk controls showing higher levels of BNP in the cardiac disease group. For differences between the two study groups, to obtain a similar result as this study, with an alpha = 0.05 and a power of 95%, we would need ten samples (five from each group).

6.4. Results

6.4.1. *Maternal characteristics and obstetric and fetal outcomes*

Table 6.4 describes the maternal characteristics and the obstetric and fetal outcomes of the participants included in the study of the levels of BNP in low-risk pregnancy and in pregnant women with CHD. Table 6.5 shows the cardiac lesions of the participants with CHD.

Table 6.4 *– Maternal characteristics and obstetric and fetal outcomes of participants included in the study of B-type natriuretic peptide (BNP) levels in low-risk pregnant women and in pregnant women with congenital heart disease'. LR – low-risk pregnant women; CHD – pregnant women with congenital heart disease; BMI – body mass index; P – parity; min – minimum; max – maximum. Significance level: p<0.05*

	LR N = 43	**CHD** N = 30	**p value**
Age, years, median (min – max)	33 (24-44)	31.5 (25-39)	0.07
Race, n (%)			0.94
White	37 (86.0)	26 (86.7)	
Non-white	6 (14.0) [1 Black, 4 Asian, 1 Middle-Eastern]	4 (13.3) [3 Asian, 1 Middle-Eastern]	
Weight, kg, median (min – max)	63.0 (43.0-91.3)	65 (49.0-101.0)	0.56
BMI, kg/m^2, median (min – max)	22.5 (18.0-34.0)	24.1 (17.6-34.9)	0.24
Smoking, n (%)			0.38
Never smoked	36 (83.7)	27 (90.0)	
Current/past smoker	7 (16.3)	3 (30.0)	
Parity, n (%)			0.13
P0	31 (72.1)	17 (56.7)	
P ≥1	12 (27.9)	13 (43.3)	
Mode of delivery, n (%)			0.30
Spontaneous Vaginal Delivery	17 (39.5)	10 (33.3)	
Ventouse Vaginal delivery	3 (7.0)	2 (6.7)	
Forceps Vaginal Delivery	5 (11.6)	3 (10.0)	
Emergency Caesarean Section	13 (30.3)	7 (23.3)	
Elective Caesarean Section	5 (11.6)	8 (26.7)	
Mode of delivery, n (%)			0.49
Vaginal (all, including instrumental)	25 (58.1)	15 (50.0)	
Caesarean Section (all)	18 (41.9)	15 (50.0)	
Gestational Age at delivery, weeks+days median (min - max)	39+5 (25+6-42+0)	38+6 (34+3-41+0)	0.002**
Sex of the baby, n (%)			0.33
Female	18 (41.9)	16 (53.3)	
Male	25 (58.1)	14 (46.7)	
Birthweight, g, median (min – max)	3320 (2440-5220)	2940 (1970-3855)	0.003**
Birthweight centile, %, median (min – max)	55.0 (3.0-100.0)	30.0 (4.0-92.0)	0.005**
Estimated Blood Loss, mL, median (min – max)	350 (100-2005)	500 (100-1800)	0.020*

*p<0.05, **p<0.01, ***p<0.001.

Table 6.5 *– Cardiac lesions of pregnant women with congenital heart disease (CHD). AS – aortic stenosis; ASD – atrial septal defect; BAV – bicuspid aortic valve; AVSD – atrioventricular septal defect; CoA – coarctation of the aorta; PDA – patent ductus arteriosus PVR – pulmonary valve regurgitation; r – repaired; SA – subaortic stenosis; ToF – Tetralogy of Fallot; TCPC – total cavopulmonary connection; TGA – transposition of great arteries; TVR – tricuspid valve regurgitation; VSD – ventricular septal defect. N=30.*

CHD lesion	N	Use of medication
rTOF	**9**	One participant on LDA, two participants on beta-blockers (Bisoprolol 2.5mg once daily)
rTOF only	*8*	
rTOF + rSA stenosis	*1*	
rCoA	**5**	One participant on Bisoprolol 5mg once daily
rCoA only	*1*	
rCoA + rVSD + BAV	*1*	
rCoA + BAV	*3*	
rTGA	**3**	One participant on beta-blockers, one participant on LMWH
rTGA (arterial switch)	*2*	
rTGA (atrial switch)	*1*	
VSD	**3**	
Unrepaired VSD	2	
rVSD + rPDA + BAV	1	
rAVSD	**1**	
Fontan TCPC	**1**	On LMWH
AS + BAV	**1**	
Pulmonary Atresia + PVR + TVR	**1**	
Ebstein's Anomaly	**1**	
Right hemi-truncus	**1**	
Mixed Lesions	**4**	One participant on Sotalol 80mg twice daily
ASD + rVSD + BAV	*1*	
rVSD + rPDA + BAV	*1*	
BAV	*2*	

6.4.2. *BNP concentrations*

The results for the Human BNP assay (BNP ELISA assay: ab193694 – BNP Human ELISA kit) are not presented here as these were deemed to be incorrect. BNP concentrations of 400 to >1000pg/mL were obtained, with most values lying outside the standard curve shown below (Figure 6.1).

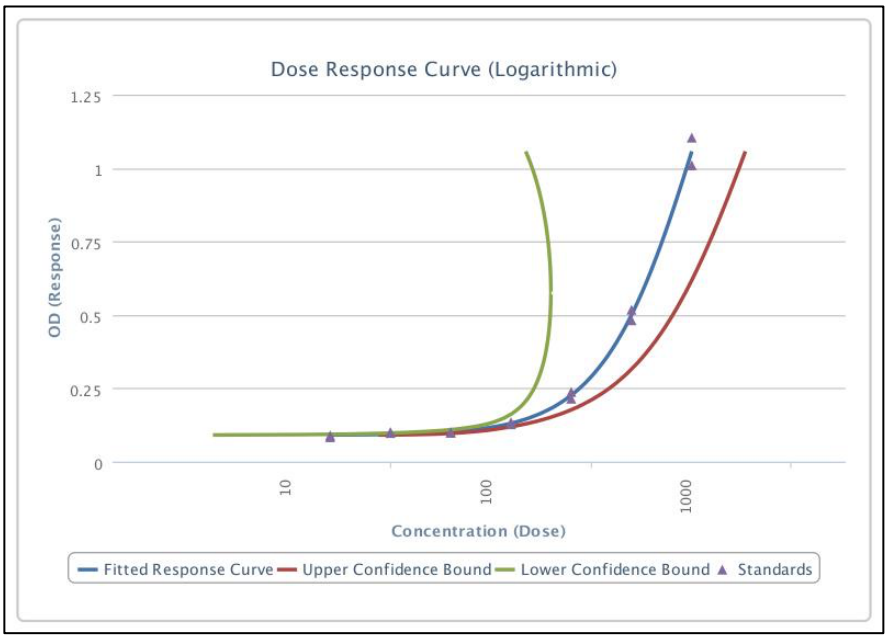


Figure 6.1 *– Standard curve for the Human B-type natriuretic peptide (BNP) enzyme-linked immunosorbent assay (ELISA), ab193694 – BNP ELISA Kit (Abcam plc, UK).*

Before repeating the assay, different dilutions were tested to establish which to use in the repeat assay (Table 6.6).

Table 6.6 *– Concentration of B-type natriuretic peptide (BNP) for three study participants (LR – low-risk pregnancy participant; CHD – pregnant participant with congenital heart disease) using different dilutions.*

	LR 1 Mean concentration, pg/mL (Confidence Interval)	**LR 2** Mean concentration, pg/mL (Confidence Interval)	**CHD 1** Mean concentration, pg/mL (Confidence Interval)
NEAT (Not Diluted)	**450.5** (193.2-707.9)	**303.0** (170.2-435.9)	**507.1** (196.7-817.5)
1:2 (50µL sample + 50µL diluent)	**321.0** (174.2-467.7)	**160.0** (118.8-201.3)	**201.3** (138.0-264.7)
1:4 (25µL sample + 75µL diluent)	**180.2** (128.8-231.6)	**79.5** (60.7-98.4)	**129.8** (101.0-158.6)
1:5 (20µL sample + 80µL diluent)	**98.4** (77.5-119.3)	**71.9** (53.5-90.4)	**105.0** (83.3-127.6)
1:10 (10µL sample + 90µL diluent)	**16.7** (3.6-26.0)	**46.0** (28.7-63.2)	**16.7** (3.6-26.0)

Absorbances for the blank wells were similar to the absorbances of samples diluted 1:10. BNP concentrations for the different dilutions were higher than expected and therefore, the test was repeated using a different methodology after discussions with the manufacturer of the assay. The high levels were attributed to a potentially unstable protein present in the standard or to a matrix effect.

6.4.2.1. Longitudinal assessment of BNP expression in low-risk pregnancy

Longitudinal BNP expression (pg/mL) in the low-risk group (43 participants with longitudinal samples; number of samples 3 * 43 = 129), measured by the *ARCHITECT Ci8200 BNP* system are presented in Figure 6.2. Table 6.7 summarises the differences in BNP levels between trimesters in the low-risk group.

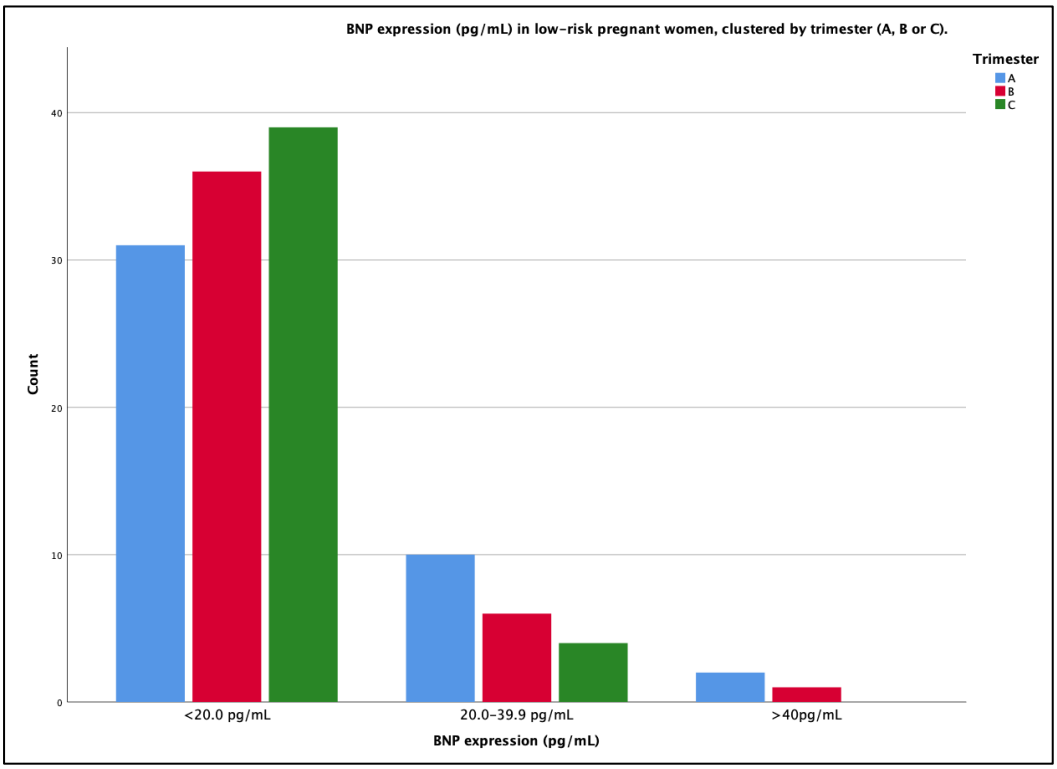


Figure 6.2 *– Representation of longitudinal B-type natriuretic peptide (BNP) concentration (pg/mL) in low-risk pregnant women across the three trimesters (A – 10-14 weeks of gestation (blue bars), B – 18-22 weeks of gestation (red bars), C – 30-34 weeks of gestation (green bars)). The Y-axis represent the number of low-risk participants.*

Table 6.7 *– Summary of Wilcoxon signed-rank test for differences in B-type natriuretic peptide (BNP) between trimesters for longitudinal samples in the low-risk (LR) group. Significance level: p<0.05. A, B and C – Differences between 1st, 2nd and 3rd trimesters.*

Differences of BNP levels (pg/mL) between trimesters in the LR group	
Trimesters	**p value**
Comparison A/B	0.11
Comparison B/C	0.018*
Comparison A/C	<0.001***

*p<0.05, **p<0.01, ***p<0.001. p values have been adjusted for multiple comparisons using Bonferroni correction.

6.4.2.2. Longitudinal assessment of BNP expression in pregnant women with CHD

BNP expression (pg/mL) in the CHD group, measured by the *ARCHITECT Ci8200 BNP* system, are presented in Figure 6.3.

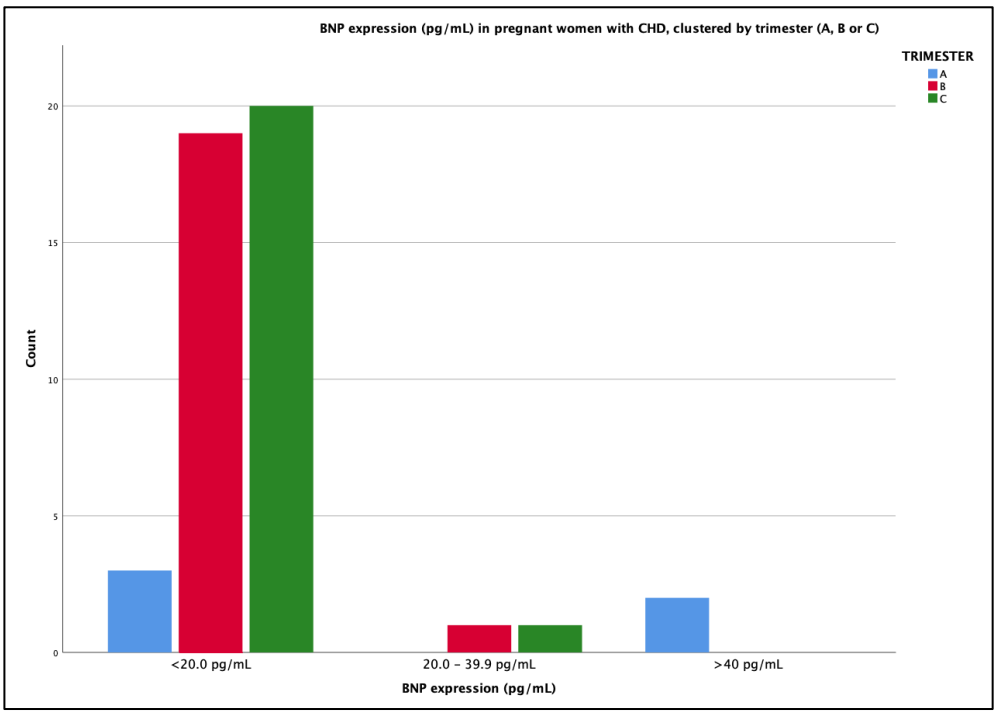


Figure 6.3 *– Longitudinal BNP concentration (pg/mL) in pregnant women with congenital heart disease (CHD) across the three trimesters: (A – 10-14 weeks of gestation (blue bars), B – 18-22 weeks of gestation (red bars), C – 30-34 weeks of gestation (green bars)).*

Only three patients in the CHD cohort had true longitudinal samples (one sample at each of the trimesters) and therefore, we decided to combine the data of all samples available at each of the trimesters (five samples for the first trimester, 20 samples for the second trimester and 21 samples for the third trimester) to be able to describe trends throughout pregnancy. The BNP profile for the three patients with longitudinal samples is presented in Table 6.8. Table 6.9 describes the BPN levels and cardiac lesions of participants in the CHD group with BNP concentrations >20.0 pg/mL. and, finally, Table 6.10 summarises the differences in BNP levels between trimesters in the CHD group.

Table 6.8 *– B-type natriuretic peptide (BNP) concentration (pg/mL) in the three pregnant patients with congenital heart disease (CHD) with longitudinal samples. r – repaired; TCPC – Total Cavopulmonary Connection; ToF – Tetralogy of Fallot; TGA – Transposition of Great Arteries; T – trimester.*

Participant ID	Condition	BNP 1st T (pg/mL)	BNP 2nd T (pg/mL)	BNP 3rd T (pg/mL)
CHD002	Fontan TCPC	14.8	<10	<10
CHD005	rToF	<10	<10	<10
CHD017	TGA (atrial switch)	59.3	<10	<10

Table 6.9 *– B-type natriuretic peptide (BNP) concentration (pg/mL) and cardiac lesion of pregnant participants with congenital heart disease (CHD) with BNP > 20.0pg/ml. ASD – atrial septal defect; BAV – bicuspid aortic valve; r – repaired; TGA – Transposition of Great Arteries; VSD – ventricular septal defect.*

Participant ID	Condition	BNP (pg/mL) – Trimester
CHD017	TGA (atrial switch)	59.3 – 1st T
CHD021	ASD + rVSD + BAV	43.5 – 1st T
CHD010	VSD	39.3 – 2nd T
CHD001	Ebstein's Anomaly	22.7 – 3rd T

Table 6.10 *– Summary of Wilcoxon signed-rank test for differences in B-type natriuretic peptide (BNP) between trimesters for longitudinal samples in the congenital heart disease (CHD) group. Significance level: p<0.05. A, B and C – Differences between 1st, 2nd and 3rd trimesters.*

Differences of BNP levels (pg/mL) between trimesters in the CHD group	
Trimesters	**p value**
Comparison A/B	0.18
Comparison B/C	0.32
Comparison A/C	0.18

p values have been adjusted for multiple comparisons using Bonferroni correction.

6.4.2.3. The differences in BNP concentration (pg/mL) at each trimester (A, B and C) between the two studied groups.

Figures 6.4, 6.5 and 6.6 illustrate the BNP concentration (pg/mL) at each trimester (A, B and C, respectively) in the two studied groups. Table 6.11 summarises the differences in BNP concentration at each trimester between the two studied groups.

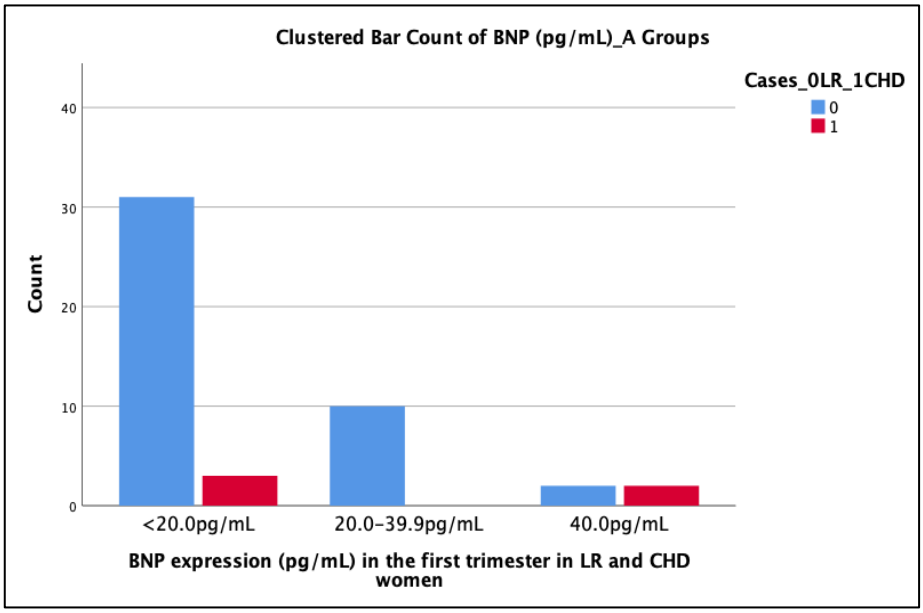


Figure 6.4 *– B-type natriuretic peptide (BNP) concentration (pg/mL) in the first trimester (A – 10-14 weeks of gestation) in low-risk pregnancy (LR, blue bars) and in pregnant women with congenital heart disease (CHD, red bars).*

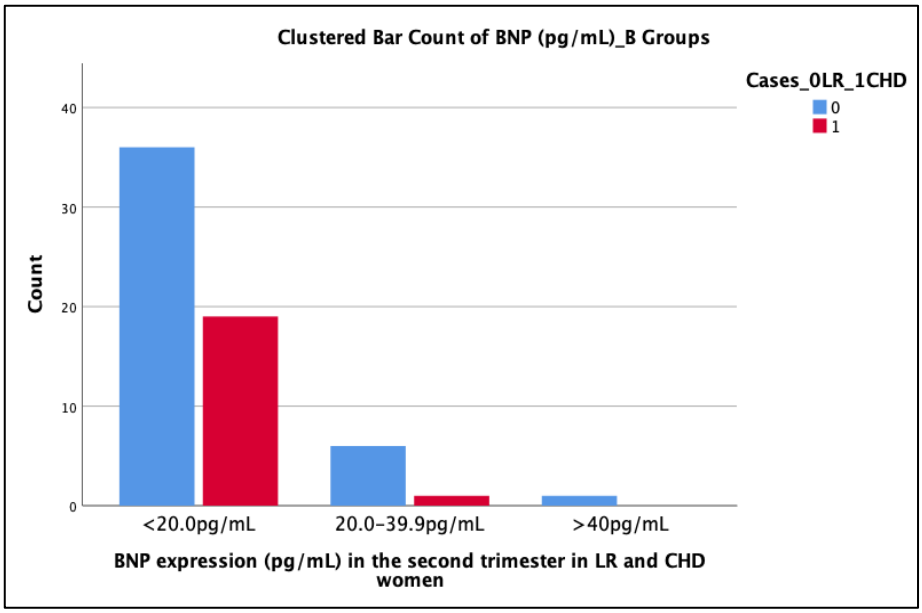


Figure 6.5 *– B-type natriuretic peptide (BNP) concentration (pg/mL) in the second trimester (B – 18-22 weeks of gestation) in low-risk pregnancy (LR, blue bars) and in pregnant women with congenital heart disease (CHD, red bars).*

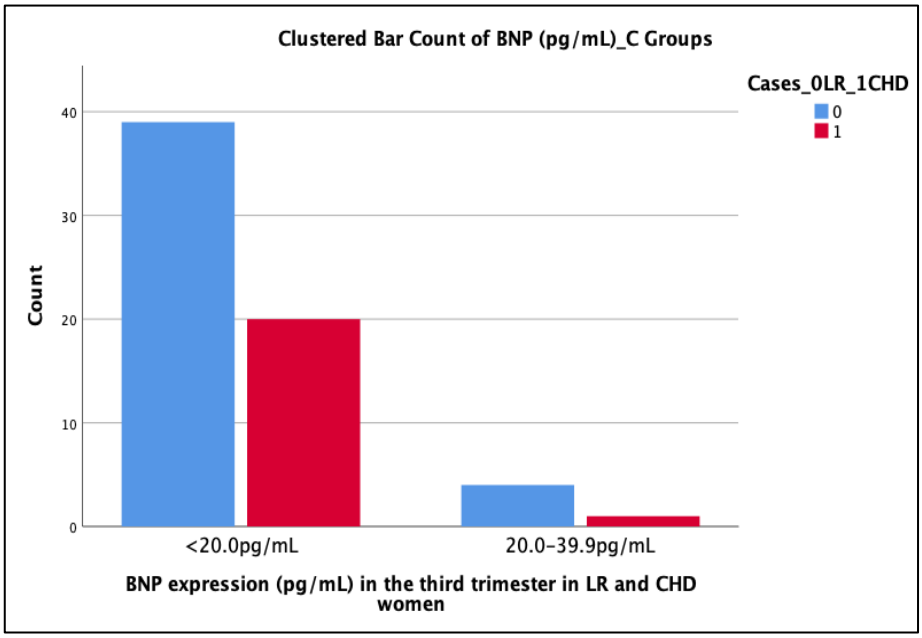


Figure 6.6 *– B-type natriuretic peptide (BNP) concentration (pg/mL) in the third trimester (C – 30-34 weeks of gestation) in low-risk pregnancy (LR, blue bars) and in pregnant women with congenital heart disease (CHD, red bars).*

Table 6.11 *– Summary of the Mann-Whitney U test results for differences in B-type natriuretic peptide (BNP) concentration (pg/mL) between the two study groups and across the three trimesters. LR – low-risk pregnant women; CHD – pregnant women with congenital heart disease. Significance level: p<0.05.*

Differences between groups LR *vs* CHD	
Trimester	**p value**
Differences **1st Trimester**	0.53
Differences **2ndTrimester**	0.09
Differences **3rd Trimester**	0.27

p values have been adjusted for multiple comparisons using Bonferroni correction.

6.4.2.4. Association between BNP concentration and percentage increase in CO and SV from rest to exercise.

The correlation between BNP and the percentage increase in CO and SV from rest to peak exercise, at each of the trimesters, is presented in Table 6.12.

Table 6.12 *– Correlation coefficient (Spearman rho, r_s) and p values for correlation between B-type natriuretic peptide (BNP) concentration (pg/mL) and percentage increase in cardiac output (CO, %) and stroke volume (SV, %) at each of the trimesters: (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation). Percentage increase of both measured as the percentage increase from rest to peak exercise ([peak exercise-rest]/peak exercise). Significance level: $p<0.05$.*

		ΔCO	**ΔSV**
BNP A (1st trimester)	r_s	0.173	0.100
	p	0.39	0.62
BNP B (2nd trimester)	r_s	0.50	0.59
	p	0.75	0.70
BNP C (3rd trimester)	r_s	-0.038	-0.009
	p	0.80	0.95

Figures 6.7 – 6.12 illustrate the relationship between the groups of BNP in each trimester with the relative increase of CO and SV measured by PhysioFlow®.

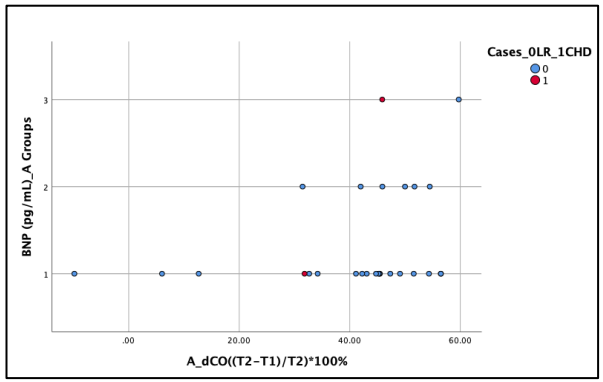


Figure 6.7 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the first trimester (A), in relation to percentage increase in cardiac output (dCO, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

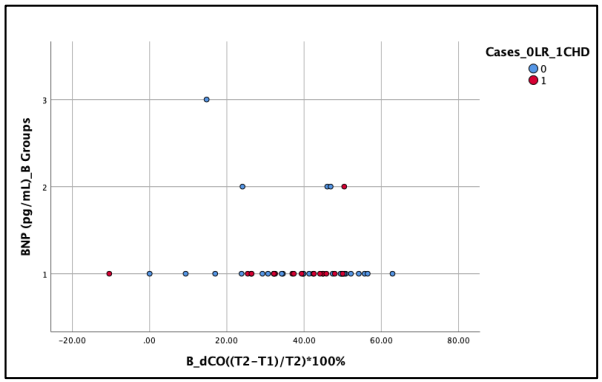


Figure 6.8 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the second trimester (B), in relation to percentage increase in cardiac output (dCO, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

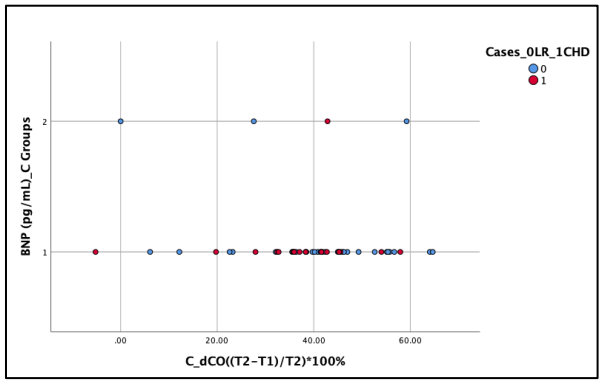


Figure 6.9 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the third trimester (C), in relation to percentage increase in cardiac output (dCO, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

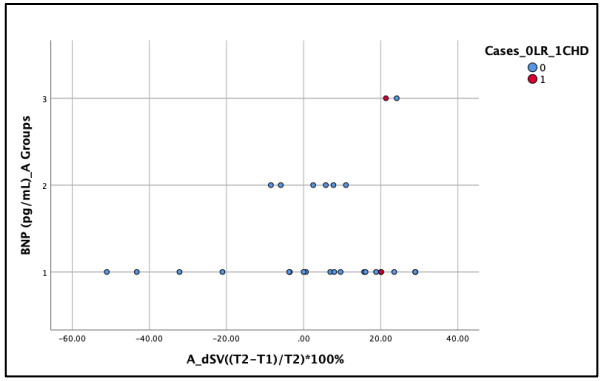


Figure 6.10 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the first trimester (A), in relation to percentage increase in stroke volume (dSV, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

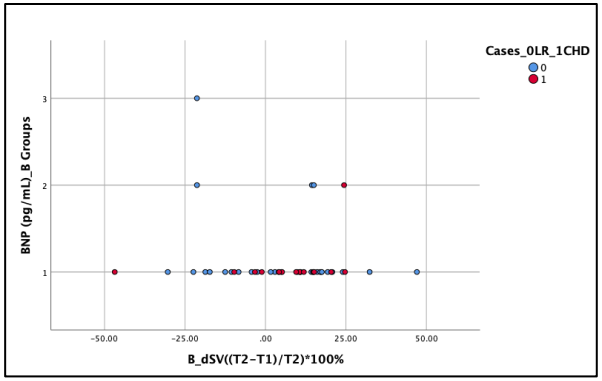


Figure 6.11 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the second trimester (A), in relation to percentage increase in stroke volume (dSV, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

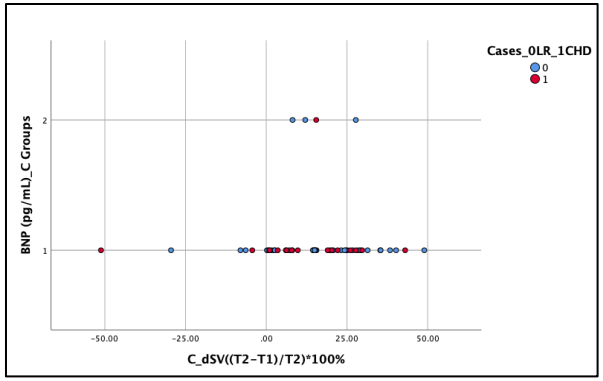


Figure 6.12 *– Type-B natriuretic peptide (BNP) concentration (pg/mL) by study group (blue dots – low-risk pregnancy, LR; red dots – pregnant women with congenital heart disease, CHD) in the third trimester (A), in relation to percentage increase in stroke volume (dSV, %) with exercise. BNP groups: (1 – BNP expression <20.0pg/mL; 2 – 20.0-39.9 pg/mL; 3 - >40.0 pg/mL).*

6.5. Discussion

6.5.1. *Demographics and obstetric and fetal outcomes*

There were no statistically significant differences between the two studied groups with regard to age, race, weight, smoking status, parity or MOD. However, pregnant women with CHD were, on average, heavier by 2kg and had higher BMI of 1.6kg/m^2 ($p > 0.05$ in both cases). There was a slightly higher proportion of parous women in the CHD group (43.3% *vs* 27.9%) and despite this, there was a higher proportion of women delivering via Caesarean section in the CHD group, a well-known fact in women with CHD.[213, 214]

The GA at delivery was statistically different between the two groups ($p = 0.002$), with women in the CHD group delivering, on average, six days earlier than the low-risk group. Pregnant women with CHD are known to deliver earlier when compared to a low-risk population and also to have a higher incidence of Caesarean section delivery, as discussed above.[214, 215] Babies of mothers with CHD were 380g lighter than the babies of low-risk mothers ($p = 0.003$) and, more importantly, the BW centiles were also statistically different. Women with CHD are known to deliver smaller babies compared to the general low-risk population[55, 57] and it is believed that the causes are multifactorial but related to an underlying poor cardiac function, and potentially associated with placental dysfunction and use of beta-blockers.[57] Once again, we did not have the power in this particular cohort to determine if beta-blockers had an effect on BW, as only five women were on beta-blockers (16.7%). There was also a statistically significant difference between the groups regarding blood loss at delivery ($p = 0.020$), with women with CHD losing an additional 150mL at birth when compared with the low-risk group. Women with CHD are known to have a higher blood loss at delivery, and although the causes for this might be similar to the causes in the general population, a more cautious use of uterotonics, due to its cardiovascular effects, might explain a higher rate of PPH.[216]

6.5.2. *Longitudinal assessment of BNP levels in low-risk pregnancy*

BNP levels declined with advancing gestation, with a higher proportion of women having BNP levels <20pg/mL in the third trimester and no women having levels >40pg/mL in the third trimester. There was no statistically significant difference in BNP levels between the first and the second trimester but there was a statistically significant difference between the second and third ($p = 0.018$) and between the first and third trimesters ($p < 0.001$).

There are only three studies[129, 132, 134] assessing the longitudinal profile of BNP in pregnancy and only two[132, 134] include data from the first trimester. Hameed *et al.*[132] included 29 low-risk pregnant women and report a non-statistically significant drop from the first to the second trimester in median BNP levels (20pg/mL to 18pg/mL, respectively), followed by a non-statistically significant increase in the third trimester (to 26pg/mL). However, the 'trimesters' in this study (1st trimester – 0-14 weeks of gestation, 2nd trimester – 14-18 weeks of gestation and 3rd trimester 28-42 weeks of gestation) were different from the ones used in our study.[132] This could explain why the authors observed higher levels in the '3rd trimester' as theirs was extended to 42 weeks of gestation. Resnik *et al.*[134] included 118 women (163 samples) with low-risk pregnancy from the first trimester to term – the authors did not specify the exact definitions of each of the trimesters or term and the median GA at recruitment was 14.5 weeks. Median BNP expression decreased with advancing GA (18.4pg/mL in the first trimester, 17.9pg/mL in the second trimester, 15.5pg/mL in the third trimester and 17.8pg/mL at term) but the differences were not statistically significant.[134] They included 74 samples in the first trimester, 49 in the second, 15 in the third and 25 at term but they did not specify if these samples were truly longitudinal (as opposed to cross-sectional or a mixture of the two) and at best, only 15 samples could be truly longitudinal. Burlingame *et al.*[129] enrolled over 100 women to assess the BNP profile during pregnancy and postpartum period but excluded data from the first trimester due to small numbers in this trimester (n=12). The authors also report a statistically non-significant drop from the second (18-24 weeks of gestation, median BNP 26.3pg/mL) to the third trimester (30-36 weeks of gestation, median BNP 24.7pg/mL).[129] Our findings are in line with the above studies, showing a decrease in BNP levels with

advancing GA, but our results indicate a statistically significant decrease from the second to the third trimester and an overall statistically significant decrease from the first to the third trimester. Our study is, to my knowledge, the largest longitudinal study of BNP levels in low-risk pregnancy.

Pregnancy is associated with evolving haemodynamic changes, with an increase in CO as soon as the first trimester when myocyte stretch in the left ventricle leads to release of BNP.[24, 115] Reported BNP levels in the first trimester of pregnancy were approximately double of those of non-pregnant subjects, however only two studies comparing this were found.[132, 137] Blood volume in the second trimester remains unchanged but the placental blood flow, which is not fully established until 12-14 weeks of gestation, continues to increase until late in the third trimester (12% of the total CO at term).[227, 245] Compression of the inferior vena cava by the growing uterus and fetus and increased placental blood flow in the third trimester can decrease venous return (preload) and, consequently SV, which will reduce the strain on the LV[24] and may explain the reduction in BNP levels with advancing gestation. As discussed previously, HR is the main driver to maintain CO in the second half of pregnancy, hence maintaining CO at adequate levels despite a drop in SV.[29] Even with the increase in blood volume, characteristic of pregnancy, BNP expression is increased when compared to non-pregnant individuals but seems to remains stable and at around 20pg/mL in pregnancy.[122, 125, 129, 132, 134]

6.5.3. *Longitudinal BNP expression in pregnant women with CHD*

We observed a similar trend in BNP expression in pregnant women with CHD, with an increasing number of women having BNP levels < 20pg/mL with increasing gestation and no women with levels >40pg/mL in the third trimester. However, we had a limited number of truly longitudinal samples (three) and could not perform longitudinal analysis of BNP expression. Participant CHD017, who had TGA (atrial switch) had high levels in the first trimester and was known to have severe RV dysfunction – however, her levels were <10pg/mL in the subsequent trimesters, which might suggest that there was less strain on the ventricle as pregnancy progressed. Tanous *et al.*[118] who included 66 pregnant women with heart disease (49 had CHD) and performed BNP measurements in the first and third trimester,

reported on variable levels of BNP ranging from 15 – 1,425pg/mL, highest in women with subaortic ventricular dysfunction and higher than the low-risk pregnant controls at any point in pregnancy. The authors did not report on any statistically significant differences between specific cardiac diagnostic subgroups.[118] Tanous *et al.*[118] also reports on 10% of women in the heart disease group having BNP levels of >100pg/mL in the first trimester. They found that this had increased to 26% in the third trimester, at the time of '*peak haemodynamic stress'*. We did not observe an increase in BNP expression with gestation and, although our samples were not truly longitudinal, we had similar numbers of samples in the second and third trimesters.

6.5.4. *Differences in BNP expression between low-risk pregnant women and in pregnant women with CHD*

We found no statistically significant difference in BNP expression between the two studied groups at any of the trimesters. However, in the first trimester we only had five samples for CHD participants and for the second and third trimesters, 20 and 21 samples, which correspond to about half the number of samples processed for the LR cohort. A higher proportion of women in the CHD group had BNP levels >40pg/mL in the first trimester: two (2/5 = 40%) CHD participants *vs* two (2/43 = 4.7%) in the low-risk group. The remainder three participants in the CHD group had BNP levels <20pg/mL.

In the second trimester, no women in the CHD group had BNP levels >40pg/mL, one (1/20 = 5%) had levels between 20.0-39.9pg/mL *vs* five (5/43 = 11.6%) in the low-risk group. In the third trimester, one participant with CHD (1/21 = 4.8%) had BNP levels between 20.0-39.9pg/mL *vs* four (4/43 = 9.3%) in the low-risk group, suggesting that overall the low-risk group had a higher proportion of women with higher BNP concentrations. Women with CHD have a suboptimal haemodynamic adaptation to pregnancy, with reduction in systolic function and progression of diastolic dysfunction with pregnancy[33] and we would, therefore, expect higher BNP concentrations. Our sample size was, however, small, which could explain our findings.

Outside pregnancy, BNP levels tend to be higher in simple and complex corrected CHD compared to healthy controls, even in asymptomatic patients, but most studies are of cross-sectional design and a wide range of BNP levels are reported, which makes it impossible to draw firm conclusions.[116, 117]

Malhamé *et al.*[126] recently proposed a BNP cut-off of 111pg/mL to diagnose HF in pregnancy and in their study, the median BNP levels for patients with heart failure were 326pg/mL compared to 75.5pg/mL for women without heart failure. All of our participants had levels well below the proposed threshold and none had heart failure.

6.5.5. *Correlation of CO and SV with BNP expression*

We found no correlation between BNP concentrations and percentage increase of CO or SV with exercise. In the first trimester, women with higher relative increase in CO/SV had BNP levels in groups 2 and 3 (BNP 20.0-39.9pg/mL; BNP>40pg/mL, respectively) but we could not identify a similar pattern in the second or third trimesters. As discussed above, the initial increase in circulating volume in the first trimester leads to ventricular dilation which stimulates BNP release and after 14 weeks, with the establishment of the placental circulation, the load to the ventricles is reduced which may explain lower levels of BNP.

6.6. Conclusion

BNP concentration decreases with advancing GA in singleton low-risk pregnancy, with no women having levels >40.0pg/mL in the third trimester. This is the largest study assessing BNP profiling in the first, second and third trimester, in singleton low-risk pregnancy to date. Although pregnant women with CHD showed proportionally higher BNP levels in the first trimester, this was not statistically significant. We found no correlation between circulating levels of BNP and cardiac function, measured as a percentage increase in CO with exercise, measured by ICG.

Chapter 7.

Maternal cytokine and cellular adhesion molecules expression

in low-risk pregnancy and in pregnant women with congenital heart disease

Chapter 7. Maternal cytokine and cellular adhesion molecules expression in low-risk pregnancy and in pregnant women with congenital heart disease

7.1. Introduction

Pregnancy is a unique immunological state where the maternal immune system has to tolerate an allogenic fetus but, at the same time, be able to protect herself and the fetus from infection.[142] Overall, there is a skew towards a more prominent Th2 response (IL-10) and a decrease in the Th1 response (IL-2, IFNγ), although there are variations across the different trimesters of pregnancy. Also, the concept of Th1/Th2 balance has matured with the recognition of other cell types which contribute to the overall immune balance.[142]

Various cytokines are involved at different stages of pregnancy, from placentation to labour.[142, 143] The first trimester of pregnancy is associated with higher levels of proinflammatory cytokines (IL-1, IL-6, TNFα) compared to non-pregnant individuals.[246] Blastocyst implantation is mainly a proinflammatory state.[142] IL-1 is present in the cytotrophoblast, the syncytiotrophoblast and decidua and its presence has been associated with adequate implantation, normal placentation and therefore adequate offspring size.[144] Levels of plasma growth factors, such as GROα, are significantly higher in pregnancy (compared to non-pregnant individuals) and its presence believed to be essential for adequate development of the fetomaternal unit.[145] High serum levels of IL-6 have been associated with reduced fertility and recurrent miscarriages, although the mechanisms for this are unclear, and high levels of IL-11 have been associated with implantation failure and defective decidualisation in animal studies.[144] During the second trimester of pregnancy there is mainly an anti-inflammatory state and a more pronounced skew towards Th2 cytokine production.[142] In the third trimester of pregnancy there is an increase in proinflammatory cytokines such as IL-1β, IL-6 and IL-12 in preparation for labour, but the mechanisms responsible for this are unclear.[145]

Despite corrective surgery, most patients with CHD will still have residual haemodynamic abnormalities.[147] Patients with CHD show similar patterns of neurohormonal activation as seen in

patients with heart failure, although data on local and circulating cytokine levels are limited.[149] Research suggests that hypoxia can stimulate immune activation[149] and this can be responsible for some late complications of CHD. Patients with CoA, in particular, are known to have higher circulating levels of proinflammatory cytokines and adhesion molecules, suggesting a state of endothelial activation.[150] Regions of disturbed oscillatory flow, as may be induced by CoA, can induce upregulation of pro-inflammatory adhesion molecules.[151] This may be mediated through an endothelial cell response to fluid flow and fluid sheer stress (a frictional force that occurs in the cell surface).[152]

Several authors[147-149, 153] have reported significantly higher levels of inflammatory cytokines (IL-6 and TNFα) in patients with CHD (and even higher levels in those with cyanotic CHD), the levels correlating with disease severity and the degree of remodelling. GROα and IL-17E correlate with patient age and pulmonary hypertension severity.[147] Ghrelin was associated with worse heart failure and also affected compensatory myocardial hypertrophy via vasodilatory activity and sympathetic nerve stimulation.[148]

Systemic intravascular inflammation is normal in pregnancy and endothelial activation plays an important role in this physiological inflammation.[247] Changes in proinflammatory cytokines can cause endothelial dysfunction by upregulation of adhesion molecules.[247] ICAM and VCAM allow binding of leucocytes to the vascular endothelium, promoting their migration to infected/inflamed tissues.[248] ICAM/VCAM mediated leucocyte-endothelial adhesion has been and can be used to monitor endothelial function in PE.[249, 250] Increased levels of adhesion molecules are associated with infection, inflammation and cancer, and adhesion molecules are highly expressed in the decidual endothelial cells.[249] Women with PE show higher expression of VCAM, a marker of endothelial dysfunction.[247] ICAM is basally expressed and upregulated by cytokine expression, whereas VCAM is induced by cytokine activation,[249] with IL-1β driving ICAM and VCAM expression in studies of coronary artery endothelial cells.[160] In patients with repaired CoA, the levels of proinflammatory cytokines are reported to be raised, consistent with an early inflammatory activation process, although levels of ICAM and VCAM were not different between the study population and controls.[159]

The effect of beta-blockers, commonly used in patients with CHD, on the circulating levels of cytokines, has been investigated by several authors. Prabhu *et al.*[251] showed a significant reduction in circulating levels of TNFα, IL-6 and IL-1 after therapy with metoprolol in the rat, and associated improved LV remodelling and systolic function. Ohtsuka *et al.*[166] compared the levels of TNFα and IL-10 before and 12 weeks after starting beta-blocker therapy in 32 patients with dilated cardiomyopathy and ten healthy controls. The authors demonstrated a significant reduction in the levels of TNFα and a reduction in the levels of IL-10, which is responsible for the enhanced release of TNFα. Mayer *et al.*[252] postulate that decreased levels of circulating IL-6 in patients with heart failure treated with beta-blockers may be associated with changes in LV function and geometry. These authors did not show a significant reduction in the circulating levels of TNFα. Norozi *et al.*[253] failed to show a reduction in the levels of IL-6, endothelin-1 or soluble TNFα receptors in 345 patients with CHD and heart failure after six months of treatment with beta-blockers. The effects of beta-blockers on the circulating levels of cytokines are conflicting, owing to the high intra and interindividual variability of these markers.

To my knowledge, there are no studies investigating cytokine, ICAM and VCAM expression in patients with CHD during pregnancy.

7.2. Hypotheses and Aims

Hypothesis 17: *There is a change in the women's immune profile throughout pregnancy.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, at the three trimesters of pregnancy (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation).

Hypothesis 18: *Higher circulating levels of proinflammatory cytokines and cellular adhesion molecules may cause inadequate spiral artery remodelling, placental ischaemia and/or insufficiency and, therefore, causes FGR and preterm birth.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, and correlate circulating levels with BW, BW centiles and gestational age.

Hypothesis 19: *In pregnancy complicated by CHD, cardiac function is related to the circulating levels of cytokines and cellular adhesion molecules.*

Aim: To measure the circulating levels of cytokines such as IL-1β, IL-6, IL-10, IL-12, TNFα, IFNγ, ghrelin and GROα, and cellular adhesion molecules such as ICAM and VCAM, in low-risk pregnancy and in pregnancy complicated by CHD, and correlate circulating levels with CO at rest and with ΔCO.

7.3. Methodology

7.3.1. *Bio-Plex® Multiplex Immunoassay*

For this assay, custom made plates were used to test the expression of IL-1β, IL-6, IL-10, IL-12, TNFα, ghrelin, GROα, IFNγ, ICAM and VCAM. The Bio-Plex® MAGPIX system was used to read the plates. A total of six plates were used: three for IL-1β, IL-6, IL-10, IL-12, TNFα, ghrelin, GROα, IFNγ (express Assay) and three for ICAM/VCAM – these had to be run separately due to dilution incompatibility.

7.3.1.1. Preparation

Previously processed plasma samples, stored at -80ºC (please refer to Chapter 2), were thawed overnight at 4º Celsius and brought to room temperature on the day of the assay. Following calibration of the Bio-Plex® system, buffers were prepared according to the manufacturer's instructions and standards were reconstituted in 500µL of diluent and diluted 4-fold.

7.3.1.2. Layout

All samples were run in duplicate. On a 96 well plate, 18 wells were used for standards and blanks (9 * 2). Due to budget constraints, I decided to analyse all samples from pregnant participants with CHD (45 samples in 30 participants with CHD) alongside matched samples from low-risk pregnant women (45 samples in 34 low-risk participants). After inclusion of the standard, blank wells and the study samples, 54 wells were left unoccupied and these were used to analyse the longitudinal samples of participants with low-risk pregnancy. The total number of true longitudinal samples (samples at all three trimesters per participant) was two for the CHD group and 12 for the low-risk group. An example of a plate layout is presented below in Table 7.1.

Table 7.1 *– Example of the layout of a 96-well plate used for the Bio-Plex® Multiplex Immunoassay. A, B and C – trimester of sample (A – 10-14 weeks of gestation, B 18-22 weeks of gestation, C – 30-34 weeks of gestation); CHD – congenital heart disease group; LR – low-risk group; ST – Standard. All samples in duplicate.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST 1A	ST 1B	BLANK	BLANK	LR 3B	LR 3B	CHD6B	CHD6B	LR9B	LR9B	LR 12C	LR 12C
B	ST 2A	ST 2B	CHD1A	CHD1A	CHD4A	CHD4A	CHD6C	CHD6C	CHD9C	CHD9C	CHD 13B	CHD 13B
C	ST 3A	ST 3A	LR 1A	LR 1A	LR 4A	LR 4A	LR 6A	LR 6A	LR 9C	LR 9C	CHD 13C	CHD 13C
D	ST 4A	ST 4B	CHD2A	CHD2A	CHD5B	CHD5B	LR 6B	LR 6B	CHD 10B	CHD 10B	LR 13B	LR 13B
E	ST 5A	ST 5B	LR 2A	LR 2A	LR 5B	LR 5B	LR 6C	LR 6C	LR 10B	LR 10B	LR 13C	LR 13C
F	ST 6A	ST 6B	CHD3A	CHD3A	CHD5C	CHD5C	CHD8C	CHD8C	CHD 11C	CHD 11C	CHD 14B	CHD 14B
G	ST 7A	ST 7B	LR 3A	LR 3A	LR 5C	LR 5C	LR 8C	LR 8C	LR 11C	LR 11C	LR 14B	LR 14B
H	ST 8A	ST 8B	CHD3B	CHD3B	CHD6A	CHD6A	CHD9B	CHD9B	CHD 12C	CHD 12C	CHD 15B	CHD 15B

7.3.1.3. Assay

The diluted beads (50µL) were added to each well. Following this, the plates were washed twice with 100µL of the wash buffer. Samples, standards and blanks were vortexed and added to each well according to a pre-planned layout.

As per manufacturer's instructions, plasma used for human cytokine assay was diluted 1:4 and ICAM/VCAM diluted 1:100 within the wells and using the sample diluent (25µL of sample to 75µL of diluent for human cytokine assay and a consecutive dilution of 10µL of sample in 30µL of sample diluent followed by 5µL of this first dilution, diluted in 120µL of sample diluent, for ICAM/VCAM). Plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, to incubate for 30 minutes. The detection antibodies were vortexed for 5 seconds and diluted 1x:300µL of detection antibody in 2,700µL of detection antibody diluent to yield a total volume of 3,000µL for the cytokines. The plates were then washed 3x with 100µL of the wash buffer and 25µL of the detection antibodies added to each well. The plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, and allowed to incubate for 30 minutes. The Bio-Plex® Manager protocol software was prepared and the standard values from standard one entered on software. The 100x SA-PE was vortexed for 5 seconds and diluted: 60µL of 100x SA-PE diluted with 5,940µL of assay buffer to yield 6,000µL of volume. The plates were once again washed three times with 100µL of the wash buffer. The beads were resuspended in 125µL of the assay buffer. The plates were covered and protected from light with aluminium foil and sealing tape and placed on a shaker at 850rpm, at room temperature, for 30 seconds. Plates were uncovered and placed on the reader using the appropriate settings.

7.3.2. *Measurements and statistical analysis*

The demographic data collected included age, race, height, weight and BMI at booking, smoking status, type of CHD lesion (for the CHD group), gravidity and parity. The delivery data collected included GA at delivery, MOD and indications for assisted delivery or Caesarean section where applicable, sex of the newborn, BW, BW centile according to the Aberdeen Maternity and Neonatal Databank nomograms[186] and EBL.

The Kolmogoroff–Smirnoff, the Shapiro-Wilk tests and visual inspection of the histograms and Q-Q plots were used to assess normality of the distribution of the data. Mann-Whitney or Chi-square tests,

as appropriate, were performed to examine the differences in the demographic characteristics between the examined populations. Pearson and Spearman-rank tests were used, where appropriate, to study the correlations between cytokine and cellular adhesion molecule expression and the variables of interest. Independent t–test, paired t-test and repeated measures ANOVA were used to assess the longitudinal changes of maternal parameters.

7.3.3. *Sample size*

Sample size calculation was performed using the G*Power software (*G*Power for Mac Os X, v. 3.1, February 2020, Heinrich-Heine-Universität, Dusseldorf, Germany*).

There are no studies, to my knowledge, specifically looking at the longitudinal expression of cytokines and CAMs in low-risk pregnancy or in pregnancy complicated by CHD. This was a pilot study and therefore we used samples of convenience to determine if further research is worth pursuing based on our results. As an example, taking as a reference the study by Wang *et al.*[148] who compared the levels of Ghrelin, IL-6 and TNFα in patients with CHD and healthy controls, to obtain a similar difference between the groups with alpha = 0.05 and a power of 95%, we would need a total of eight samples (four in each group).

7.4. Results

The maternal characteristics, obstetric and fetal outcomes are presented in Table 7.2. The distribution of the specific cardiac lesions of participants with CHD and the use of medication are presented in Table 7.3.

Table 7.2 *– Maternal characteristics, obstetric and fetal outcomes of participants included in the study 'Maternal cytokine, vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) expression in low-risk pregnancy and in pregnant women with congenital heart disease. LR – low-risk pregnant women; CHD – pregnant women with congenital heart disease; P – parity; min – minimum; max – maximum. Significance level: p<0.05.*

	LR N = 34	CHD N = 30	p value
Age, years, median (min – max)	33 (24-44)	31.5 (25-39)	0.06
Race, n (%)			0.30
White	26 (76.5)	26 (86.7)	
Non-white	8 (23.5) [1 Black, 4 Asian, 2 Middle-Eastern, 1 Mixed]	4 (13.3) [3 Asian, 1 Middle-Eastern]	
Weight, kg, median (min – max)	62 (51-91.3)	65 (49-101)	0.28
BMI, kg/m^2, median (min – max)	22.5 (18.0-32.7)	24.1 (17.6-34.9)	0.18
Smoking, n (%)			0.82
Never smoked	30 (88.2)	27 (90.0)	
Current/past smoker	4 (11.8)	3 (10.0)	
Parity, n (%)			0.68
P0	21 (61.8)	17 (56.7)	
P ≥1	13 (38.2)	13 (43.3)	
Mode of delivery, n (%)			0.72
Spontaneous Vaginal Delivery	15 (44.1)	10 (33.3)	
Ventouse Vaginal delivery	3 (8.8)	2 (6.7)	
Forceps Vaginal Delivery	2 (5.9)	3 (10.0)	
Emergency Caesarean Section	9 (26.5)	7 (23.3)	
Elective Caesarean Section	5 (14.7)	8 (26.7)	
Mode of delivery, n (%)			0.48
Vaginal (all, including instrumental)	20 (58.8)	15 (50.0)	
Caesarean Section (all)	14 (41.2)	15 (50.0)	
Gestational Age at delivery, weeks+days median (min – max)	39+4 (35+4-42+0)	38+6 (34+3-41+0)	0.005**
Sex of the newborn, n (%)			0.33
Female	14 (41.2)	16 (53.3)	
Male	20 (58.8)	14 (46.7)	
Birthweight, g, median (min – max)	3415 (2640-5220)	2940 (1970-3855)	0.001***
Birthweight centile median (min – max)	62.5 (3.0-100)	30.0 (4.0-92.0)	0.001***
Estimated Blood Loss, mL, median (min – max)	335 (100-1700)	500 (100-1800)	0.041*

*p<0.05, **p<0.01, ***p<0.001

Table 7.3 *– Specific cardiac lesions and use of medication of pregnant women with congenital heart disease (CHD). AS – aortic stenosis; ASD – atrial septal defect; AVSD – atrioventricular septal defect; BAV – bicuspid aortic valve; CoA – coarctation of the aorta; PDA – patent ductus arteriosus PVR – pulmonary valve regurgitation; r – repaired; SA – subaortic stenosis; ToF – Tetralogy of Fallot; TCPC – total cavopulmonary connection; TGA – transposition of great arteries; TVR – tricuspid valve regurgitation; VSD – ventricular septal defect. N = 30*

CHD lesion	N	Use of medication
rTOF	**9**	One participant on LDA, two participants on beta-blockers (Bisoprolol 2.5mg once daily)
rTOF only	*8*	
rTOF + rSA stenosis	*1*	
rCoA	**5**	One participant on Bisoprolol 5mg once daily
rCoA only	*1*	
rCoA + rVSD + BAV	*1*	
rCoA + BAV	*3*	
rTGA	**3**	One participant on beta-blockers, one participant on LMWH
rTGA (arterial switch)	*2*	
rTGA (atrial switch)	*1*	
VSD	**3**	
Unrepaired VSD	2	
rVSD + rPDA + BAV	1	
rAVSD	**1**	
Fontan TCPC	**1**	On LMWH
AS + BAV	**1**	
Pulmonary Atresia + PVR + TVR	**1**	
Ebstein's Anomaly	**1**	
Right hemi-truncus	**1**	
Mixed Lesions	**4**	One participant on Sotalol 80mg twice daily
ASD + rVSD + BAV	*1*	
rVSD + rPDA + BAV	*1*	
BAV	*2*	

The analysis of IL-1β, IL-12 and IFNγ expressions were excluded as the levels were below the detection range of the assay (87.2%, 70.1% and 86.3% of levels below the range, respectively). Expression of IL-6, IL-10, TNFα, GROα and Ghrelin did not follow a Gaussian distribution. Expression of ICAM and VCAM followed a Gaussian distribution.

An example of a standard curve for each of the cytokines studied is presented below in Figures 7.1-7.5.

The standard curves for ICAM and VCAM for each plate are presented in Figures 7.6 – 7.11.

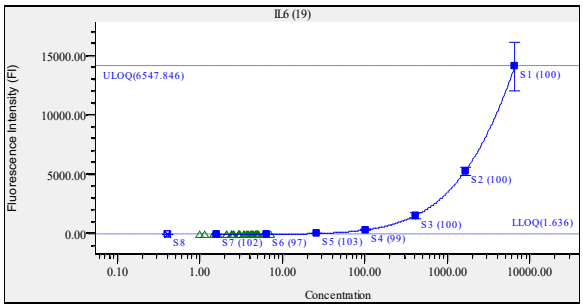


Figure 7.1 *– Standard Curve for IL-6. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

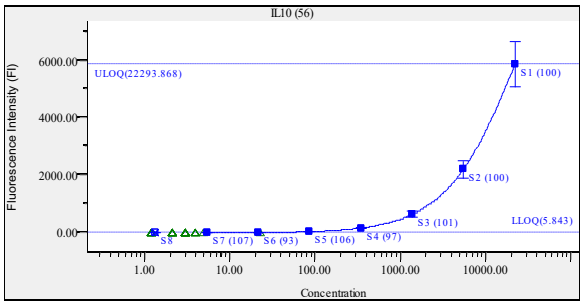


Figure 7.2 *– Standard Curve for IL-10. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

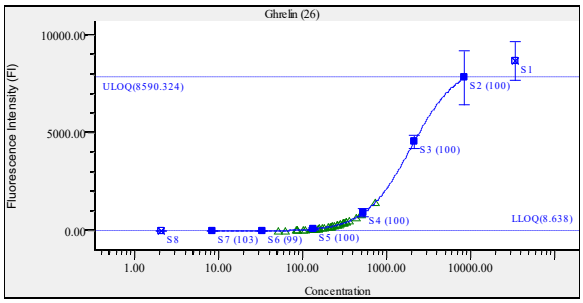


Figure 7.3 *– Standard Curve for TNFα. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

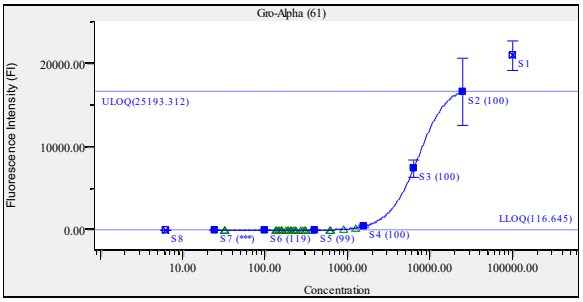


Figure 7.4 *– Standard Curve for GROα. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings).*

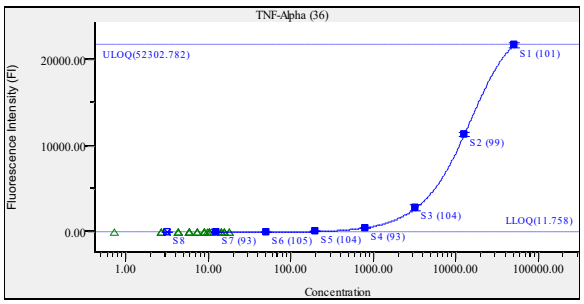


Figure 7.5 *– Standard Curve for Ghrelin. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings).*

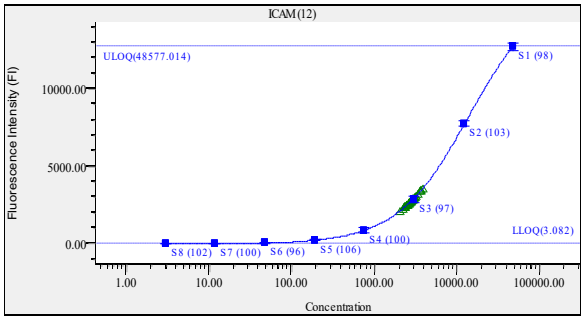


Figure 7.6 *– Standard Curve for ICAM, Plate 1. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

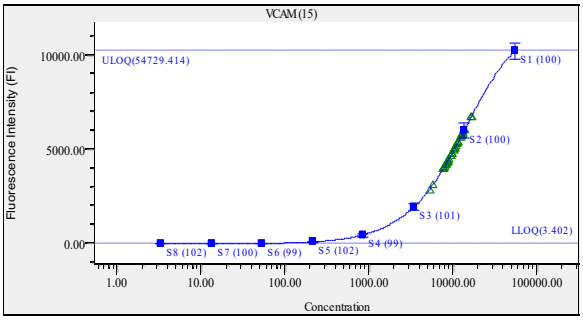


Figure 7.7 *– Standard Curve for VCAM, Plate 1. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

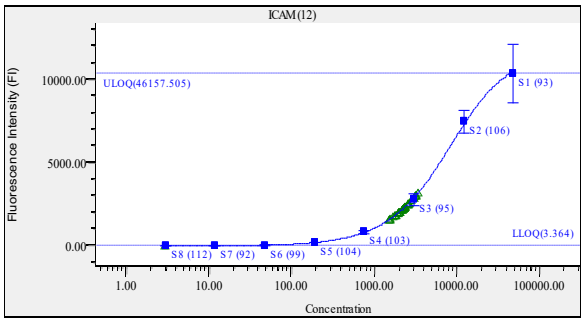


Figure 7.8 *– Standard Curve for ICAM, Plate 2. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

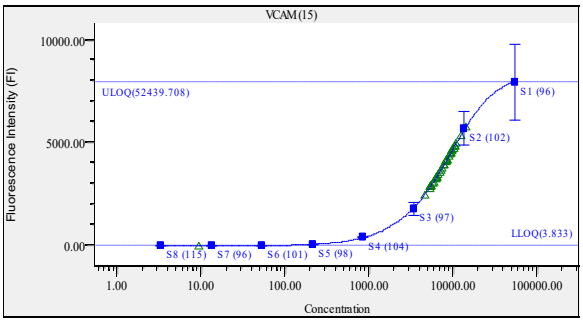


Figure 7.9 *– Standard Curve for VCAM, Plate 2. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings)*

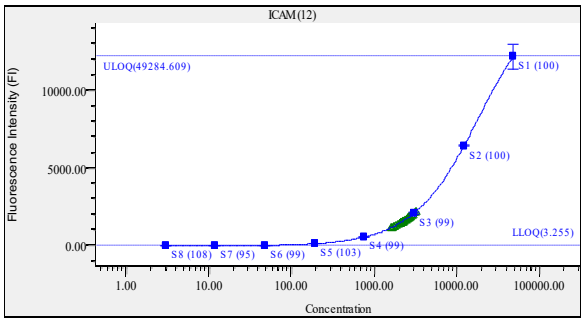


Figure 7.10 *– Standard Curve for ICAM, Plate 3. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings*

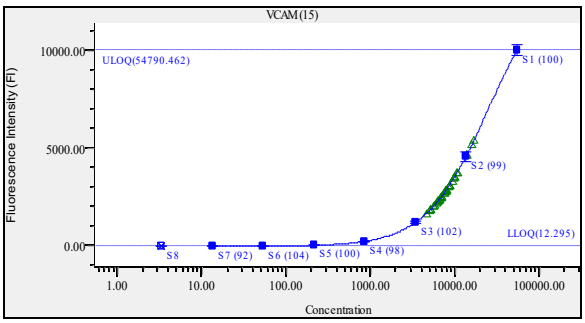


Figure 7.11 *– Standard Curve for VCAM, Plate 3. (S1-S8: standards 1 to 8, U/LLOQ – upper and lower levels of quantification, green triangles – sample readings*

7.4.1. *Longitudinal assessment of cytokine and cellular adhesion molecules throughout pregnancy*

True longitudinal samples were available for 12 of the low-risk participants and for two of the CHD participants. Cytokine expression across the three trimesters are presented in Figures 7.12-7.16 and ICAM/VCAM expression in Figures 7.17-7.18. The summary of the Wilcoxon signed-rank test for

differences in cytokine expression between trimesters in the low-risk group are presented in Table 7.4 and Paired t-test summary for differences across trimesters in ICAM/VCAM expression are presented in Table 7.5. Due to the small number of true longitudinal samples in the CHD group, data are not presented for this group. Differences in cytokine and ICAM/VCAM expression for both studied groups using all samples available at each trimester are presented in Table 7.6.

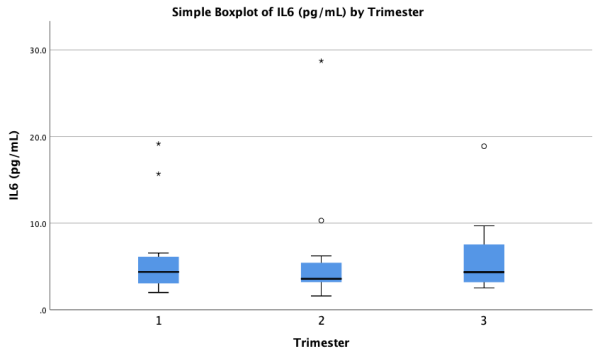


Figure 7.12 *– Expression of IL-6 in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

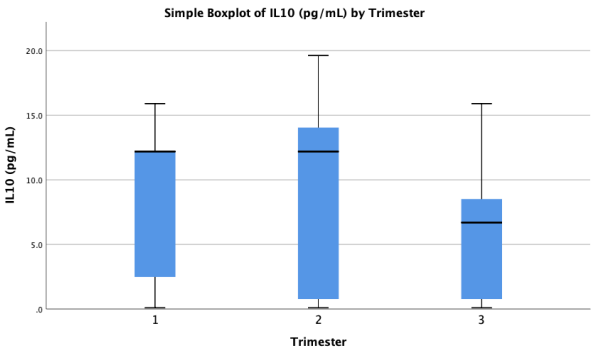


Figure 7.13 *– Expression of IL-10 in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

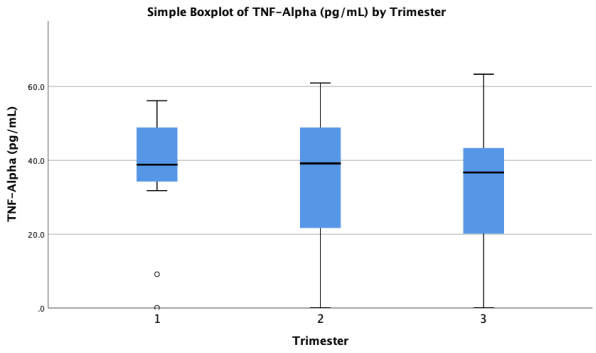


Figure 7.14 *– Expression of TNFα in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

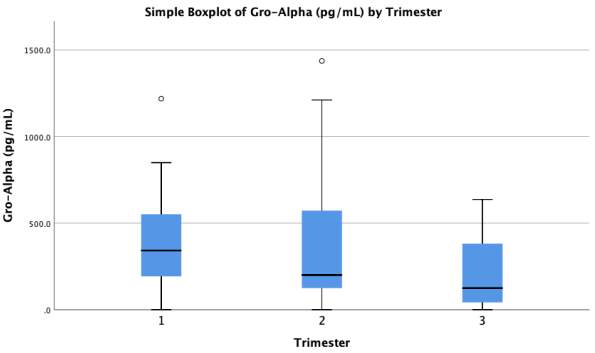


Figure 7.15 *– Expression of GROα in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

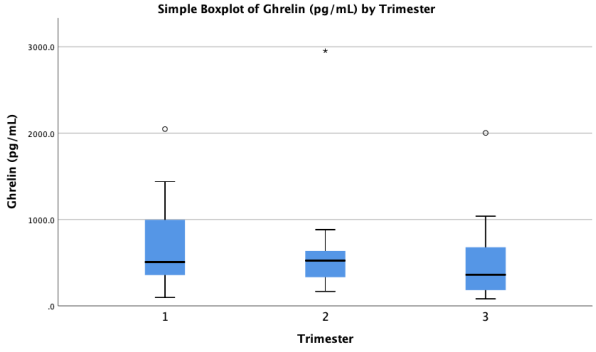


Figure 7.16 *– Expression of Ghrelin in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

Table 7.4 *– Summary of Wilcoxon signed-rank test for differences in cytokine expression between trimesters (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation) for truly longitudinal samples in the low-risk group. Significance level: p<0.05.*

	IL-6	IL-10	TNFα	Groα	Ghrelin
Comparison A/B	0.76	0.60	0.61	0.44	0.64
Comparison B/C	0.84	0.08	0.29	0.11	0.10
Comparison A/C	0.78	0.09	0.21	**0.024***	**0.006***

*p<0.05, **p<0.01, ***p<0.001. p values have been adjusted for multiple comparisons using Bonferroni correction.

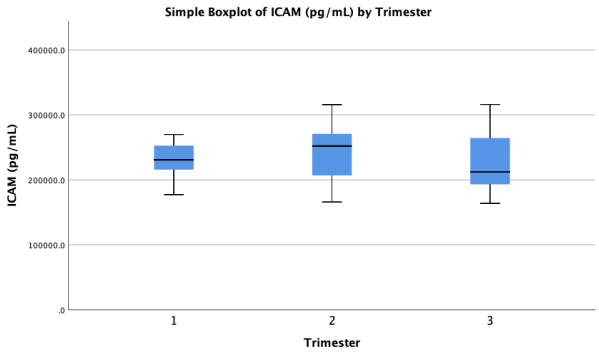


Figure 7.17 *– Expression of ICAM in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

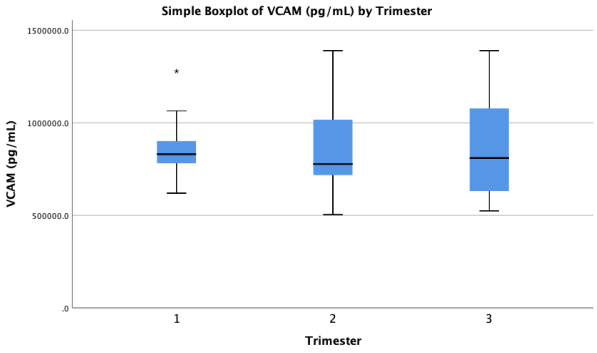


Figure 7.18 *– Expression of VCAM in low-risk pregnancy across trimesters. 1 – 1st trimester, 2 – 2nd trimester, 3 – 3rd trimester.*

Table 7.5 *– Summary of Wilcoxon signed-rank test for differences in ICAM and VCAM expression between trimesters (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation) for truly longitudinal samples in the low-risk group. Significance level: p<0.05.*

	ICAM	VCAM
Comparison A/B	0.42	0.38
Comparison B/C	0.20	0.59
Comparison A/C	0.47	0.27

p values have been adjusted for multiple comparisons using Bonferroni correction.

Table 7.6 *– Summary of Wilcoxon matched-paired signed-rank test for differences between trimesters (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation) for IL-6, IL-10, TNFα, GROα and Ghrelin and summary of the Paired-samples t-test for ICAM and VCAM for all samples available at each of the trimesters and for both study groups (LR – low-risk pregnancy; CHD – pregnant women with congenital heart disease). Significance level: p<0.05. Numbers for each comparison between brackets.*

	Comparison A/B		**Comparison B/C**		**Comparison A/C**	
	LR (n = 13)	**CHD** (n = 2)	**LR** (n = 24)	**CHD** (n = 12)	**LR** (n = 14)	**CHD** (n = 3)
IL-6 (pg/mL)	0.69	0.18	0.70	0.51	0.85	0.11
IL-10 (pg/mL)	0.40	1.0	0.11	0.07	0.31	1.0
TNFα (pg/mL)	0.89	0.18	0.90	0.48	0.35	1.0
GROα (pg/mL)	0.33	0.18	0.19	0.93	**0.009****	1.0
Ghrelin (pg/mL)	0.42	0.18	**0.003****	**0.015***	**0.003****	0.11
ICAM (pg/mL)	0.55	**<0.001*****	0.09	0.28	0.57	0.14
VCAM (pg/mL)	0.20	**<0.001*****	0.06	**0.045***	**0.024***	0.18

*p<0.05, **p<0.01, ***p<0.001. p values have been adjusted for multiple comparisons using Bonferroni correction

We could not investigate the effect of beta-blockers in the CHD group due the small numbers in this cohort.

7.4.2. *Differences between the two studied groups and changes throughout pregnancy in both groups*

Table 7.7 shows the median cytokine and ICAM/VCAM concentrations in both study groups at each of the trimesters, when all available samples per trimester were analysed together. The median concentrations of the studied cytokines across the three trimesters (A, B and C) and between the two studied populations (low-risk pregnant women and pregnant women with CHD) are presented below in Figures 7.19-7.33.

Table 7.7 *– Cytokine, ICAM and VCAM concentrations of all samples available for the low-risk group (LR) and for the congenital heart disease (CHD) group across the different trimesters (A – 10-14 weeks of gestation, B – 18-22 weeks of gestation, C – 30-34 weeks of gestation). Summary of the Mann-Whitney U test for differences between the two groups for IL-6, IL-10, TNFα, GROα and Ghrelin and summary of the Independent-samples t-test for ICAM and VCAM. Data are presented as Median (Min-Max) for IL-6, IL-10, TNFα, GROα and Ghrelin and as Mean ± standard deviation of ICAM and VCAM. Significance level: p<0.05.*

	A			B			C		
	LR (n = 14)	CHD (n = 5)	p value	LR (n = 29)	CHD (n = 19)	p value	LR (n = 29)	CHD (n = 21)	p value
IL-6 (pg/mL)	4.37 (1.9-19.2)	19.17 (5.7-20.6)	**0.005****	6.22 (1.6-28.7)	9.86 (2.8-23.7)	0.10	6.22 (2.5-23.4)	7.95 (3.0-38.3)	0.28
IL-10 (pg/mL)	10.35 (0.1-15.9)	0.10 (0.1-21.7)	0.34	4.60 (0.1-19.6)	0.10 (0.1-16.2)	0.18	4.60 (0.1-15.9)	0.10 (0.1-92.4)	**0.018***
TNFα (pg/mL)	38.83 (0.1-56.9)	34.53 (26.8-63.0)	1.00	36.72 (0.1-61.0)	35.84 (9.2-73.2)	0.58	30.01 (0.1-65.7)	32.37 (9.2-117.5)	0.69
GROα (pg/mL)	360.33 (1.0-4204.6)	636.99 (307.4-5078.9)	0.19	540.53 (1.0-1490.4)	309.46 (1.0-3592.9)	0.58	437.16 (1.0-1498.3)	524.99 (1.0-2513.3)	0.67
Ghrelin (pg/mL)	622.03 (97.9-2047.3)	1238.09 (515.6-1954.2)	0.19	684.21 (167.6-2952.4)	939.87 (131.7-1958.4	0.16	453.58 (81.7-2002.4)	538.91 (174.0-1355.7)	0.40
ICAM (pg/mL)	232,339.0 ± 23,940.4	277,163.2 ± 20,898.1	**0.002****	241,354.0 ± 63,150.3	273,843.8 ± 53,839.6	0.07	241,301.3 ± 53,909.7	258,345.4 ± 59,280.9	0.29
VCAM (pg/mL)	852,167.7 ± 183,329.2	858,153.8 ± 141,171.5	0.95	805,386.5 ± 271,196.8	899,604.4 ± 308,486.7	0.27	893,297.6 ± 273,032.9	984,818.8 ± 365,827.0	0.32

*p<0.05, **p<0.01, ***p<0.001

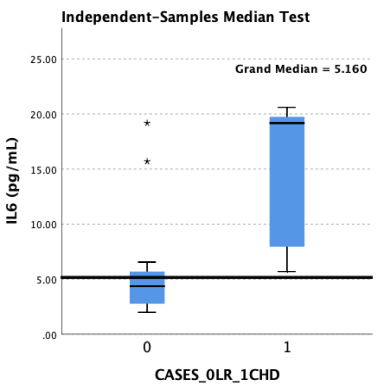


Figure 7.19 *– Expression of IL-6 (pg/mL) in the first trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

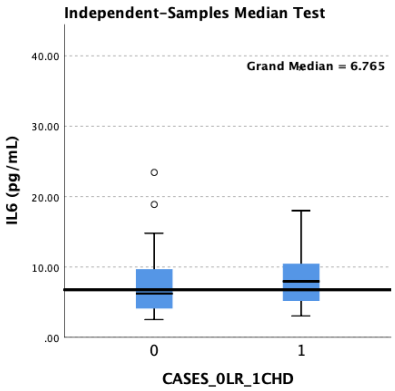


Figure 7.20 *– Expression of IL-6 (pg/mL) in the second trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

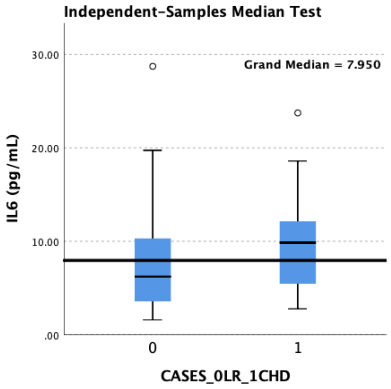


Figure 7.21 *– Expression of IL-6 (pg/mL) in the third trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

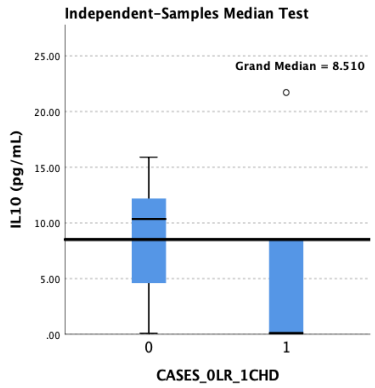


Figure 7.22 *– Expression of IL-10 (pg/mL) in the first trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

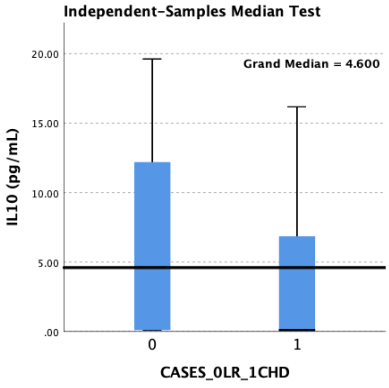


Figure 7.23 *– Expression of IL-10 (pg/mL) in the second trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

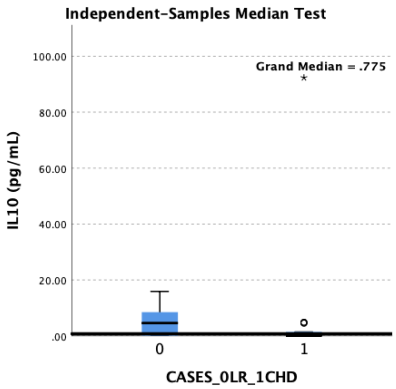


Figure 7.24 *– Expression of IL-10 (pg/mL) in the third trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

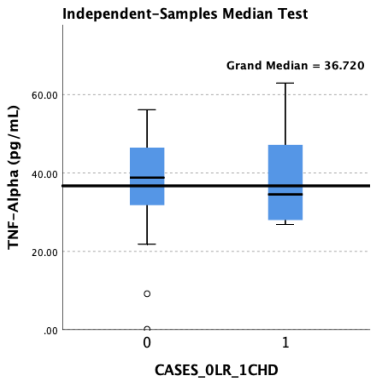


Figure 7.25 *– Expression of TNFα (pg/mL) in the first trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

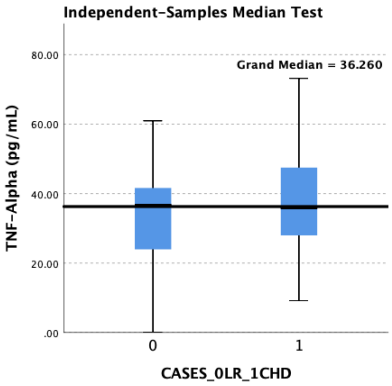


Figure 7.26 *– Expression of TNFα (pg/mL) in the second trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

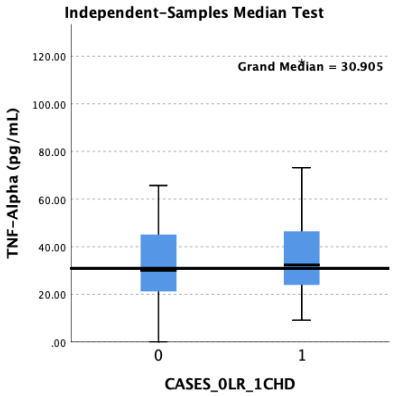


Figure 7.27 *– Expression of TNFα (pg/mL) in the third trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

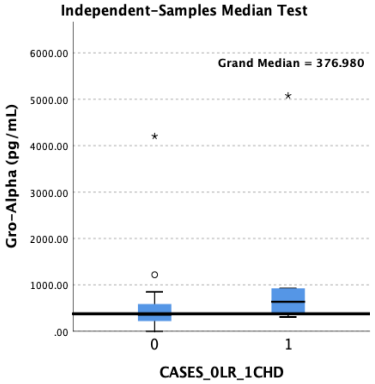


Figure 7.28 *– Expression of GROα (pg/mL) in the first trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease - CHD (1).*

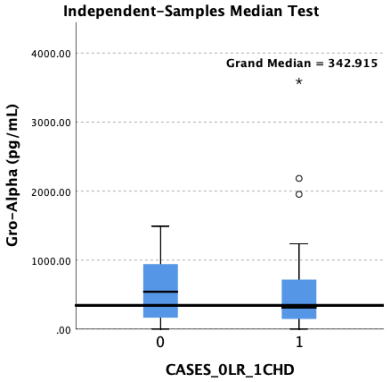


Figure 7.29 *– Expression of GROα (pg/mL) in the second trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease - CHD (1).*

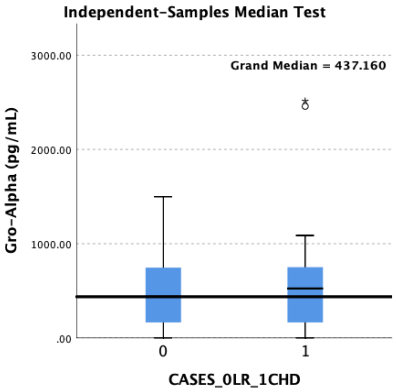


Figure 7.30 *– Expression of GROα (pg/mL) in the third trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

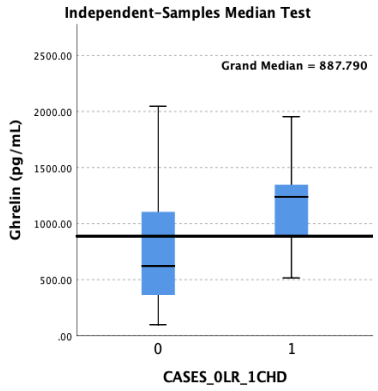


Figure 7.31 *– Expression of Ghrelin (pg/mL) in the first trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

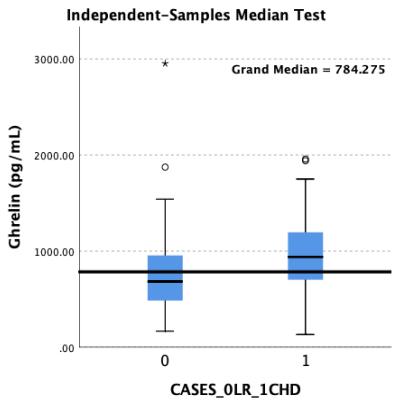


Figure 7.32 *– Expression of Ghrelin (pg/mL) in the second trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

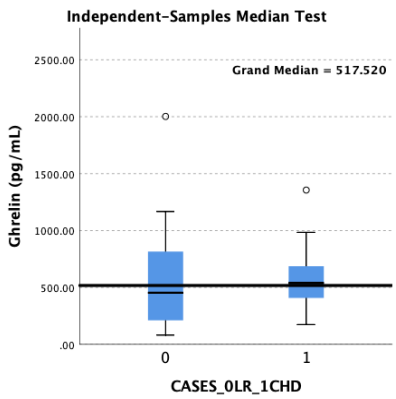


Figure 7.33 *– Expression of Ghrelin (pg/mL) in the third trimester in low-risk pregnant women (0) and in pregnant women with congenital heart disease – CHD (1).*

7.4.3. *Correlation of cytokine and cellular adhesion molecules with BW, BW centile and GA at delivery in low-risk pregnancy and in pregnancy complicated by CHD*

Pearson's and Spearman's correlations (and associated significance values) between cytokine expression and cellular adhesion molecules (ICAM/VCAM) with birthweight (BW), birthweight centile (BW centile) and gestational age at delivery (GA), at each trimester and in each of the studied groups are presented in Table 7.8. There was only a statistically significant correlation between ICAM concentrations in the third trimester in the CHD group and BW. IL-10 concentration in the second trimester in the CHD group was associated with BW and GA at delivery, although statistical significance was not achieved. There was an association between TNFα in the third trimester in the low-risk group and BW. These associations are shown in Figures 7.34-7.37. When samples were analysed as a whole (low-risk and CHD samples together), there were statistically significant correlations between the concentration of IL-6 and BW and BW centile in the first trimester (trimester A) and a statistically significant correlation between ICAM and BW and BW centile in the first trimester (trimester A) – Figures 7.38-7.41. No statistically significant correlations were found with any other cytokines or VCAM at any of the trimesters.

Table 7.8 *– Pearson's and Spearman-rank correlations (and associated significance values) between cytokine expression and cellular adhesion molecules (ICAM/VCAM) with birthweight (BW), birthweight centile (BW cent) and gestational age at delivery (GA), at each trimester (T) and in each of the studied groups (LR – low-risk pregnancy; CHD – pregnant women with congenital heart disease).*

	T	Group	r_s/r	IL-6	IL-10	TNFα	Groα	Ghrelin	ICAM	VCAM
BW	**A**	LR (N=14)	r_s/r	-0.42	0.20	0.41	0.22	-0.03	-0.36	-0.42
			p	0.13	0.49	0.15	0.45	0.92	0.21	0.14
		CHD (N=5)	r_s/r	0.10	-0.11	-0.60	-0.50	0.00	-0.38	-0.23
			p	0.87	0.86	0.29	0.39	1.00	0.53	0.71
	B	LR (N=29)	r_s/r	0.22	-0.02	0.11	0.08	-0.02	0.27	-0.004
			p	0.25	0.93	0.58	0.69	0.93	0.16	0.98
		CHD (N=19)	r_s/r	-0.11	0.42	-0.13	-0.08	-0.17	-0.05	0.16
			p	0.67	0.07	0.61	0.74	0.49	0.84	0.52
	C	LR (N=29)	r_s/r	0.17	0.09	0.32	0.18	-0.19	0.15	-0.05
			p	0.37	0.65	0.09	0.35	0.33	0.45	0.81
		CHD (N=21)	r_s/r	0.02	-0.18	-0.28	-0.17	-0.06	-0.44	-0.26
			p	0.92	0.44	0.22	0.45	0.78	**0.047***	0.25
BW Cent	**A**	LR (N=14)	r_s/r	-0.31	0.24	0.41	0.12	-0.09	-0.34	-0.32
			p	0.28	0.40	0.15	0.70	0.75	0.24	0.26
		CHD (N=5)	r_s/r	1.00	-0.78	0.00	0.70	0.30	-0.47	-0.20
			p	‡‡	0.12	1.00	0.19	0.62	0.43	0.75
	B	LR (N=29)	r_s/r	0.27	-0.02	0.04	-0.13	-0.06	0.27	0.08
			p	0.17	0.92	0.85	0.51	0.76	0.16	0.70
		CHD (N=19)	r_s/r	0.08	0.17	-0.24	-0.28	-0.24	0.25	0.39
			p	0.74	0.48	0.31	0.25	0.32	0.31	0.10
	C	LR (N=29)	r_s/r	0.26	0.05	0.26	0.07	-0.28	0.17	-0.03
			p	0.18	0.79	0.17	0.71	0.14	0.37	0.88
		CHD (N=21)	r_s/r	0.19	-0.09	0.07	-0.11	-0.11	-0.35	-0.12
			p	0.41	0.69	0.78	0.63	0.63	0.12	0.62
GA	**A**	LR (N=14)	r_s/r	-0.36	0.07	0.32	0.39	-0.04	-0.09	-0.33
			p	0.20	0.82	0.26	0.16	0.90	0.77	0.25
		CHD (N=5)	r_s/r	-0.20	0.22	-0.30	-0.70	0.20	-0.16	-0.10
			p	0.75	0.72	0.62	0.19	0.75	0.79	0.88
	B	LR (N=29)	r_s/r	0.04	0.11	0.14	0.12	-0.09	0.08	-0.20
			p	0.83	0.56	0.47	0.53	0.64	0.68	0.29
		CHD (N=19)	r_s/r	-0.33	0.44	0.09	0.04	-0.10	-0.34	-0.24
			p	0.17	0.06	0.71	0.87	0.67	0.15	0.32
	C	LR (N=29)	r_s/r	-0.09	0.15	0.11	0.17	-0.16	-0.15	-0.29
			p	0.65	0.43	0.58	0.39	0.40	0.43	0.12
		CHD (N=21)	r_s/r	-0.02	0.04	-0.30	-0.07	-0.04	-0.36	-0.28
			p	0.92	0.88	0.18	0.77	0.85	0.11	0.22

*p<0.05, **p<0.01, ***p<0.001

‡‡ No p value

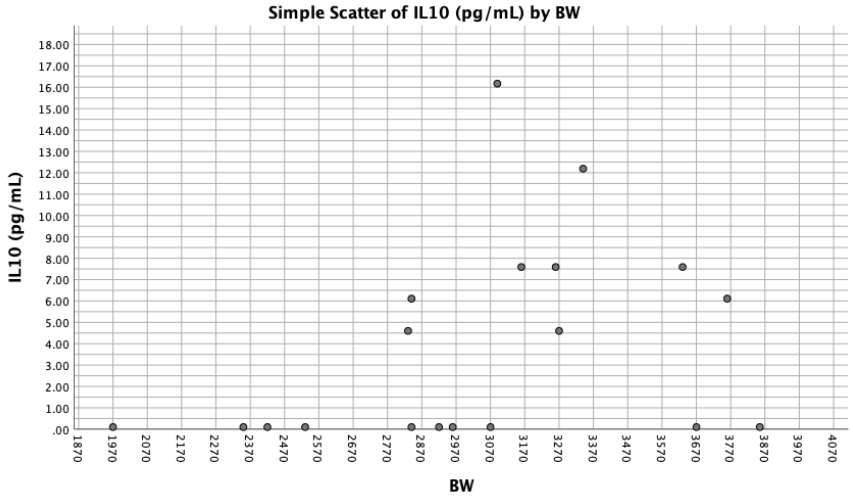


Figure 7.34 *– Scatter plot of IL-10 concentrations in the second trimester and birthweight (BW) in the congenital heart disease (CHD) group. rs – 0.42, p – 0.07.*

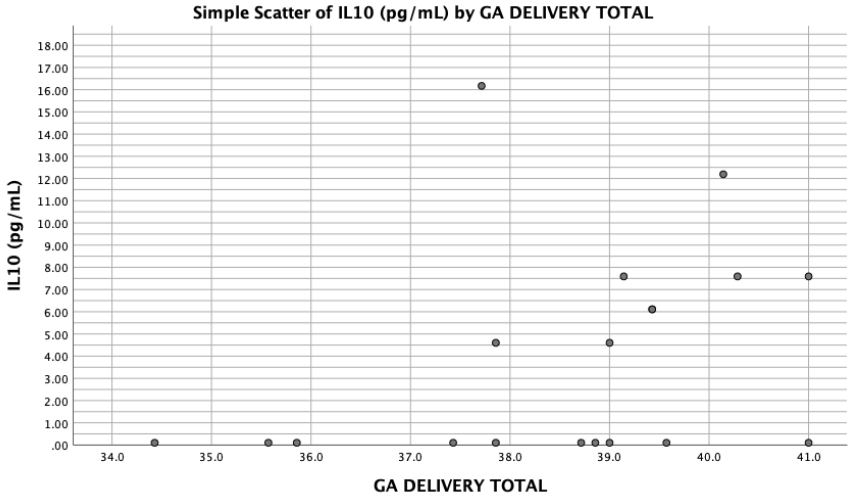


Figure 7.35 *– Scatter plot of IL-10 concentrations in the second trimester and gestational age (GA) at delivery in the congenital heart disease (CHD) group. rs – 0.44, p – 0.06.*

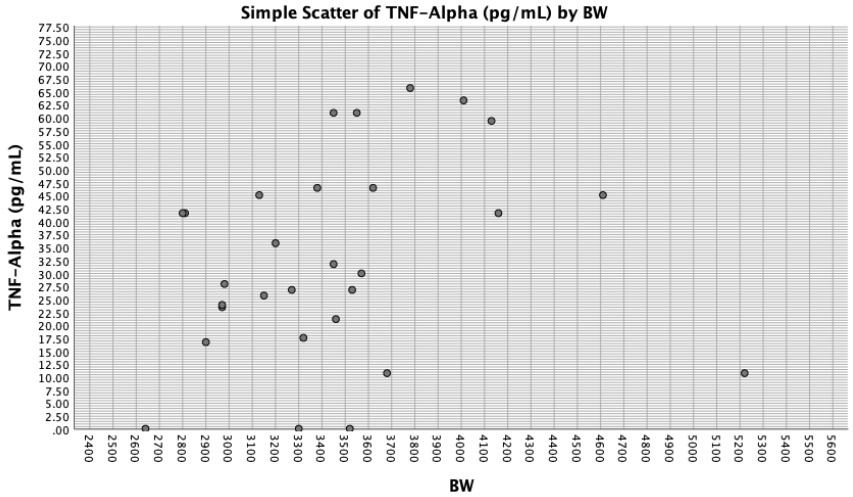


Figure 7.36 *– Scatter plot of TNFα concentrations in the third trimester in the low-risk group and birthweight (BW). rs – 0.32, p – 0.09*

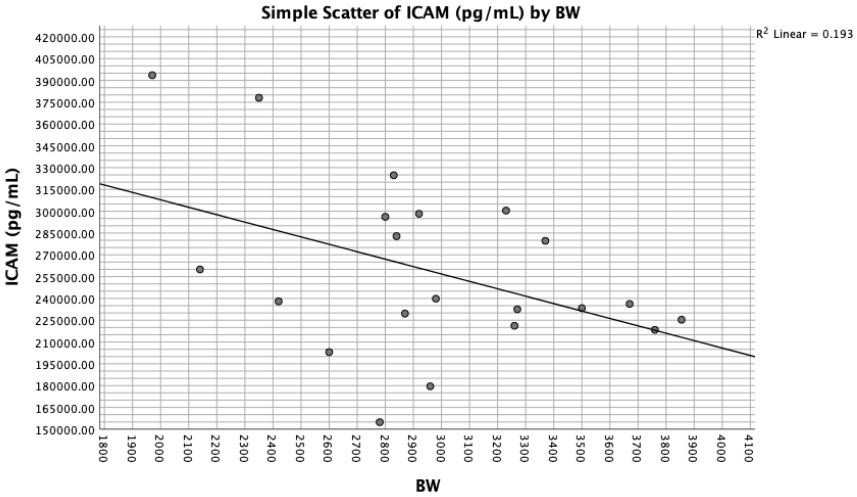


Figure 7.37 *– Scatter plot of ICAM concentrations in the third trimester and gestational age (GA) at delivery in the congenital heart disease (CHD) group. r – -0.44, p – 0.047*.*

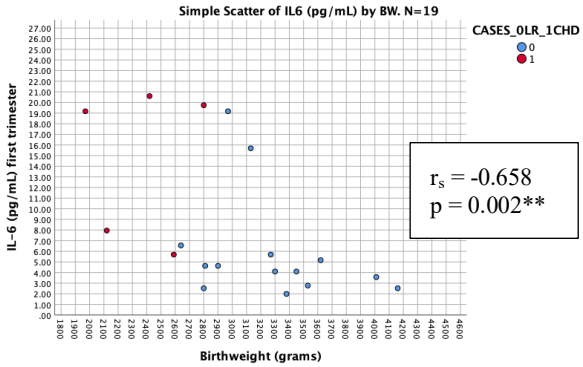


Figure 7.38 *– Scatter plot of IL-6 concentration (pg/mL) in the first trimester and birthweight (BW). The blue dots (n=14) represent the low-risk (LR) group and the red dots (n=5) represent the congenital heart disease (CHD) group.*

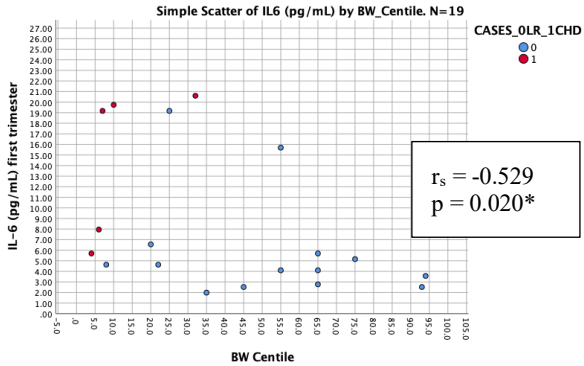


Figure 7.39 *– Scatter plot of IL-6 concentration (pg/mL) in the first trimester and birthweight (BW) centile. The blue dots (n=14) represent the low-risk (LR) group and the red dots (n=5) represent the congenital heart disease (CHD) group.*

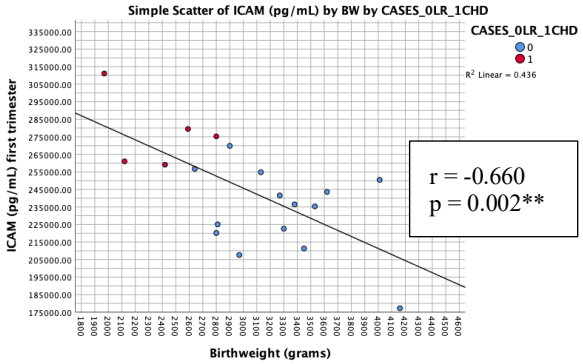


Figure 7.40 *– Scatter plot of ICAM concentration (pg/mL) in the first trimester and birthweight (BW). The blue dots (n=14) represent the low-risk (LR) group and the red dots (n=5) represent the congenital heart disease (CHD) group.*

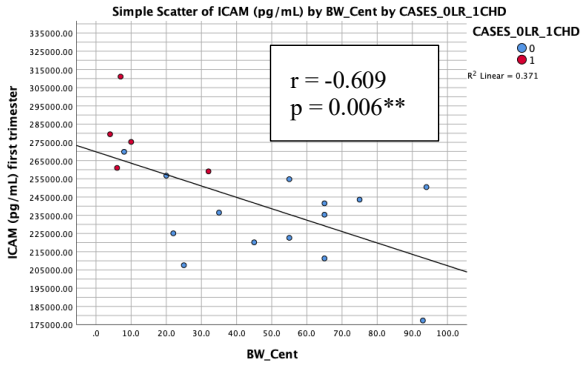


Figure 7.41 *– Scatter plot of ICAM concentration (pg/mL) in the first trimester and birthweight (BW) centile. The blue dots (n=14) represent the low-risk (LR) group and the red dots (n=5) represent the congenital heart disease (CHD) group.*

7.4.4. *Correlation of cytokine and cellular adhesion molecules with CO at rest and ΔCO in low-risk pregnancy and in pregnancy complicated by CHD*

Table 7.9 shows the correlations (and associated p values) between each of the cytokines and ICAM/VCAM at each of the trimesters and CO in the same trimesters. Table 7.10 shows the same correlations, but with ΔCO.

Table 7.9 *– Correlation between cytokines and ICAM/VCAM and cardiac output (CO) at rest (T1), measured by PhysioFlow®, stratified by trimester and by study group (LR – low-risk pregnancy; CHD – pregnant women with congenital heart disease). r – Pearson correlation coefficient; r_s – Spearman rho. Significance level: p<0.05.*

		CO (T1) A		**CO (T1) B**		**CO (T1) C**	
Cytokines		**CHD** (n=4)	**LR** (n=14)	**CHD** (n=17)	**LR** (n=29)	**CHD** (n=20)	**LR** (n=29)
IL-6	r_s	0.60	0.12	-0.25	0.02	0.11	0.16
	p	0.40	0.68	0.34	0.92	0.66	0.39
IL-10	r_s	-0.26	-0.014	-0.32	0.08	0.002	0.06
	p	0.74	0.96	0.22	0.66	0.99	0.78
TNFα	r_s	-0.60	0.098	0.04	0.17	-0.09	0.28
	p	0.40	0.74	0.89	0.37	0.70	0.14
Groα	r_s	-0.40	0.23	0.03	-0.19	-0.18	0.05
	p	0.60	0.42	0.92	0.34	0.45	0.79
Ghrelin	r_s	0.40	0.32	-0.14	-0.11	-0.30	0.16
	p	0.60	0.27	0.60	0.60	0.19	0.41
ICAM	r	-0.19	**-0.60**	0.09	0.24	-0.22	0.10
	p	0.81	**0.02***	0.73	0.22	0.36	0.61
VCAM	r	0.31	-0.43	0.21	0.28	0.02	-0.02
	p	0.69	0.12	0.41	0.15	0.92	0.90

*p<0.05, **p<0.01, ***p<0.001

Table 7.10 *– Correlation between cytokines and ICAM/VCAM and percentage increase in cardiac output (ΔCO), measured by PhysioFlow®, stratified by trimester and by study group (LR – low-risk pregnancy; CHD – pregnant women with congenital heart disease). r – Pearson correlation coefficient; r_s – Spearman rho. Significance level: p<0.05.*

		ΔCO (T2-T1/T2) **A**		**ΔCO** (T2-T1/T2) **B**		**ΔCO** (T2-T1/T2) **C**	
Cytokines		**CHD** (n=4)	**LR** (n=10)	**CHD** (n=15)	**LR** (n=24)	**CHD** (n=17)	**LR** (n=26)
IL-6	r_s	0.20	0.02	-0.38	0.25	-0.02	-0.06
	p	0.80	0.95	0.16	0.24	0.94	0.78
IL-10	r_s	0.26	0.07	-0.05	-0.24	0.14	-0.21
	p	0.74	0.85	0.86	0.26	0.58	0.30
TNFα	r_s	-0.2	-0.33	0.21	-0.37	-0.16	-0.23
	p	0.80	0.35	0.47	0.08	0.55	0.25
Groα	r_s	0.00	-0.43	0.03	-0.06	-0.25	-0.22
	p	1.00	0.21	0.91	0.79	0.33	0.28
Ghrelin	r_s	0.00	-0.22	**-0.64***	0.05	-0.03	0.02
	p	1.00	0.53	**0.01****	0.82	0.93	0.92
ICAM	r	-0.54	0.03	-0.39	-0.09	0.05	-0.32
	p	0.45	0.94	0.15	0.68	0.85	0.11
VCAM	r	-0.66	-0.14	0.02	0.01	0.25	-0.17
	p	0.34	0.70	0.95	0.96	0.33	0.42

*p<0.05, **p<0.01, ***p<0.001

7.5. Discussion

7.5.1. *Maternal characteristics and obstetric and fetal outcomes*

There were no statistically significant differences between the groups with regard to age, race, weight and BMI at booking, smoking status, parity, MOD and sex of the newborn. Most women were white, had normal BMI, were non-smokers and in their first pregnancy, which is in line with the demographic characteristics of the population at CW. As discussed in Chapters 4, 5 and 6, despite a higher proportion of parous women in the CHD group (43.3% *vs* 38.2%), these women were delivered via Caesarean section more often (50.0% *vs* 41.2%), reflecting the tendency to deliver women with CHD via Caesarean section despite little evidence this improves maternal outcomes.[213, 214]There were statistically significant differences regarding GA at delivery, BW, BW centiles and EBL at delivery. The GA at delivery was shorter by five days (p = 0.005) in women with CHD and their babies' BW was lower by nearly 500g (p = 0.001). More importantly, the BW centiles were also significantly different (30.0 *vs* 62.5, p = 0.001). Women with CHD are known to deliver earlier (mainly for iatrogenic reasons) than women who have an uncomplicated pregnancy, and are also known to have smaller babies.[55, 57, 215] There are multiple potential reasons to explain the difference in BW, including maternal demographics and smoking status (which were not different between the two studied groups) and also use of medication (namely, beta-blockers) and cardiac function. A relatively reduced maternal cardiac adaptation to pregnancy is associated with lower birthweight.[55, 57] Women with CHD are also more likely to have increased blood loss at delivery[215, 216]. The reasons behind increased blood loss and increased rates of PPH are multifactorial and associated with increased operative vaginal delivery (forceps in particular), increased Caesarean section rates, increased use of general anaesthetic and higher rates of women on anticoagulation in the CHD cohort.

7.5.2. *Longitudinal assessment of cytokine and cellular adhesion molecules throughout pregnancy*

In the low-risk group, the median concentrations of IL-6 increased from the first to the second trimester (from 4.37pg/ml to 6.22pg/ml [median]) and then increased slightly in the third trimester. In the CHD group, IL-6 concentrations decreased throughout gestation (from 19.17pg/ml to 9.86pg/ml and then to 7.95pg/ml [medians]). None of the differences were statistically significant in either groups, however, the results from the CHD group should be interpreted with caution as there were only two participants with true longitudinal samples. There is conflicting evidence regarding changes of IL-6 expression during pregnancy with some authors reporting an increase with GA[254, 255] and others reporting a decrease[256] or, no difference[246]. However, longitudinal data on cytokine expression in pregnancy are limited.[246] Brewster *et al.*[257] who compared cytokine levels in women with early and late PE (seven women ≤34 weeks' gestation, 13 women >34 weeks' gestation) with 20 matched controls, reported increasing levels of IL-6 with advancing gestation in both groups and that the levels of IL-6 were higher in the PE group, although not statistically significant. The authors suggest that an increase in the inflammatory response with increasing gestation is likely a preparation for labour. Gillespie *et al.*[255] also showed an increase in IL-6 with advanced GA and suggest an increased inflammatory environment at term in preparation for labour. Christian *et al.*[254] reported similar findings to these studies above. Our findings are consistent with the above studies, showing an increase throughout gestation in the low-risk group suggesting preparation for labour. Kraus *et al.*[256] including a higher number of participants than the above studies (n = 50), reported opposite results. The authors suggest that analysing individual cytokines might not be the best approach in studying complex conditions and that due to high intersubject variation in cytokine expression, comparison to each woman's (pregnant) individual background might be of more use. In the CHD group we observed a trend to that of Kraus *et al.*[256], with IL-6 levels decreasing with gestation. The number of participants is, however, different in each of the trimesters and only five in the first trimester, therefore no final conclusions can be made. Pregnancy is associated with lower levels of IL-6 compared to non-pregnant subjects,[145] although IL-6 is required

for implantation and responsible for decidual vascularisation.[258] Different methodologies in the different studies may explain the conflicting information available.

IL-10 decreased with advancing gestation, in the low-risk group, although none of the differences between trimesters reached statistical significance. IL-10 is a pleiotropic cytokine with both stimulation and supressing immune functions.[259] Several studies imply mainly an anti-inflammatory effect of IL-10 in pregnancy and it is believed that it may be involved in maintaining pregnancy by corpus luteum stimulation and production of progesterone.[260, 261] Our findings are consistent with the literature: it is suggested that IL-10 is down-regulated with advanced gestational age, in preparation for labour, an inflammatory state.[260] Holtan *et al.*[145] and Kraus *et al.*[256] showed, however, no statistically significant change in the IL-10 expression with advanced GA. In the CHD cohort, IL-10 remained stable throughout pregnancy and levels were lower than in the low-risk group (please see comparison between the two studied groups below) suggesting that, in the CHD group, there was a more proinflammatory environment.

Levels of TNFα decreased with gestation in the low-risk group. TNFα levels in pregnancy are usually similar to those of non-pregnant subjects.[145] TNFα exhibits a proinflammatory function and increased levels of TNF are associated with implantation failure, fetal loss, hypertensive disease in pregnancy and gestational diabetes.[262] Once again, evidence is conflicting regarding levels of TNFα in pregnancy. Kraus *et al.*[256] and Curry *et al.*[258] reported an increase in pregnancy, Denney *et al.*[259] reported a decrease in levels and suggest this is necessary to tolerate the allogenic fetus and to carry the pregnancy to term and Brewster *et al.*[257] also reported a decrease in levels with advancing gestation. The decrease in TNFα expression was not statistically significant, which is in keeping with other studies.[145] In the CHD group, TNFα levels were similar throughout pregnancy suggesting, once again, a more proinflammatory environment.

The median concentration of Groα also increased from the first to the second trimester from 360.3pg/mL to 540.5pg/mL and then decreased in the third trimester to 437.2pg/mL, in the low-risk group. These changes were not statistically significant. Levels of Groα are known to be raised in pregnancy in comparison to the non-pregnant state.[145] Trophoblastic cells are known to produce Groα to recruit monocytes and facilitate the growth and invasion into the decidua.[263] In this cohort, the expression of Groα decreased as pregnancy progressed but there were only significant differences between the first and third trimesters (significant decrease). In the CHD group, the pattern was similar. Expression of Groα is, however, known to remain elevated even at the end of pregnancy when compared to non-pregnant subjects.[145] Groα has potent neutrophil-activating and -chemoattracting properties.[264] The onset of labour is characterised by an infiltration of immunological cells and secretion of multiple cytokines in the maternal-fetal interface, and this is independent of the presence of infection or timing of onset of labour – Groα is thought to play a role in this.[265] Previous studies have not identified a change with time in the expression of Groα.[145] Cytokine levels are known to vary greatly between individuals, even if the study population is considered homogeneous[145, 256] and this might explain why we have detected a difference.

Ghrelin levels increased slightly from the first to the second trimester and then decreased significantly in the third trimester (622.0pg/mL in the first trimester to 453.2pg/mL in the third trimester, $p = 0.006$), in the low-risk group. Ghrelin is a potent growth hormone stimulant[155] and levels tend to reach their peak at around 18 weeks of gestation and decline thereafter, with lowest levels observed in the late third trimester.[266] We observed the same trend with a gradual decrease in the Ghrelin expression with advanced GA (lowest levels in the third trimester) and the differences between trimesters were statistically significant. During pregnancy the growth hormone axis changes from pituitary to placental; ghrelin stimulates the production of growth hormone and it seems that ghrelin does not play an important role of maintaining growth hormone levels during pregnancy as its levels decline.[266, 267] It is speculated that, like in obesity where ghrelin levels are reduced, ghrelin levels in pregnancy can reflect the nutritional status and a positive energy balance in the third trimester, with decreasing levels in the

third trimester at a time of maternal weight gain.[266-268] The role of ghrelin in pregnancy has not been fully established but its presence has been reported in the human placenta.

No statistically significant differences were found between trimesters for ICAM/VCAM in the low-risk group, for true longitudinal samples. When all samples available per trimester were analysed as if longitudinal, there was a statistically significant increase in VCAM from the first to the third trimester, from 852,167.7pg/mL to 893,297.6pg/mL ($p = 0.024$). The sample numbers were, however, different in each of the trimesters which may have affected the results. There is, once again, conflicting evidence regarding ICAM/VCAM expression in pregnancy. Clark *et al*.[269] reports increased levels of ICAM in pregnant subjects (compared to non-pregnant) secondary to a physiological increase in thrombin expression, but did not investigate longitudinal changes. Austgulen *et al*.[249] reports similar levels (of both ICAM and VCAM) in the non-pregnant and pregnant population. The same authors reported no change in ICAM expression throughout pregnancy, which is consistent with our findings for the low-risk group and the CHD group. Our findings regarding VCAM are also consistent with those of Austgulen *et al.*[249], who showed increasing VCAM levels with gestation. These authors also report higher levels of VCAM and ICAM in patients with PE and suggest that this may be secondary to higher levels of circulating proinflammatory cytokines, such as IL-6 and TNFα. We found statistically significantly higher levels of ICAM in the CHD group (as well as IL-6), which is in line with those findings.

7.5.3. *Differences between LR and CHD cohorts and association with BW, BW centiles and GA at delivery*

IL-6 expression was statistically significantly higher in the CHD cohort in the first trimester (19.17pg/mL *vs* 4.37pg/mL, $p = 0.005$) and remained higher throughout pregnancy, although the difference was not statistically significant for the second and third trimesters, suggesting an underlying inflammatory state. This is further supported by lower levels of IL-10 (anti-inflammatory cytokine), a difference that was statistically significant in the third trimester (0.10pg/mL *vs* 4.60pg/mL, $p = 0.018$),

and slightly higher levels of TNFα (proinflammatory) in the CHD cohort. Higher levels of IL-6 expression (when samples of low-risk women and of women with CHD were analysed together to yield a higher *n* number) were associated with lower BW (r_s = -0.658, p = 0.002) and lower BW centiles (r_s = -0.529, p = 0.020) in the first trimester. However, when the two studied populations were analysed separately, no statistical difference was found. The reasons for low BW in the offspring of women with CHD are multifactorial[57] and our findings suggest that IL-6 expression in the first trimester may play a role in this, probably due to its effect on placentation. There was no statistically significant association between any other cytokines, but in the CHD group, in the second trimester, IL-10 expression was associated with BW (r_s = 0.42, p = 0.07) and with GA (r_s = 0.44, p = 0.06), suggesting that higher circulating levels of this anti-inflammatory cytokine may be associated with longer GA and higher BW.

ICAM, which is constitutively expressed[249], was higher in women with CHD across all trimesters but the difference was only statistically significant in the first trimester. ICAM expression in low-risk pregnancy was similar to that in healthy subjects.[270] There was a statistically significant inverse relationship between ICAM expression and BW for the CHD group in the third trimester (r_s = -0.44, p= 0.047). Exposure to increased blood flow and pressure can lead to endothelial damage with subsequent release of inflammatory cytokines and overexpression of adhesion molecules, such as ICAM and VCAM.[270] ICAM can induce and promote inflammation by facilitating leucocyte adhesion.[271] Placental macrophages in early gestation are unable to express ICAM but maternal cytokines overexpression due to inflammation may cause expression of ICAM and affect trophoblast and local placental haemodynamics, which may affect placentation and consequently fetal growth.[271] No correlation was found between BW centiles or GA.

VCAM expression was not statistically significantly different between the two study groups. VCAM plays an important role in leucocyte recruitment mediated through cytokines and elevated levels have been reported in PE.[272] There was no correlation between VCAM and BW, BW centile or GA.

7.5.4. *Association with CO and ΔCO measured by ICG*

There were no significant correlations between any of the cytokines and CO at rest. Levels of ICAM in the low-risk group in the first trimester were negatively associated with CO at rest (r_s = -0.60, p = 0.020). No correlation was found between CO at rest and VCAM. We cannot explain the correlation between the ICAM levels in the low-risk cohort with CO at rest and assume this was a chance result.

The only statistically significant correlation between ΔCO and cytokine and ICAM/VCAM expression was found in the CHD group for ghrelin in the second trimester.[267] As discussed above, ghrelin stimulates the release of growth hormone but its levels decrease with advancing gestation (after peaking at around 16-20 weeks of gestation[266]), suggesting it does not play a role in maintaining growth hormone in pregnancy.[267] The effects of ghrelin in the cardiovascular system are largely unknown but it has been demonstrated that it reduces MAP and increases CO, without increasing the HR.[273] High levels of ghrelin are, however, associated with hypertensive disorders of pregnancy.[266] The impact of ghrelin in pregnancy, on maternal physiology and placenta remains unknown.[266] In CHD, elevated levels of ghrelin may represent an imbalance between anabolic/catabolic states and be an indirect marker of malnutrition and caquexia.[274]

7.6. Conclusion

The expression of IL-6, IL-10, TNFα and ghrelin, ICAM and VCAM followed the same pattern described by other authors in normal pregnancy. We found that expression of Groα decreased with advancing gestation, which has not been reported before, but this difference might be due to small sample numbers and/or heterogeneous population.

IL-6 expression was statistically significantly higher in women with CHD compared to low-risk pregnancy, and expression of IL-10 was lower, suggesting the existence of a proinflammatory state. There was a statistically significant association between high levels of IL-6 and lower BW centiles. ICAM expression was also statistically significantly higher in the CHD cohort in the first trimester,

suggesting endothelial activation, and there was also a statistically significant association between higher ICAM expression and lower BW centiles.

We did not find any association between cardiac output increase and circulating levels of cytokines and ICAM/VCAM, except from a statistically significant association between ghrelin expression and CO in the CHD cohort in the second trimester.

This was the first time that expression of certain cytokines and ICAM/VCAM was performed in pregnant women with congenital heart disease.

Chapter 8.

Principal Findings, Novel Findings and Implications, Limitations and Future Work

Chapter 8. Principal Findings, Novel Findings and Implications, Limitations and Future Work

8.1. Principal Findings

The number of women with adult congenital heart disease who reach reproductive age and wish to become pregnant will likely continue to increase.[12] Despite multidisciplinary coordinated care and the efforts of dedicated cardiac obstetric centres, cardiac disease in pregnancy remains the main cause of maternal mortality in developed countries and, specifically in the United Kingdom, the rate of maternal deaths due to congenital heart disease has not decreased since the year 2000.[6, 12]

The high risk of adverse pregnancy and cardiac events in pregnant women with congenital heart disease is mainly due to the strain that pregnancy imposes on the cardiovascular system: an increase in blood volume, heart rate, stroke volume and cardiac output.[215] Cardiac output is one of the most useful haemodynamic parameters in obstetrics and being able to measure this easily would allow improved monitoring, more rapid diagnosis and more timely intervention.[25] Several devices are available to measure cardiac output in pregnancy.[25, 83] However, many are used in the pregnant population without having been previously validated.[25] There is therefore a need to validate non-invasive cardiac output monitors in pregnancy.[25]

Uteroplacental blood flow (as measured by uterine artery and umbilical artery Doppler studies) is impaired in pregnant women with congenital heart disease and seems to be associated with maternal cardiac function prior to pregnancy, although data are limited.[11, 32] The relationship between uteroplacental blood flow and cardiac function remains largely unknown.[32] Research in the context of cardiac disease in pregnancy is difficult and most available data are from observational studies.[12] Being able to monitor cardiac output non-invasively during pregnancy without the need to resort to a skilled operator and correlating the haemodynamic findings with those of uteroplacental flow could help with risk stratification and appropriate counselling of pregnant women with congenital heart disease wishing to become pregnant.

In addition to maternal haemodynamic and fetal ultrasound parameters, biochemical markers might also aid in the risk stratification. The incidence of heart failure in pregnancy has increased over the years.[126] There are limited data on the use of B-type natriuretic peptide in pregnancy, specially data on longitudinal assessment throughout pregnancy. Data on cytokine and cellular adhesion molecules in pregnancy are even more limited. Further research on both B-type natriuretic peptide and cytokine/cellular adhesion molecules in pregnancy and particularly in pregnancy complicated by heart disease could provide important insights into the multifactorial causes responsible for the higher incidence of adverse cardiovascular and pregnancy outcomes in pregnant women with heart disease.

Below is a summary of the results of this work and whether each of the hypotheses proposed in the first chapter were supported by the results.

Hypothesis 1: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably measure CO at any trimester.*
Not supported

In low-risk pregnancy, CO measured by PhysioFlow® at any of the trimesters did not correlate with CO measured by TTE at the same time points and therefore, PhysioFlow® did not provide an accurate measure of absolute value of CO. CO at rest, measured by ICG (PhysioFlow®), was higher by an average of 1.5L/min compared to CO measured by TTE.

Hypothesis 2: *In low-risk pregnancy, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*
Supported

PhysioFlow® showed equally good trending ability when compared to TTE in low-risk pregnancy, as demonstrated by a concordance rate of 95.4%, angular bias of 4.5° and radial limits of agreement ≤30° when a central exclusion zone of 15% was used and when measurements with a variation of >150% were excluded.

Hypothesis 3: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably measure CO at any trimester.*
Not supported

In pregnancy complicated by CHD, CO measured by PhysioFlow® during the second and third trimesters, did not correlate with CO measured by TTE at the same time points and therefore, PhysioFlow® did not provide an accurate measure of absolute CO in these circumstances (data from the first trimester was not analysed as n = 4). PhysioFlow® performed worse in terms of absolute values of CO in the CHD group compared to the low-risk group as demonstrated by higher percentage errors (percentage errors of 46.2%-95.2% in the CHD group *vs* 38.1%-70.8% in the low-risk group). CO at rest, measured by ICG (PhysioFlow®), was higher by an average of 1.5L/min compared to CO measured by TTE.

Hypothesis 4: *In pregnancy complicated by CHD, ICG (PhysioFlow®) can reliably detect CO changes with exercise, at any of the trimesters of pregnancy (PhysioFlow® has good trending ability).*
Supported

PhysioFlow® showed good trending ability, compared to TTE in pregnant women with CHD with a concordance rate of 100%, angular bias of 4.1^{o} and radial limits of agreement of ≤30^{o} when a central exclusion zone of 10% is used. Importantly, in the CHD group, PhysioFlow® showed good trending ability irrespective of the central exclusion used (10% or 15%) even without excluding larger variations (>150%) in CO. Angular bias was below the acceptable threshold of 5^{o} and radial limits of agreement remained < ±30^{o}, irrespective of exclusions applied. A possible explanation is that in the CHD group the CO variation was lower and therefore most values could have fallen within the optimal band of 20-50% variation in CO.

Hypothesis 5: *There is a second trimester drop in BP in normal low-risk pregnancy and this is not demonstrated in pregnant women with CHD.*
Supported

In low-risk pregnancy, systolic and diastolic BP decreased significantly from the first to the second trimester (p = 0.009 for systolic BP and, p = 0.003 for diastolic BP) and then in the third trimester, BP

returned to levels similar to those in the first trimester. Although differences in BP between trimesters in the CHD group were not statistically significant, the BP pattern was similar to low-risk pregnancy with a small drop in the second trimester. However, the number of participants in the CHD group in the first trimester was small (n = 4) which could explain the lack of statistical significance in the CHD group.

Hypothesis 6: *In pregnancy, there is a progressive increase in the HR with advancing gestation.*
Supported

Heart rate increased with advancing gestational age in both study groups. Differences were statistically significant in the low-risk group ($p < 0.001$ from the second to the third trimester and $p < 0.001$ between the first and third trimesters). The differences in HR between trimesters were not statistically significant in the CHD group. However, the number of participants available for comparison between trimesters was small and there were two participants on beta-blocker therapy. HR was higher in the CHD group than in the low-risk group, across all three trimesters.

Hypothesis 7: *In pregnancy, there is a decrease in SpO_2 saturations with advancing gestation*
Supported

Peripheral oxygen saturations decreased with advancing gestational age in both the study groups. In the low-risk group, the decrease was statistically significant between the second and third trimesters ($p = 0.012$) and between the first and third trimesters ($p < 0.001$). In the CHD group, differences were not statistically significant. The number of participants available for comparison between trimesters was small in the CHD group which could explain the results. Peripheral oxygen saturations were similar between the two studied groups at all trimesters.

Hypothesis 8: *In pregnancy, there is a progressive increase in CO until 34 weeks of gestation.*
Not supported

In the low-risk group, when CO was measured with ICG (PhysioFlow®), CO remained stable throughout pregnancy (no statistically significant differences between trimesters). CO measured by TTE, at the exact same time points and in the same low-risk participants as ICG, increased slightly in the second trimester with a subsequent decrease in the third trimester although once again, there were no statistically significant differences between trimesters. In the CHD group, CO measured with ICG (PhysioFlow®) increased progressively with advancing gestation, although the differences between trimesters were not statistically significant. When CO was measured by TTE at the same time points and in the same CHD participants, it increased in the second trimester with a subsequent decrease in the third trimester (similar to the low-risk participants) although, once again, differences were not statistically significant.

Hypothesis 9: *In pregnancies complicated by heart disease, there is a suboptimal increase in cardiac output and stroke volume and/or a suboptimal decrease in systemic vascular resistance.*
Not supported

When measurements of CO and SV were taken using ICG (PhysioFlow®), there were no differences at rest between the two studied populations in the second or third trimesters. There was a difference in the CO at rest in the first trimester (CO for low-risk group 6.3L/min *vs* 4.8Lmin in the CHD group, p = 0.044) although this should be interpreted with caution due to the small number of CHD participants in the first trimester (n = 4 in the CHD group *vs* n = 45 in the low-risk group). At peak exercise (exercise targeted at >70% predicted maximum HR for age, calculated as 0.7*[220-age]), there were no differences between groups in CO or SV in the second or third trimesters. There was a statistically significant difference in the first trimester (CO for low-risk group 13.7L/min *vs* 10.7Lmin in the CHD group, p = 0.003) although, as discussed above, these results should be interpreted with caution due to the difference in the number of participants in each group in the first trimester (n = 4 in the CHD group *vs* n = 45 in the low-risk group). When CO and SV were measured using TTE, there were no differences between the two groups in the first and second trimesters in CO and SV at rest. In the third trimester, CO was different between groups (CO for low-risk group 4.1L/min *vs* 5.3Lmin in the CHD group, p = 0.012). This was an unexpected and isolated finding that could have occurred by chance. Overall, there

were no major differences between the two studied populations with regard to CO/SV at rest or CO/SV at peak exercise, suggesting an adequate cardiovascular adaptation to pregnancy in the CHD group.

An important finding was that participants in the CHD group were unable to increase their HR with exercise as much the participants in the low-risk group, a sign of chronotropic incompetence.

Hypothesis 10: *Fetal growth is related to maternal cardiac output and impaired cardiovascular adaptation to pregnancy can lead to defective placentation and, therefore, growth restriction.*
Not supported

There was no association between EFW in the third trimester (between 30-34 weeks of gestation), BW or BW centile and CO at rest or percentage increase in CO with exercise measured by ICG, in a low-risk pregnant population. There was a statistically significant negative association between percentage increase in CO with exercise in the low-risk group, when this was measured by TTE and EFW in the third trimester (r_s = -0.298, p = 0.042), a higher increase in CO was associated with lower birthweight. There was also a statistically significant association between percentage increase in CO with exercise in the low-risk group, when this was measured by TTE and the AC/EFW ratio (r_s = 0.304, p = 0.038). A possible explanation for this is that women with larger percentage increases in CO with exercise start from a lower baseline CO and, low CO, is associated with lower BW.

There was no association between EFW in the third trimester (between 30-34 weeks of gestation), BW or BW centile and CO at rest measured by ICG, in the CHD group. There was a statistically significant correlation (r_s = 0.559, p = 0.024) between percentage increase in CO with exercise measured by ICG in the third trimester in the CHD group and BW. This may be because those with the ability to increase their CO with exercise by a greater degree have better cardiac function and therefore larger BW (this contrasts with the results in the low risk group, where the most important variable was the baseline cardiac output). There was no association between EFW in the third trimester (between 30-34 weeks of gestation), BW or BW centile and CO at rest or percentage increase in CO with exercise, measured by ICG, in the CHD group.

Hypothesis 11: *Defective placentation can be measured indirectly using Doppler indices of UtA and an increased UtA PI in the third trimester is associated with lower EFW on ultrasound and, consequently, with lower BWs and associated BW centiles.*
Not supported

No association was found between UtAD PI and EFW, BW and BW centiles in both populations. UtAD indices were higher in CHD population although not statistically significantly different. However, there were few participants in the CHD group were however less (n = 3 in the CHD group *vs* n = 62 in the low-risk group) and these results need to be interpreted with caution.

Hypothesis 12: *Defective placentation can be secondary to cardiovascular maladaptation in pregnancy and there is an association between UtAD PI and CO and MAP.*
Not supported

No association was found between UtAD PI and CO, ΔCO or MAP in either population. However there were few participants in the CHD group (n = 3 in the CHD group *vs* n = 62 in the low-risk group) and therefore, results need to be interpreted with caution.

Hypothesis 13: *UAD is a marker of placental vascular resistance and increased PI is associated with lower EFW, BW and BW centiles.*
Supported

In the low-risk group, UAD was negatively associated with BW (r_s = -365, p = 0.004) and BW centile (r_s = -0.346, p = 0.006) suggesting that a lower PI and therefore, less resistance in the uteroplacental circulation is associated with higher BW. This association was further reinforced by the correlation found between CPR and BW (r_s = 0.383, p = 0.002) and BW centile (r_s = 0.377, p = 0.003).

No associations were found between UAD PI and EFW, BW or BW centile in the CHD group. However, the number of participants in the CHD group was significantly smaller (n = 16 in the CHD group *vs* n = 62 in the low-risk group).

Women with CHD had lower UAD indices when compared to the low-risk group, which contradicts existing literature. However, CHD women had Doppler studies at a later gestation (UAD PI decreases with advancing gestation) and, therefore, these findings need to be interpreted with caution.

Hypothesis 14: *CPR in the third trimester is a surrogate marker for placental insufficiency and a low CPR is associated with high UtAD PI and lower EFW, BW and BW centiles.*
Supported

There was a positive and statistically significant association between CPR in the third trimester (between 30-34 weeks of gestation) and BW ($r_s = 0.383$, $p = 0.002$) and BW centile ($r_s = 0.377$, $p = 0.003$) in the low-risk group. Women with higher UtAD PIs had lower CPRs, suggesting that increased UtAD PI, a marker of placental perfusion, is associated with blood flow redistribution. This association did not, however, reach statistical significance ($p = 0.82$).

Hypothesis 15: *Pregnancy is associated with fluid overload which stimulates the release of BNP. However, the levels of BNP do not change significantly across the three trimesters of pregnancy.*
Supported

BNP concentrations remained stable throughout pregnancy in the low-risk group. BNP levels decreased with advanced gestational age in low-risk pregnancy with no women having levels >40.0pg/mL in the third trimester. The decrease in BNP concentrations between the first and second trimesters was not statistically significant but the differences between the second and third trimesters was statistically significant ($p < 0.001$). In pregnant women with CHD, a similar trend was observed (albeit with no statistically significant differences) although samples were not truly longitudinal.

Hypothesis 16: *Pregnancy is associated with fluid overload which causes release of BNP and suboptimal increase in CO/SV and/or suboptimal decrease of TVR in CHD is associated with higher levels of BNP.*
Not supported

We found no statistically significant difference in BNP concentrations between the two studied groups at any of the trimesters. We found no correlation between BNP concentrations and percentage increase of CO or SV with exercise.

Hypothesis 17: *There is a change in the women's immune profile throughout pregnancy.*
Supported

In the low-risk group, no statistically significant differences were found between trimesters for IL-6, IL-10 and TNFα. Groα decreased significantly from the first to the second trimester ($p = 0.024$) and Ghrelin decreased significantly from the first to the third trimester ($p = 0.006$). ICAM and VCAM levels remained stable throughout pregnancy in the low-risk group. In the CHD group, no statistically significant differences were found between trimesters for IL-6, IL-10 and TNFα and Groα. Ghrelin decreased significantly from the first to the third trimester ($p = 0.015$). ICAM and VCAM decreased significantly between the first and second trimesters ($p < 0.001$for both) and VCAM then increased significantly in the third trimester ($p = 0.045$).

We found that expression of Groα decreased with advancing gestation, which has not been reported before but this difference might be due to small sample numbers and/or heterogeneous population.

Hypothesis 18: *Higher circulating levels of proinflammatory cytokines and cellular adhesion molecules may cause inadequate spiral artery remodelling and placentation therefore causing FGR and preterm labour.*
Supported

IL-6 expression was statistically significantly higher in pregnant women with CHD compared to low-risk pregnancy (in the first trimester), and expression of IL-10 was lower (in the third trimester), suggesting the existence of a proinflammatory state in the CHD group. There was a statistically significant association between high levels of IL-6 in the first trimester and lower BW ($r = -0.658$, $p = 0.002$) and lower BW centiles ($r = -0.592$, $p = 0.020$). ICAM expression was also statistically significantly higher in the CHD cohort in the first trimester, suggesting endothelial activation. There

was also a statistically significant association between higher ICAM expression and lower BW (r = -0.660, p = 0.002) and BW centiles (r = -0.609, p = 0.006).

Hypothesis 19: *In CHD, cardiac function is related to the circulating levels of cytokines and cellular adhesion molecules.*
Not supported

There was no association between CO at rest and any of the cytokines studied in either study groups. There was an association (r = -0.060, p = 0.020) between ICAM levels in the low-risk participants in the first trimester and CO at rest. There was no association between percentage increase in CO with exercise and IL-6, IL-10, TNFα, Groα and ICAM/VCAM. There was a statistically significant association (r_s = -0.064, p = 0.010) between Ghrelin expression and CO in the CHD cohort in the second trimester.

8.2. Novel Findings and Implications

This is the first time, to my knowledge, that PhysioFlow® has been validated for use in pregnancy. Our findings suggest that PhysioFlow® can safely and reliably be used in low-risk pregnancy to study trends in CO and SV change. Although we had a heterogeneous pregnant population with CHD, our findings also suggest that PhysioFlow® can safely and reliably be used in the CHD population. However, most of the participants in the CHD group had repaired lesions and/or lesions without significant haemodynamic compromise so we suggest not extending the use of PhysioFlow® to patients with CHD who have significant haemodynamic changes secondary to their lesions. The clinical implications of the validation of the PhysioFlow® in pregnancy are that it is easy-to-use, non-invasive, operator independent, cost-effective and reproducible as a technique for measuring HR, SV and CO in a continuous manner. This could be particularly useful in situations with large haemodynamic shifts where continuous measurement of HR, SV and CO are important such as during insertion of loco-regional anaesthesia, during the second stage of labour, during the third stage of labour or during Caesarean section delivery. Real-time monitoring in these situations could aid in real-time diagnosis and guide management. In the particular case of pregnant women with CHD, the use of PhysioFlow®

would allow non-invasive and continuous monitoring of HR, SV and CO during the second stage of labour, a time where active pushing is often limited to prevent cardiovascular complications.[3]

Another novel finding from this work was that the CO percentage increase with exercise was similar between the low-risk participants and the participants with CHD. Although further research is needed, our data suggest that in pregnant women with CHD (those with repaired lesions and/or lesions with no major haemodynamic compromise), the response to exercise was similar to low-risk pregnant women and that the BP increase with exercise was also similar between the groups. Perhaps limiting the second stage of labour for these women might not need to be as prescriptive as it currently is.

An unexpected finding, lower UAD indices in the CHD group, could probably be explained by the fact that Doppler studies were performed at a later gestation in the CHD group and the number of participants was also considerably lower in this group. We suggest this novel finding be interpreted with caution.

One of the strengths of this work is that it is the largest longitudinal study of BNP concentrations in pregnancy including samples from the first trimester. Only two studies[132, 134] report on the longitudinal assessment of BNP from the first through to the third trimester and both were performed over 10 years ago and included fewer participants than in my study. We found declining levels of BNP throughout pregnancy. Studies reporting on BNP concentrations, specifically in CHD, are also lacking and while our samples for the CHD group were not truly longitudinal, we showed a similar trend in the CHD group, with decreasing levels of BNP with advancing gestation.

Studies on cytokine and cellular adhesion molecules in pregnancy are also scarce and, to my knowledge, there are no studies reporting on cytokine and CAMs expression in pregnant women with CHD. We report on important differences between the two studied groups, specifically a more proinflammatory environment in the CHD group characterised by an increased expression of IL-6 and low expression of

IL-10. These findings could guide future research into how cytokines can affect placentation and the effects of inadequate placentation in pregnancy outcomes.

8.3. Limitations

This work has a number of limitations. First of all, there are a limited number of participants with CHD and, particularly, a limited number of participants who underwent the study procedures in the first trimester. Due to delays in triaging referrals in the NHS and arranging first time appointments in the Joint Cardiac Obstetric Medicine Clinic, many patients were past the first trimester by the time that they were approached by me and asked to participate, and of those who were still within the first study window, many could not complete the study visit on the same day. Pregnant patients with CHD have many appointments during pregnancy and occasionally, on the same day, they could be having a TTE at the Royal Brompton Hospital, a consultation at Chelsea and Westminster Hospital and a fetal ultrasound. This would take up an average of three hours of their time and some participants were reluctant to have an extra study visit which would take, on average, an extra hour. In addition to this, some participants were out of area and had long commutes to attend the Hospital and therefore were unwilling to rearrange appointments and attend the Hospital for an extra visit for the purpose of research, which is understandable. Another limitation relating to the CHD participants is that they had a variety of lesions, often with important differences structurally and haemodynamically. In the Joint Cardiac Obstetric Medicine Clinic, an average of 115 new patients are seen per annum (of which 85% are patients with ACHD). To be able to recruit an adequate number of patients for each individual lesion, recruitment would have to be extended for a longer period of time. Due to delays in obtaining a favourable opinion from the REC, I initiated recruitment later than anticipated which contributed to the relatively smaller number of participants with CHD recruited compared with normal controls.

Another limitation of this study is the number of participants with truly longitudinal data. Throughout the duration of my study, I was dependent on a senior cardiac physiologist who performed the TTEs and due to other commitments, they could not perform TTE at all visits, hence the small number of participants with longitudinal TTE data. Although I was trained to perform the TTE measurements

required for the study, I would always need a second operator to simultaneously operate the PhysioFlow® software. Also, due to my own clinical commitments, I was unable to follow-up every woman recruited into the study and see them at every time point studied. Although the TTE measurements required for this particular study are simple and easy to learn, for the CHD cohort, an experienced cardiac physiologist or a cardiologist trained in TTE in CHD would be required to be able to perform TTEs in this group.

Initially, I planned to recruit non-pregnant women and follow them up as they became pregnant and I also planned to see women six weeks postpartum so that each participant acted as their own control. This was not possible due to time constraints and therefore I do not report on preconception (non-pregnant) data or postpartum data. I cannot, therefore, report on the haemodynamic parameters (BP, HR, SV and CO) pre-pregnancy which would have useful when using a new device (PhysioFlow®).

Due to budget constraints, we could not perform an extra fetal ultrasound in the CHD group in the third trimester. Because of this, the number of CHD participants with Doppler studies is very limited and the results should be interpreted with caution.

Although the results of the BNP assay are novel and provide important information on BNP expression throughout pregnancy, the assay used had a range between 10-5000pg/mL. As many samples produced readings < 10pg/mL, it would have been useful to use an assay with more discriminative power so the actual concentration could have been obtained.

8.4. Future Work

Using PhysioFlow®, large numbers of participants could be followed up without the need to have the assistance of an experienced TTE technician. Individual studies could be performed for a wide variety of CHD lesions.

It would be particularly valuable to recruit more women with CHD in order to obtain larger numbers for the main CHD lesions seen in the Joint Cardiac Obstetric Medicine Clinic (ASD/VSD, ToF, TGA

and CoA) in order to create reference values for CO/SV measured by the PhysioFlow® device in all three trimesters of pregnancy. This could be started by my successor in the cardiac disease in pregnancy research post, and I could take part in the study once I have completed my training programme further, provided the necessary funding could be obtained. My doctoral work would provide a strong foundation for grant applications and I would be keen to explore this possibility in the future.

An important part of my study protocol included the recruitment of pregnant women with SGA/FGR. I am particularly interested in the differences in cardiac function in women deemed to be low-risk at booking who then develop SGA/FGR babies and would be keen to investigate this further. For my next level of training, I am interested in applying for subspecialty training in maternal-fetal medicine and this could potentially be a project to do alongside training. Women referred for growth scans from 24 weeks of gestation could be included in the study and the PhysioFlow® used to monitor HR, SV and CO at the same time as having a growth scan.

Following the validation of PhysioFlow® against TTE to measure trends in SV and CO in pregnancy, it can now be employed to study these trends in a variety of situations by other investigators. Measurement of haemodynamic shifts during labour (both first and second stage) are lacking and the PhysioFlow® can be used to monitor HR, SV and CO during this period which will provide novel information. PhysioFlow® can also safely be used during regional anaesthesia insertion and during Caesarean section (I have started collecting these data but, due to insufficient numbers, it was not presented in this thesis) which would, once again provide novel data. My study protocol, approved by the REC, includes permission to use the PhysioFlow® in labour and during Caesarean section.

My study protocol also includes a Part 2 in which women who with SGA/FGR babies who participated in Part 1 would be invited to attend a follow-up visit at around 22-26 months after delivery and have the same study procedures as per Part 1. Their children would also be invited to attend and measurements of height, weight and other anthropometric measurements, such as assessing the distribution of fat in the infants (ponderal index and subscapular skinfold thickness) would also be

recorded. The development and catchup growth of infants of all women studied would be by a paediatric neurologist. The development of the infants is to be carried out using the Bayley Scales of Infant and Toddler Development, version III, a recognised method of assessing the development of infants and toddlers aged 0-42 months. Each test consists on a series of developmental play tasks and takes between 45-60 minutes to complete, depending on the child.

Participants with CHD could be recruited from the preconception clinic at the Royal Brompton Hospital and those who agree to take part, could undergo the same study procedures including measurements of BNP pre pregnancy. Although this would require the inclusion of a significant proportion of women who would then either not become pregnant or miscarry, pre pregnancy data in this cohort would provide invaluable insights on the haemodynamic changes in pregnancy.

Finally, further cytokine/CAMs studies including not only plasma samples but potentially the local expression of these in the placenta could elucidate the potential role of these in pregnancy outcomes.

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Appendices.

APPENDIX 1 – Sponsorship Letter

Imperial College London

Imperial College Healthcare NHS Trust

Joint Research Compliance Office
Academic Health Science Centre
Imperial College London and
Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
Norfolk Place
London, W2 1PG
Tel: +44 (0)207 594 9459
Becky.ward@imperial.ac.uk/
www.ic.ac.uk/clinicalresearchgovernanceoffice

Becky Ward
Research Governance Manager

04/05/2017

Dr Francois Dos Santos
Imperial College London
Department of Surgery and Cancer
Chelsea and Westminster Hospital
369 Fulham Road
London SW10 9NH

Dear Dr Francois Dos Santos

RE: Heart Disease in Pregnancy - Maternal Cardiovascular Adaptation and Fetal Outcomes

Joint Research Compliance Office Reference number: 17IC3946

This is to confirm that the above named research project utilises human participants, their organs, tissue and/or data as defined under the sponsorship requirements of the Research Governance Framework for Health and Social Care 2005, incorporating the Medicines for Human Use (Clinical Trials) Regulations 2004.

On behalf of Imperial College of Science, Technology and Medicine, we undertake to act as the identified Research Sponsor for this project.

This letter confirms:

- The research proposal has been discussed, assessed and registered with the Joint Research Compliance Office, Imperial College Academic Health Science Centre, Imperial College London and provisional sponsor approval granted.
- The Chief Investigator has undergone a process of scientific critique commensurate with the scale of the project.
- Indemnity and insurance arrangements have been put in place to cover the project.
- Resources and support are available to the research team to aid delivery of the research as proposed.
- Management, monitoring and reporting responsibilities for the research have been approved.
- Imperial College will undertake and enforce those sponsor duties set out in the NHS Research Governance Framework for Health and Social Care.

Imperial College Sponsorship is conditional on the project receiving applicable ethical and regulatory approval for all research related aspects of its conduct. It is also conditional on successful contract and agreement negotiations and sign off via the Joint Research Office, where relevant, and before the study commences.

A copy of the ethics approval letter **must** be sent to the Research Governance Manager prior to the study commencing. Sponsorship is dependant on obtaining R&D Office approval for all NHS sites where the research is being conducted.

Yours sincerely

Ms Becky Ward
Research Governance Manager

APPENDIX 2 – Health Research Authority (HRA) Approval Letter

NHS
Health Research Authority

Dr Francois Dos Santos
Obstetric Cardiac Medicine Research Fellow - ST4
Imperial College London
Department of Surgery and Cancer - Academic O&G (3rd Floor), Chelsea and Westminster Hospital
369 Fulham Road, London
SW10 9NH

Email: hra.approval@nhs.net

14 July 2017

Dear Dr Dos Santos

Letter of HRA Approval

Study title:	**Heart Disease in Pregnancy - Maternal Cardiovascular Adaptation and Fetal Outcomes**
IRAS project ID:	**222431**
REC reference:	**17/LO/0970**
Sponsor	**Imperial College London**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators",* issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application

procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **222431**. Please quote this on all correspondence.

Yours sincerely

Rekha Keshvara
Assessor

Email: hra.approval@nhs.net

Copy to: *Ms Becky Ward*
Mr Damon Foster, Chelsea and Westminster Hospital NHS Foundation Trust

IRAS project ID	222431

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Poster SGA]	1.0	09 December 2016
Copies of advertisement materials for research participants [Poster non-SGA]	1.0	09 December 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Insurance]		19 July 2016
IRAS Application Form [IRAS_Form_25052017]		25 May 2017
IRAS Checklist XML [Checklist_25052017]		25 May 2017
Letter from funder [Funding Letter]		01 February 2017
Letter from sponsor [Sponsorship Letter]		04 May 2017
Letters of invitation to participant	1.0	06 January 2017
Other [Addendum to Q A27]		04 June 2017
Other [HRA SoE]	1	13 July 2017
Other [HRA SoA]	1	13 July 2017
Participant consent form [Consent Part 1]	1.1	03 May 2017
Participant consent form [Consent Part 2]	1.0	09 December 2016
Participant information sheet (PIS) [Heart Disease]	1.4	12 July 2017
Participant information sheet (PIS) [Low Risk Women]	1.4	12 July 2017
Participant information sheet (PIS) [SGA]	1.4	12 July 2017
Participant information sheet (PIS) [Part 2]	1.4	12 July 2017
Referee's report or other scientific critique report [peer review Dr Von Klemperer]	2.0	21 October 2014
Referee's report or other scientific critique report [Peer review - Ms Savvidou]	2.0	21 October 2014
Research protocol or project proposal	1.4	17 May 2017
Summary CV for Chief Investigator (CI)		15 February 2017
Summary CV for supervisor (student research)		06 May 2017
Summary CV for supervisor (student research) [Dr Sonya Babu Narayan]		31 May 2017

IRAS project ID	222431

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Ms Becky Ward
Email: becky.ward@imperial.ac.uk
Tel: 0207 594 9459

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	Statement of activities will act as agreement of an NHS organisation to participate.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical

IRAS project ID	222431

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			defence organisation covers the activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	The applicant has confirmed in the statement of activities that the sponsor will not provide any funding to sites
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is one site type for the research; all sites will undertake the same activity. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.
Participating NHS organisations in England **will be expected to formally confirm their capacity and capability to host this research**. • Following issue of this letter, participating NHS organisations in England may now confirm to the sponsor their capacity and capability to host this research, when ready to do so. How capacity and capacity will be confirmed is detailed in the *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* section of this appendix. • The Assessing, Arranging, and Confirming document on the HRA website provides further information for the sponsor and NHS organisations on assessing, arranging and confirming capacity and capability.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).
It is expected that a Principal Investigator will be in place at the participating NHS sites. GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

IRAS project ID	222431

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
Use of identifiable patient records held by an NHS organisation to identify potential participants should be undertaken by a member of the direct care team for the patient, so it would not normally be acceptable for this to be done by staff not employed by that organisation. An Honorary Research Contract (or equivalent) would be expected for any external NHS/research staff undertaking all of the other activities for the study once consent from the participant is in place. The pre-engagement checks should include an enhanced DBS check (including a check against the DBS 'barred list' for adults), and Occupational Health Clearance.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
• The applicant has indicated that they intend to apply for inclusion on the NIHR CRN Portfolio.

APPENDIX 3 – Ethics Committee Favourable Opinion Letter

NHS
Health Research Authority

London - South East Research Ethics Committee
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

29 June 2017

Dr Francois Dos Santos
Obstetric Cardiac Medicine Research Fellow - ST4
Imperial College London
Department of Surgery and Cancer - Academic O&G (3rd Floor), Chelsea and Westminster Hospital
369 Fulham Road, London
SW10 9NH

Dear Dr Dos Santos

Study title:	**Heart Disease in Pregnancy - Maternal Cardiovascular Adaptation and Fetal Outcomes**
REC reference:	**17/LO/0970**
IRAS project ID:	**222431**

The Research Ethics Committee reviewed the above application at the meeting held on 14 June 2017. Thank you for attending to discuss the application along with Professor Phil Steer.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request.
Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1) Please amend the Participant Information Sheet as follows:
   a. Please insert a sentence at the beginning of the document to state that this is an education project
   b. Paragraph 3, line 2 please correct the spelling mistake from 'who's' and change to 'whose'.
   c. Under the heading 'Why have I been chosen?' please change the word 'chosen' to 'invited'.
   d. Under the heading 'Why have I been chosen? Do I have to take part? Please begin the first sentence with 'No, you do not have to take part in this study'.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Summary of discussion at the meeting

Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)

The Committee raised a concern with regard to the exercise that pregnant women would be asked to do, and if there was any risk of exercising so close to delivery of their baby. As the women would possibly be in active labour, the Committee were concerned especially for women that may go on to have a caesarean and the muscle contractions involved having any impact on that.

The researchers explained that there was research that has been conducted on over 1000 women who had completed 5km runs and there had been no adverse responses, in fact exercise in pregnancy was shown to be beneficial.

The Committee accepted that and asked if this would be different for women who were already in labour and experiencing contractions, and if there was any risk of exercise interfering in the labour process or bringing it forward.

The researchers explained that the vast majority of women that would be presenting at the hospital would have had an elective caesarean planned in, and therefore it would be unlikely that they would actually be in labour.

The Committee acknowledged that would be the case for women with heart disease, however for the healthy women this may not apply.

The researchers explained that research had shown that being ambulatory during labour decreased the rates of caesarean births and the midwives actually encouraged this. For those who go on to have a caesarean deliver there was no additional risk at all.

The Committee were satisfied with the response.

Informed consent process and the adequacy and completeness of participant information

The Committee requested that the PIS should state that this was an Educational project. There were also some other minor changes requested as outlined below.

Please contact the REC Manager if you feel that the above summary is not an accurate reflection of the discussion at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Poster SGA]	1.0	09 December 2016

Copies of advertisement materials for research participants [Poster non-SGA]	1.0	09 December 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Insurance]		19 July 2016
IRAS Application Form [IRAS_Form_25052017]		25 May 2017
IRAS Checklist XML [Checklist_25052017]		25 May 2017
Letter from funder [Funding Letter]		01 February 2017
Letter from sponsor [Sponsorship Letter]		04 May 2017
Letters of invitation to participant	1.0	06 January 2017
Other [Addendum to Q A27]		04 June 2017
Participant consent form [Consent Part 1]	1.1	03 May 2017
Participant consent form [Consent Part 2]	1.0	09 December 2016
Participant information sheet (PIS) [PIS Heart Disease]	1.2	03 May 2017
Participant information sheet (PIS) [PIS - Low-risk Women]	1.2	03 May 2017
Participant information sheet (PIS) [PIS SGA]	1.2	03 May 2017
Participant information sheet (PIS) [PIS Part 2]	1.0	12 April 2017
Referee's report or other scientific critique report [peer review Dr Von Klemperer]	2.0	21 October 2014
Referee's report or other scientific critique report [Peer review - Ms Savvidou]	2.0	21 October 2014
Research protocol or project proposal	1.4	17 May 2017
Summary CV for Chief Investigator (CI)		15 February 2017
Summary CV for supervisor (student research)		06 May 2017
Summary CV for supervisor (student research) [Dr Sonya Babu Narayan]		31 May 2017

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and

the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

17/LO/0970	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

pp
Professor David Caplin
Chair

E-mail: nrescommittee.london-southeast@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers"

Copy to: *Ms Becky Ward*
Mr Damon Foster, Chelsea and Westminster Hospital NHS Foundation Trust

London - South East Research Ethics Committee

Attendance at Committee meeting on 14 June 2017

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Ashok Bhiman	Consultant Psychiatrist	Yes	
Professor David Caplin	Physicist	Yes	Chair
Ms Stephanie Cooper	Retired Solicitor	No	
Mr Ron Driver	Retired University Lecturer/Statistician	Yes	
Professor John Eastwood	Consultant Renal Physician	Yes	
Professor Anthony Fox	Pharmaceutical Medicine	Yes	
Mr Guy Gardener	Retired Assistant Chief Constable	Yes	
Ms Janelle Hill	Former Banking Administrator	Yes	
Professor Atholl Johnston	Professor of Clinical Pharmacology	Yes	
Dr Morven Leese	Reader in Biostatistics	Yes	
Prof Eleni Palazidou	Consultant Psychiatrist	Yes	
Mr Graham Smith	Business Consultant	No	
Ms Vanda Taylor	Senior Cancer Information Nurse	No	
Ms Brigid Tucker	Head of Policy & Communications, General Osteopathic Council	Yes	
Professor Zahur Zaman	Retired Clinical Pathologist	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Julie Acourt	REC Assistant
Mrs Margaret Hutchinson	REC Manager

APPENDIX 4 – Confirmation of Capacity and Capability at Chelsea and Westminster Hospital NHS Foundation Trust

Chelsea and Westminster Hospital NHS
NHS Foundation Trust

Research and Development
Unit G2, Harbour Yard
Chelsea Harbour
London SW10 0XD

Francois Sousa Dos Santos
Obstetric Cardiac Medicine Research Fellow
Department of Surgery and Cancer - Academic O&G (3rd Floor)
Chelsea and Westminster Hospital
369 Fulham Road, London, SW10 9NH

3rd August 2017

Our reference: C&W17/058

Dear Francois,

RE: Confirmation of Capacity and Capability at Chelsea and Westminster Hospital NHS Foundation Trust

Study title: Heart Disease in Pregnancy - Maternal Cardiovascular Adaptation and Fetal Outcomes
IRAS reference: 222431
REC reference: 17/LO/0970

This letter confirms that Chelsea and Westminster Hospital NHS Foundation Trust has the capacity and capability to deliver the above referenced study.

We agree to start this study on a date to be agreed when your Sponsor gives the green light to begin.

As you may be aware, the Trust is committed to achieving the national metric of recruiting the first participant promptly and finishing recruitment on time and on target. These metrics are reported to the Department of Health and published on our website, and there are financial penalties if we fail to achieve them.

Therefore, your targets are:

Recruitment of first participant by: 2nd September 2017
Recruitment of 100 participants by: 19th December 2018

Please let me know the date you enrol your first participant. If you are not able to meet these targets, please contact Damon Foster, R&D Operations Manager (damon.foster@chelwest.nhs.uk), who will be happy to discuss support available, and/or take note of the reasons why these targets

cannot be achieved. If you have any queries about these targets or any point throughout your project, please do not hesitate to contact me.

Meanwhile, may I take this opportunity to wish you well with your research.

We look forward to hearing the progress and outcomes for the study.

Kind regards



Damon Foster, R&D Operations Manager

APPENDIX 5 – Funding Letter

Research to prevent premature birth

Chelsea & Westminster Hospital
Academic Dept. of Obstetrics & Gynaecology
Room H 3.32 | 369 Fulham Road
London SW10 9NH
T: +44 (0)20 3315 3184

Damon Foster
Research Delivery Operations Manager
Chelsea and Westminster Hospital NHS Foundation Trust

1st February 2017

Dear Damon,

On behalf of Professor Mark Johnson, I would like to confirm that Borne intends to fund the following:

Name of student and research group: Dr Francois Sousa Dos Santos, Professor Mark R Johnson Research Group
Name of research study: HDiP-MCAFO: Heart Disease in Pregnancy - Maternal Cardiovascular Adaptation and Fetal Outcomes
Name of funding source; amount of funding provision; duration/ time period: £10k p/yr for consumables for 2.5 years
Who will hold and manage these funds throughout this time: The funds will be held by Borne, who will be invoiced periodically and as needed by Imperial College

Please do let me know if you need any further detail or confirmation.

Best wishes,

Jenny Palmer
Operations Manager

T: +44 (0)203 315 7930
E: jenny@borne.org.uk

Borne aims to identify the causes of premature birth to save lives, prevent disability and create lifelong health for mothers and babies.

Registered Charity no. 1167073

APPENDIX 6 – Letter of Confirmation of NIHR Clinical Research Network Support

FOR INFORMATION – Study eligible for NIHR CRN support IRAS ID 222431

sumera.galaria@nihr.ac.uk <sumera.galaria@nihr.ac.uk>
on behalf of
Portfolio Applications Team <portfolio.applications@nihr.ac.uk>
Wed 13/09/2017 12:29

To: dos Santos, Francois <f.dos-santos@imperial.ac.uk>
Cc: Ward, Becky <becky.ward@imperial.ac.uk>; Regimantas Pestininkas <Regimantas.Pestininkas@nihr.ac.uk>; CSP CRN North West London <studysupport.crnnwlondon@nihr.ac.uk>

Dear Mr Dos Santos,

RE: HDiP - MCAFO IRAS ID 222431

I am pleased to inform you that your study has been deemed eligible for NIHR Clinical Research Network support.

Further information about Eligibility for Studies to receive CRN support, is available on our web site.

Please note your study has provisionally been assigned to the CRN: Reproductive health & Childbirth Specialty. A member of the Specialty Portfolio team will be in touch to collect any additional information required for inclusion of your study on the Central Portfolio Management System (CPMS). However if you have any queries please don't hesitate to contact CRN: Reproductive health & Childbirth at crncc.portfolioa@nihr.ac.uk

Your application for HRA Approval will continue; should you have any questions about HRA Approval, contact the HRA at hra.approval@nhs.net

The Local CRN for each participating site is now able to provide support to undertake NHS Support activities for this study as per their local model. These models may vary and therefore agreement with the Local CRN for each participating site is required. To note: Provision of NHS Support Activities is informed by the attribution of study specific activities as NHS Support Activities. This is agreed once on behalf of the CRN by the Lead Local CRN AcoRD Specialist at the Lead Local CRN in line with AcoRD policy.

Kind regards,

Sumera

Portfolio Eligibility

CRN Research Delivery | CRN National Coordinating Centre (CRNCC) | NIHR Clinical Research Network (CRN)



t. 0203 328 6722 (Jane); 0113 3430329 (Richard) | **e.** portfolio.applications@nihr.ac.uk
a. 21 Queen Street, Leeds, LS1 2TW

Confidential information may be contained in this message.If you are not the intended recipient, any reading, printing, storage, disclosure, copying or any other action taken in respect of this e-mail is prohibited and may be unlawful. If you are not the intended recipient, please notify the sender immediately by using the reply function and then permanently delete what you have received.

APPENDIX 7 – Participant Information Sheet: Participant with Congenital Heart Disease

Imperial College London

HDiP Heart Disease in Pregnancy

NHS Chelsea and Westminster Hospital NHS Foundation Trust

Participant Information Sheet
Participant with Congenital Heart Disease

Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation & Fetal Outcomes

Chief Investigator – Dr Francois Dos Santos | IRAS Project ID: 222431 | REC: 17/LO/0970

We would like to invite you to take part in our research study which is an education project. Before you decide to participate, we would like you to understand why this research project is being undertaken and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We anticipate this should take about 30 minutes. Please do not hesitate to ask us if there is anything that is not clear.

What is the purpose of this study and why is it important?

In normal pregnancy, the cardiovascular system (the heart and the blood vessel network) undergoes a series of physiological changes to meet the increased metabolic demands of the mother and the baby. There is limited information on how the cardiovascular system adapts to pregnancy in women with congenital heart disease and little is known about the reversibility of these changes after pregnancy. Women with congenital heart disease are also known to deliver small babies. It is yet to be established if this is a consequence of poor adaptation of the cardiovascular system to pregnancy or if there is an underlying problem with the placenta caused by the heart disease itself.

In addition to this, heart disease in pregnancy is the single largest cause of indirect maternal deaths in the UK. The number and complexity of congenital heart disease survivors continues to grow and obstetricians need to be able to safely manage women who can tolerate pregnancy. Research in this field is therefore imperative.

Our aim is to study the adaptation of the cardiovascular system to pregnancy in women with **congenital heart disease** but also in women without any heart disease and in women whose babies are found to be small during pregnancy.

In order to do this, we plan to check your blood pressure and saturations (level of oxygen in the blood using a finger probe), perform a scan of your heart (known as *'ECHO'*) and take a reading of your heart using electrodes (*'Trans-thoracic impedance cardiography*' - similar to an *'ECG').* These tests would be conducted at rest and after a short period of exercise on a stationary bicycle. We would also like to perform some scans of your baby to monitor the growth and blood flow. Finally we will also take some blood samples from you and samples from your placenta after birth.

We will do our best to minimise the number of extra visits to the Hospital and the number of extra blood samples. We will aim to see you when you were already planning to come in for your routine visits and take extra samples of blood when you were meant to have bloods taken as part of the normal investigations in pregnancy thereby minimising any disruption to your normal life.

Why have I been invited? Do I have to take part?

No, you do not have to take part in this study. We are inviting you to take part in this project because you are planning a pregnancy/pregnant and have a known heart condition. It is up to you whether you take part in the study. If you decide to not take part in the study, this will not affect the standard of care you receive. Before you sign up, we will describe the process to you and talk you through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This will not affect the standard of care you receive. If you withdraw from the study we will destroy all your identifiable samples but we will need to use the data collected up to your withdrawal.

What will happen to me if I take part?

Below is a diagram of the planned visits and the investigations we would like to undertake if you agree to participate:

	Timing of Visits	Investigations
Pre-conception	Routine visit to pre-conception clinic	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra)
10-14 weeks gestation	Same day as booking appointment or booking scan	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test
18-22 weeks gestation	Same day as anomaly scan or fetal echocardiogram or maternal echocardiogram	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra) • Check blood flow of the baby at the time of the scan
28-32 weeks gestation	Same day as growth scan or maternal echocardiogram	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test • Check blood flow of the baby at the time of the scan
Labour	Around the time of admission: in labour, for induction or caesarean section.	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test
6-10 weeks postpartum	Routine post-partum visit	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra)

What will happen to me if I take part? (continued)

If you decide to join the study just let your midwife or doctor in antenatal clinic at Chelsea & Westminster Hospital know.

Alternatively you can contact the research team led by Dr Francois Dos Santos directly on mobile: 07771515471.

We would like to see you ideally once before pregnancy and then at different stages of your pregnancy, in labour and once after pregnancy (please see diagram above). We will arrange all necessary investigations at the same time as your routine antenatal appointments so no further Hospital visits will be required.

What do I have to do? What to expect during the investigations

Once you arrive for your appointment, we would attach the electrodes of the *'Impedance Cardiography'* (see description below) to your chest and perform a baseline reading along with a baseline *'ECHO'*, blood pressure and saturations. After this, we will ask you to exercise on a stationary bike to increase your heart rate and perform the same measurements immediately after you exercise. We would then repeat the same readings after you've rested for about 20 minutes. The entire visit shouldn't take more than 60 minutes.

'Impedance Cardiography'

This assessment is painless and not invasive - the electrodes are similar to those used for an *'ECG'*. Once attached, the electrodes will be recording the blood flow in your heart from the time we start, throughout your 6-min walking test and during your rest time. Chaperons will be available.

'ECHOs'

You would have probably had a heart *'ECHO'* before due to your heart condition. We will perform a simplified *'ECHO'* that will take less time than your usual *'ECHO'*. Scans of your heart are painless and not invasive. You will be lying on your left side and wearing a gown. A doctor or specialist technician (a person specially trained to do heart scans) will apply some gel and a wand-like device called a 'transducer' on your chest. This transducer is able to create images of your heart on a screen next to you. This test takes about 5-10 minutes throughout which the lights will be dimmed so the images are easier to be seen. Chaperons will be available.

Scans of the baby

You will not need extra scans for this study but we would like to look into the blood flow of the baby in more detail when you have your scans – this can add about 5 minutes to your appointment.

Blood Tests/Samples from the placenta

We will try and coincide taking the blood samples required for the study at the same time you would have your routine pregnancy blood taken. However, to participate in this study, you would have to have additional bloods samples taken on three occasions: pre-pregnancy, at 18-22 weeks and after pregnancy. We will be taking 20-25ml (about two tablespoons) of extra blood on each occasion. We will take two small samples from the placenta (about the size of a £2 coin)

What are the disadvantages and risks of participating in this study?

This study requires you to attend the hospital regularly for appointments which you will attend in any case for your antenatal care. Extra time may be required. The blood samples required from you could cause some discomfort and bruising which are however minor. If you wish not to have blood taken, you can still take part in this study. Baby scans, *'ECHOs'* and the *'Impedance Test'* are safe and cause no harm. Taking samples from the placenta will not put you or the baby at any increased risk and does not lengthen the delivery time.

What are the advantages of participating?

There will be no direct benefit to you from participating in this project, however, your participation will help to improve the knowledge of how heart disease affects pregnancy and help improve the care of women.

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Francois Dos Santos, email: f.dos-santos@imperial.ac.uk, mobile: 07771515471). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Should any of your test results be or become abnormal, such as an abnormality during your *ECHO*, we will immediately inform you and refer you to the appropriate specialist for further management.

What happens when the research stops? What happens to the samples taken?

All the information collected on you will be treated as confidential and only the research team will have access to your details. The samples taken will be given a study ID, stored and analysed in a laboratory. Blood samples and samples from the placentas will be stored in the Imperial College London Academic O&G laboratory at Chelsea and Westminster Hospital NHS Trust. Access is restricted to authorised personnel (protected by access card) and the samples will be under the custody of the Chief Investigator. The samples will not be stored for future use in other studies.

What about confidentiality?

All the information collected on you will be treated as confidential and only the research team will have access to your details. Personal data will first be pseudoanonymised by allocating each participant a trial specific ID number. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Electronic data containing participant personal details will be stored on a password protected file on a password protected computer. Hard copies of the documents will be stored in a locked cabinet within the Academic O&G Department at Chelsea and Westminster Hospital NHS Trust. Personal data will be stored in line with *Imperial College London* policy to enable full reconstruction of the study up to 10 years after its completion.

What will happen to the results of the research study?

The intention is to publish the results of the study in peer review journals. The participants will not be identified in any report/publication.

Who is organising and who has reviewed the study?

The project is organised by the Academic Department of Obstetrics and Gynaecology of Chelsea & Westminster Hospital. All research in the NHS is looked at by independent group of people, called the *Health Research Authority (HRA)* and *Research Ethics Committee (REC)* to protect your interests. This study has been reviewed and given favourable opinion by HRA. If you agree to participate, you will be given a copy of the information sheet and a signed consent form to keep. Thank you for considering taking part and taking time to read this sheet.

Further info?

Please contact:

Dr Francois Dos Santos
email: f.dos-santos@imperial.ac.uk
mobile: 07771515471

APPENDIX 8 – Participant Information Sheet: Low-risk Women

Imperial College London

HDiP Heart Disease in Pregnancy

NHS Chelsea and Westminster Hospital NHS Foundation Trust

Participant Information Sheet

Participant without Heart Disease (Low-risk Women)

Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation & Fetal Outcomes

Chief Investigator – Dr Francois Dos Santos | IRAS Project ID: 222431 | REC: 17/LO/0970

We would like to invite you to take part in our research study which is an education project. Before you decide to participate, we would like you to understand why this research project is being undertaken and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We anticipate this should take about 30 minutes. Please do not hesitate to ask us if there is anything that is not clear.

What is the purpose of this study and why is it important?

In normal pregnancy, the cardiovascular system (the heart and the blood vessel network) undergoes a series of physiological changes to meet the increased metabolic demands of the mother and the baby. There is limited information on how the cardiovascular system adapts to pregnancy in women with congenital heart disease and little is known about the reversibility of these changes after pregnancy.

In addition to this, heart disease in pregnancy is the single largest cause of indirect maternal deaths in the UK. The number and complexity of congenital heart disease survivors continues to grow and obstetricians need to be able to safely manage women who can tolerate pregnancy. Research in this field is therefore imperative.

Our aim is to study the adaptation of the cardiovascular system to pregnancy in women with congenital heart disease but also in **women without any heart disease** and in women whose babies are found to be small during pregnancy. **Your participation is extremely important to us and will help us understand how a 'normal' heart adapts to pregnancy compared to someone with an 'abnormal heart'.**

In order to do this, we plan to check your blood pressure and saturations (level of oxygen in the blood using a finger probe), perform a scan of your heart (known as *'ECHO'*) and take a reading of your heart using electrodes (*'Trans-thoracic impedance cardiography'* - similar to an 'ECG'). These tests would be conducted at rest and after a short period of exercise on a stationary bicycle. We would also like to perform some scans of your baby to monitor the growth and blood flow. Finally we will also take some blood samples from you and samples from your placenta after birth.

We will do our best to minimise the number of extra visits to the Hospital and the number of extra blood samples. We will aim to see you when you were already planning to come in for your routine visits and take extra samples of blood when you were meant to have bloods taken as part of the normal investigations in pregnancy thereby minimising any disruption to your normal life.

Why have I been invited? Do I have to take part?

No, you do not have to take part in this study. We are inviting you to take part in this project because you are planning a pregnancy/pregnant and will book your pregnancy at Chelsea & Westminster Hospital. It is up to you whether you take part in the study. If you decide to not take part in the study, this will not affect the standard of care you receive. Before you sign up, we will describe the process to you and talk you through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This will not affect the standard of care you receive. If you withdraw from the study we will destroy all your identifiable samples but we will need to use the data collected up to your withdrawal.

What will happen to me if I take part?

Below is a diagram of the planned visits and the investigations we would like to undertake if you agree to participate:

	Timing of Visits	Investigations
Pre-conception	Extra Visit	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra)
10-14 weeks gestation	Same day as booking appointment or booking scan	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test
18-22 weeks gestation	Same day as anomaly scan or fetal echocardiogram or maternal echocardiogram	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra) • Check blood flow of the baby at the time of the scan
28-32 weeks gestation	Extra Scan	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test • Extra growth scan + blood flow of the baby at the time of the scan
Labour	Around the time of admission: in labour, for induction or caesarean section.	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test
6-10 weeks postpartum	Extra Visit	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra)

What will happen to me if I take part? (continued)

If you decide to join the study just let your midwife or doctor in antenatal clinic at Chelsea & Westminster Hospital know.

Alternatively you can contact the research team led by Dr Francois Dos Santos directly on mobile: 07771515471.

We would like to see you ideally once before pregnancy and then at different stages of your pregnancy, in labour and once after pregnancy (please see diagram above). We will arrange all necessary investigations at the same time as your routine antenatal appointments to limit the number of extra Hospital visits.

What do I have to do? What to expect during the investigations

Once you arrive for your appointment, we would attach the electrodes of the *'Impedance Cardiography'* (see description below) to your chest and perform a baseline reading along with a baseline *'ECHO'*, blood pressure and saturations. After this, we will ask you to exercise on a stationary bike to increase your heart rate and perform the same measurements immediately after you exercise. We would then repeat the same readings after you've rested for 20 minutes. The entire visit shouldn't take more than 60 minutes.

'Impedance Cardiography'

This assessment is painless and not invasive - the electrodes are similar to those used for an *'ECG'*. Once attached, the electrodes will be recording the blood flow in your heart from the time we start, throughout your 6-min walking test and during your rest time. Chaperons will be available.

'ECHOs'

We will perform a simplified *'ECHO'* that will take less time than your usual *'ECHO'*. Scans of your heart are painless and not invasive. You will be lying on your left side and wearing a gown. A doctor or specialist technician (a person specially trained to do heart scans) will apply some gel and a wand-like device called a 'transducer' on your chest. This transducer is able to create images of your heart on a screen next to you. This test takes about 5-10 minutes throughout which the lights will be dimmed so the images are easier to be seen. Chaperons will be available.

Scans of the baby

We would perform an extra scan of your baby around 28-32 weeks. We would also like to look into the blood flow of the baby in more detail when you have your scans – this can add about 5 minutes to your appointment.

Blood Tests/Samples from the placenta

We will try and coincide taking the blood samples required for the study at the same time you would have your routine pregnancy blood taken. However, to participate in this study, you would have to have additional bloods samples taken on three occasions: pre-pregnancy, at 18-22 weeks and after pregnancy. We will be taking 20-25ml (about two tablespoons) of extra blood on each occasion. We will take two small samples from the placenta (about the size of a £2 coin)

What are the disadvantages and risks of participating in this study?

This study requires you to attend the hospital regularly for appointments which you will attend in any case for your antenatal care. Extra time may be required. The blood samples required from you could cause some discomfort and bruising which are however minor. If you wish not to have blood taken, you can still take part in this study. Baby scans, *'ECHOs'* and the *'Impedance Test'* are safe and cause no harm. Taking samples from the placenta will not put you or the baby at any increased risk and does not lengthen the delivery time.

What are the advantages of participating?

There will be no direct benefit to you from participating in this project, however, your participation will help to improve the knowledge of how heart disease affects pregnancy and help improve the care of women.

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Francois Dos Santos, email: f.dos-santos@imperial.ac.uk, mobile: 07771515471). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Should any of your test results be or become abnormal, such as an abnormality during your *ECHO*, we will immediately inform you and refer you to the appropriate specialist for further management.

What happens when the research stops? What happens to the samples taken?

All the information collected on you will be treated as confidential and only the research team will have access to your details. The samples taken will be given a study ID, stored and analysed in a laboratory. Blood samples and samples from the placentas will be stored in the Imperial College London Academic O&G laboratory at Chelsea and Westminster Hospital NHS Trust. Access is restricted to authorised personnel (protected by access card) and the samples will be under the custody of the Chief Investigator. The samples will not be stored for future use in other studies.

What about confidentiality?

All the information collected on you will be treated as confidential and only the research team will have access to your details. Personal data will first be pseudoanonymised by allocating each participant a trial specific ID number. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Electronic data containing participant personal details will be stored on a password protected file on a password protected computer. Hard copies of the documents will be stored in a locked cabinet within the Academic O&G Department at Chelsea and Westminster Hospital NHS Trust. Personal data will be stored in line with *Imperial College London* policy to enable full reconstruction of the study up to 10 years after its completion.

What will happen to the results of the research study?

The intention is to publish the results of the study in peer review journals. The participants will not be identified in any report/publication.

Who is organising and who has reviewed the study?

The project is organised by the Academic Department of Obstetrics and Gynaecology of Chelsea & Westminster Hospital. All research in the NHS is looked at by independent group of people, called the *Health Research Authority (HRA)* and *Research Ethics Committee (REC)* to protect your interests. This study has been reviewed and given favourable opinion by HRA. If you agree to participate, you will be given a copy of the information sheet and a signed consent form to keep. Thank you for considering taking part and taking time to read this sheet.

Further info?

Please contact:

Dr Francois Dos Santos
email: f.dos-santos@imperial.ac.uk
mobile: 07771515471

APPENDIX 9 – Consent Form: Part 1

Imperial College London

NHS Chelsea and Westminster Hospital NHS Foundation Trust

HDiP Heart Disease in Pregnancy

CONSENT FORM – Part 1

HDiP | Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation and Fetal Outcomes

Centre Number:

Study Number:

Participant ID

Name of Researcher: _____ **Please initial box**

1. I confirm that I have read and understand the participant information sheet dated 12 of Jul 2017 (Version 1.2, REC Ref 17/LO/0970) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I give consent for the following samples to be taken throughout specified points in pre-pregnancy and during pregnancy: maternal blood and two samples from the placenta. ☐
5. I give consent to the following tests to be undertaken throughout specified points in pre-pregnancy and during pregnancy: blood pressure, peripheral oxygen saturations, echocardiogram and trans-thoracic impedance cardiography. ☐
6. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
_____	_____	_____

Once completed: 1 copy for participant; 1 copy for researcher site file; 1 (original) to be kept in medical notes

APPENDIX 10 – Participant Information Sheet: Participant with SGA Baby

Imperial College London

HDiP Heart Disease in Pregnancy

NHS Chelsea and Westminster Hospital NHS Foundation Trust

Participant Information Sheet
Participant with SGA Baby

Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation & Fetal Outcomes

Chief Investigator – Dr Francois Dos Santos | IRAS Project ID: 222431 | REC: 17/LO/0970

We would like to invite you to take part in our research study which is an education project. Before you decide to participate, we would like you to understand why this research project is being undertaken and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We anticipate this should take about 30 minutes. Please do not hesitate to ask us if there is anything that is not clear.

What is the purpose of this study and why is it important?

In normal pregnancy, the cardiovascular system (the heart and the blood vessel network) undergoes a series of physiological changes to meet the increased metabolic demands of the mother and the baby. There is limited information on how the cardiovascular system adapts to pregnancy in women with congenital heart disease and little is known about the reversibility of these changes after pregnancy. Women with congenital heart disease are also known to deliver small babies. It is yet to be established if this is a consequence of poor adaptation of the cardiovascular system to pregnancy or if there is an underlying problem with the placenta caused by the heart disease itself. In addition to this, heart disease in pregnancy is the single largest cause of indirect maternal deaths in the UK. The number and complexity of congenital heart disease survivors continues to grow and obstetricians need to be able to safely manage women who can tolerate pregnancy. Research in this field is imperative.

Our aim is to study the adaptation of the cardiovascular system to pregnancy in women with congenital heart disease but also in women without any heart disease and in women **whose babies are found to be small during pregnancy.** Your participation is extremely important to us and will help us understand the factors that cause **babies to be small in patients with heart disease and in patients without heart disease.**

In order to do this, we plan to check your blood pressure and saturations (level of oxygen in the blood using a finger probe), perform a scan of your heart (known as *'ECHO'*) and take a reading of your heart using electrodes (*'Trans-thoracic impedance cardiography'* - similar to an 'ECG'). These tests would be conducted at rest and after a short period of exercise on a stationary bicycle. We would also like to perform some scans of your baby to monitor the growth and blood flow. Finally we will also take some blood samples from you and samples from your placenta after birth. We will do our best to minimise the number of extra visits to the Hospital and the number of extra blood samples. We will aim to see you when you were already planning to come in for your routine visits and take extra samples of blood when you were meant to have bloods taken as part of the normal investigations in pregnancy thereby minimising any disruption to your normal life.

Why have I been invited? Do I have to take part?

No, you do not have to take part in this study. We are inviting you to take part in this project because you are pregnant and your baby had been found to be small-for-gestational-age. It is up to you whether you take part in the study. If you decide to not take part in the study, this will not affect the standard of care you receive. Before you sign up, we will describe the process to you and talk you through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This will not affect the standard of care you receive. If you withdraw from the study we will destroy all your identifiable samples but we will need to use the data collected up to your withdrawal.

What will happen to me if I take part?

If you decide to join the study just let your midwife or doctor in antenatal clinic at Chelsea & Westminster Hospital know.

Alternatively you can contact the research team led by Dr Francois Dos Santos directly on mobile: 07771515471.

We would like to see you from the time you have been told your baby was small and then 2-4 weekly depending on how often your growth scans are organised. We would then see you when you come in to deliver and once after pregnancy (please see diagram below). We will arrange all necessary investigations at the same time as your routine antenatal appointments to limit the number of extra Hospital visits.

Below is a diagram of the planned visits and the investigations we would like to undertake if you agree to participate:

	Timing of Visits	Investigations
Anytime after 24 weeks (2-4 weekly from then)	Routine Growth Scans	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test • Extra growth scan + blood flow of the baby at the time of the scan
Labour	Around the time of admission: in labour, for induction or caesarean section.	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test
6-10 weeks postpartum	Extra Visit	• Blood Pressure, Saturations, Heart ECHO, Impedance test – all before and after exercise – stationary bike. • Blood test (extra)

What do I have to do? What to expect during the investigations

Once you arrive for your appointment, we would attach the electrodes of the *'Impedance Cardiography'* (see description below) to your chest and perform a baseline reading along with a baseline *'ECHO'*, blood pressure and saturations. After this, we will ask you to to exercise on a stationary bike to increase your heart rate and perform the same measurements immediately after you exercise. We would then repeat the same readings after you've rested for 20 minutes. The entire visit shouldn't take more than 60 minutes.

'Impedance Cardiography'

This assessment is painless and not invasive - the electrodes are similar to those used for an *'ECG'*. Once attached, the electrodes will be recording the blood flow in your heart from the time we start, throughout your 6-min walking test and during your rest time. Chaperons will be available.

'ECHOs'

We will perform a simplified *'ECHO'* that will take less time than your usual *'ECHO'*. Scans of your heart are painless and not invasive. You will be lying on your left side and wearing a gown. A doctor or specialist technician (a person specially trained to do heart scans) will apply some gel and a wand-like device called a 'transducer' on your chest. This transducer is able to create images of your heart on a screen next to you. This test takes about 5-10 minutes throughout which the lights will be dimmed so the images are easier to be seen. Chaperons will be available.

Scans of the baby

If your baby is small-for-gestational-age, you will be having regular scans (every 2 to 4 weeks, depending on how small the baby is) and whoever is performing the scan will also look into the blood flow of the baby. We would not organise any extra scans for the purpose of the study, but we would need to look into the blood flow of the baby in more detail. This can add about 5 minutes to your appointment.

Blood Tests/Samples from the placenta

We will try and coincide taking the blood samples required for the study at the same time you would have your routine pregnancy blood taken. However, to participate in this study, you might need some extra blood samples taking. We will be taking 20-25ml (about two tablespoons) of extra blood on each occasion. We will take two small samples from the placenta (about the size of a £2 coin)

What are the disadvantages and risks of participating in this study?

This study requires you to attend the hospital regularly for appointments which you will attend in any case for your antenatal care. Extra time may be required. The blood samples required from you could cause some discomfort and bruising which are however minor. If you wish not to have blood taken, you can still take part in this study. Baby scans, *'ECHOs'* and the *'Impedance Test'* are safe and cause no harm. Taking the placenta will not put you or the baby at any increased risk and does not lengthen the delivery time.

What are the advantages of participating?

There will be no direct benefit to you from participating in this project, however, your participation will help to improve the knowledge of how heart disease affects pregnancy and help improve the care of women.

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Francois Dos Santos, email: f.dos-santos@imperial.ac.uk, mobile: 07771515471). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Should any of your test results be or become abnormal, such as an abnormality during your *ECHO*, we will immediately inform you and refer you to the appropriate specialist for further management.

What happens when the research stops? What happens to the samples taken?

All the information collected on you will be treated as confidential and only the research team will have access to your details. The samples taken will be given a study ID, stored and analysed in a laboratory. Blood samples and samples from the placentas will be stored in the Imperial College London Academic O&G laboratory at Chelsea and Westminster Hospital NHS Trust. Access is restricted to authorised personnel (protected by access card) and the samples will be under the custody of the Chief Investigator. The samples will not be stored for future use in other studies.

What about confidentiality?

All the information collected on you will be treated as confidential and only the research team will have access to your details. Personal data will first be pseudoanonymised by allocating each participant a trial specific ID number. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Electronic data containing participant personal details will be stored on a password protected file on a password protected computer. Hard copies of the documents will be stored in a locked cabinet within the Academic O&G Department at Chelsea and Westminster Hospital NHS Trust. Personal data will be stored in line with *Imperial College London* policy to enable full reconstruction of the study up to 10 years after its completion.

What will happen to the results of the research study?

The intention is to publish the results of the study in peer review journals. The participants will not be identified in any report/publication.

Who is organising and who has reviewed the study?

The project is organised by the Academic Department of Obstetrics and Gynaecology of Chelsea & Westminster Hospital. All research in the NHS is looked at by independent group of people, called the *Health Research Authority (HRA)* and *Research Ethics Committee (REC)* to protect your interests. This study has been reviewed and given favourable opinion by HRA. If you agree to participate, you will be given a copy of the information sheet and a signed consent form to keep. Thank you for considering taking part and taking time to read this sheet.

Further info?

Please contact:

Dr Francois Dos Santos
email: f.dos-santos@imperial.ac.uk
mobile: 07771515471

APPENDIX 11 – Participant Information Sheet: Part 2

Imperial College London

HDiP Heart Disease in Pregnancy Maternal Cardiovascular Adaptation & Fetal Outcomes

Royal Brompton & Harefield NHS Foundation Trust

NHS Chelsea and Westminster Hospital NHS Foundation Trust

Participant Information Sheet

Participation in Part 2

Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation & Fetal Outcomes

Chief Investigator – Dr Francois Dos Santos | IRAS Project ID: 222431 | REC: 17/LO/0970

What is the purpose of this study and why is it important?

This is an education project. Little is known about the later development of babies that were diagnosed with FGR (Fetal Growth Restriction) whilst they were inside the womb. We are looking into the suggestion that FGR in women with pre-existing cardiac disease does not persist as slow growth in childhood nor is it related to any delay in development.

Our aim is to assess the growth and the development of infants in two groups of women:

1 – babies born to women with pre-existing cardiac disease, especially in those with FGR

2 – babies born to women without cardiac disease but whose fetuses were diagnosed with FGR.

In order to do this, we plan to invite you and your child to a follow-up appointment when your child is 22-26 months old and perform a few measurements like height, weight, subscapular skinfold thickness (the thickness of

Why have I been invited? Do I have to take part?

No, you do not have to take part in this study. We are inviting you to take part in this project because when you were pregnant, your baby was found to be measuring small (FGR).

We are very grateful that you have participated in Part 1 and it is completely up to you whether you take part in the second part of the study. Before you sign up, we will describe the process to you and talk you through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

We will contact you when your child is about 18 months old and invite you to attend a follow-up appointment with a paediatric neurologist.

The follow-up appointment will be at the time of your child becoming 2 years old (between 22 and 26 months of age)

What will happen to me if I take part? (continued)

At the appointment, the Paediatrician will make a note of:

- Height
- Weight
- Subscapular skinfold thickness

This is measured to determine the amount of body fat. It is painless and non-invasive. A pinch is made using the natural fold of the skin at the back and a special instrument measures the thickness of that pinch.

- Overall neurodevelopment

The development of the infants will be carried out using the Bayley Scales of infant and Toddler Development, version III - each test consists on a series of developmental play tasks and takes between 45-60 minutes to complete, depending on the child.

What are the disadvantages and risks of participating in this study?

This study requires you to attend the hospital for an extra appointment. The appointment time may vary from 45-60 minutes depending on the child.

What are the advantages of participating?

There will be no direct benefit to you from participating in this project, however, your participation will help to improve the knowledge of how children do after birth if they were small inside the uterus..

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr Francois Dos Santos, email: f.dos-santos@imperial.ac.uk, mobile: 07771515471). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Should any of your test results be or become abnormal, we will immediately inform you and refer you to the appropriate specialist for further management.

What happens when the research stops? What happens to the test results taken?

All the information collected on you will be treated as confidential and only the research team will have access to your details. All the tests taken will be given a study ID, stored and analysed. Personal information is kept in a locked cabinet in an area with restricted access to staff.

What about confidentiality?

All the information collected on you will be treated as confidential and only the research team will have access to your details. Personal data will first be pseudoanonymised by allocating each participant a trial specific ID number. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Electronic data containing participant personal details will be stored on a password protected file on a password protected computer. Hard copies of the documents will be stored in a locked cabinet within the Academic O&G Department at Chelsea and Westminster Hospital NHS Trust. Personal data will be stored in line with *Imperial College London* policy to enable full reconstruction of the study up to 10 years after its completion.

What will happen to the results of the research study?

The intention is to publish the results of the study in peer review journals. The participants will not be identified in any report/publication.

Who is organising and who has reviewed the study?

The project is organised by the Academic Department of Obstetrics and Gynaecology of Chelsea & Westminster Hospital. All research in the NHS is looked at by independent group of people, called the *Health Research Authority (HRA)* to protect your interests. This study has been reviewed and given favourable opinion by HRA. If you agree to participate, you will be given a copy of the information sheet and a signed consent form to keep. Thank you for considering taking part and taking time to read this sheet.

Further info?

Please contact:

Dr Francois Dos Santos
email: f.dos-santos@imperial.ac.uk
mobile: 07771515471

APPENDIX 12 – Consent Form: Part 2

CONSENT FORM – Part 2

HDiP | Heart Disease in Pregnancy

Maternal Cardiovascular Adaptation and Fetal Outcomes

Centre Number:

Study Number:

Participant ID

Name of Researcher: _____ **Please initial box**

1. I confirm that I have read and understand the participant information sheet dated xx of XXX 2016 (Version x.x, REC Ref XXX) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I give consent for me and my child to be seen at the age of 22-26 months and for their height, weight, subscapular skinfold thickness and overall development to be recorded. ☐

Name of Participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
_____	_____	_____

Once completed: 1 copy for participant; 1 copy for researcher site file; 1 (original) to be kept in medical notes

APPENDIX 13 – Invitation Letter

Invitation Letter

HDiP | Heart Disease in Pregnancy Study
Maternal Cardiovascular Adaptation and Fetal Outcomes

Dear (Names),

I am writing to you because you had your baby at Chelsea & Westminster. I would like to invite you to participate in our upcoming research study if you are planning another pregnancy in the next year.

We are running a study entitled *Heart Disease in Pregnancy: Maternal Cardiovascular Adaptation and Fetal Outcomes,* looking at how the heart and blood flow adapt to pregnancy both in normal women and in women with heart disease. Professor Mark Johnson is the lead researcher for this project.

We need to recruit women without any heart disease (women who are usually fit and well) as our 'controls'. We can then compare them with women with heart disease both before they become pregnant and during pregnancy. This will give us a better understanding of how pregnancy affects the heart and the blood flow of pregnant women, and how this differs in women with heart disease.

Our aim is to study women before they fall pregnant and then at different time points during pregnancy. If you are willing to help us, this will take up some of your time, but you will benefit from having a pre-conception appointment (to check your health before you become pregnant) and extra appointments and baby scans during your pregnancy.

Heart disease in pregnancy has remained the leading cause of maternal death in the United Kingdom for the past decade and therefore your contribution to our study would be extremely valuable.

You can find more information about the study on the Participant Information Sheet attached (PIS – Low-risk women, vx.x, xx xxx xx) but feel free to contact me via email or telephone (contact details on the PIS) to discuss this further or to arrange a meeting face-to-face.

Thank you for taking your time to read this letter and we are hoping to hear from you.

Kind Regards,

Francois Dos Santos
Clinical Research Fellow in Obstetric Cardiac Medicine
Chelsea and Westminster Hospital, 369 Fulham Rd, SW10 9NH, London, UK

APPENDIX 14 – Recruitment Poster: Non-SGA



APPENDIX 15 – Recruitment Poster: SGA



APPENDIX 16 – HDiP-MCAFO Data Collection Sheet

HDiP – MCAFO: Data collection sheet

	Pt ID: EDD:
	Date: Current GA:
	Visit: 12/40 A 20/40 B 32/40 C Delivery Second stage Postpartum
	Bloods taken – Time:
	Height (booking):
	Weight (booking):
	Arm circumference:
	Attach physioflow + electrodes for HR with ECHO
	Left lateral:
	BP -
	HR -
	Sats -
	Left lateral:
	Mark start ECHO
	Mark finish ECHO
	Target HR: 220 age x 0.7 - Age:
	Bicycle
	Mark start exercise
	Mark achieved target HR
	Keep target HR for one minute
	Start:
	Finish:
	Record Sats at finished
	Mark stop exercise
	Transfer to bed
	Mark start ECHO
	Mark finish ECHO
	Mark BP
	HR/Sats at finish ECHO
	Time to achieve baseline HR
	Post exercise, L lateral
	Mark start ECHO
	Mark BP
	Mark stop ECHO
	Record HR
	Record Sats

APPENDIX 17 – PhysioFlow® Enduro™ EC Certificate

LNE
Le progrès, une passion à partager

Certification
Médical-Santé

Notified Body N° 0459

ATTESTATION / *CERTIFICATE* **N° 11826 rev. 5**
Délivrée à Paris le 21 décembre 2016
Issued in Paris on December 21st, 2016

ATTESTATION CE / *EC CERTIFICATE*

Approbation du Système Complet d'assurance Qualité/ *Approval of full Quality Assurance System*
ANNEXE II excluant le point 4 Directive 93/42/CEE relative aux dispositifs médicaux
ANNEX II excluding section 4 Directive 93/42/EEC concerning medical devices
Pour les dispositifs de classe III, un certificat CE de conception est requis
For class III devices, a EC design certificate is required

Fabricant / *Manufacturer*

MANATEC
21 rue du Stade - Petit Ebersviller
57730 FOLSCHVILLER FRANCE

Catégorie du(des) dispositif(s) / *Device(s) category*

Dispositifs médicaux de mesures hémodynamiques Non-Invasifs par analyse du signal d'impédance transthoracique.

Non-invasive medical devices for hemodynamic measurement by transthoracic impedance signal analysis.

Voir détails sur addendum / See attachment for additional information

Le LNE/G-MED atteste qu'à l'examen des résultats figurant dans le rapport référencé P110793, le système d'assurance qualité - pour la conception, la production et le contrôle final - des dispositifs médicaux énumérés ci-dessus est conforme aux exigences de l'annexe II excluant le point 4 de la Directive 93/42/CEE.

LNE/G-MED certifies that, on the basis of the results contained in the file referenced P110793, the quality system - for design, manufacturing, and final inspection - of medical devices listed here above complies with the requirements of the Directive 93/42/EEC, annex II excluding section 4

La validité du présent certificat est soumise à une vérification périodique ou imprévue
The validity of the certificate is subject to periodic or unexpected verification

Début de validité / *Effective date :* **December 21st, 2016 (included)**
Valable jusqu'au / *Expiry date :* **October 2nd, 2017 (included)**

Laboratoire national de métrologie et d'essais LNE

On behalf of the Certification Director
Cécile VAUGELADE
G-MED Certification Division Manager

GMED_D2p3-V3-01-2013

LNE - 11826 rev. 5
Modifie le certificat 11826-4

Laboratoire national de métrologie et d'essais • Établissement public à caractère industriel et commercial
LNE/G-MED • Organisme notifié n° 0459
1, rue Gaston Boissier - 75724 Paris Cedex 15 • Tél. : 01 40 43 37 00 • Fax : 01 40 43 37 37 • www.lne.fr • www.gmed.fr

LNE
Le progrès, une passion à partager

Certification
Médical-Santé

Addendum au certificat n° 11826 rev. 5 page 1/1
Addendum of the certificate n° 11826 rev. 5
Dossier / *File* N° P110793

Identification des dispositifs / *Identification of devices*

Désignation du dispositif *Device designation*	**Nom commercial du dispositif ou code article** *Device commercial name or article code*	**Classe du DM** *MD class*
PhysioFLow PF07 Enduro	**PhysioFLow Enduro**	**IIa**
PhysioFLow PF07 Q-Link	**PhysioFLow Q-Link**	**IIa**

Ce certificat couvre les activités et les sites suivants :
This certificate covers the following activities and sites:

- **21 rue du stade - Petit Ebersviller - 57730 FOLSCHVILLER – France**
  Siège social /*Headquarters*
- **44 rue De Laborde - 75008 PARIS – France**
  Commercialisation / *Sales*
- **10 bis rue Jacob Courant - 78300 POISSY – France**
  Conception, fabrication, commercialisation, service /*Design, manufacture, sales, service*

Laboratoire national de métrologie et d'essais • LNE

LNE/G-MED	0459

On behalf of the Certification Director
Cécile VAUGELADE
G-MED Certification Division Manager

Laboratoire national de métrologie et d'essais • Établissement public à caractère industriel et commercial
LNE/G-MED • Organisme notifié n° 0459
1, rue Gaston Boissier - 75724 Paris Cedex 15 • Tél. : 01 40 43 37 00 • Fax : 01 40 43 37 37 • www.lne.fr • www.gmed.fr

APPENDIX 18 - PhysioFlow® Enduro™ EC Declaration of Conformity

Manatec Biomedical
Headquarters :
21, rue du stade Petit Ebersviller ▪ 57730 *FOLSCHVILLER* ▪ France
tel : +33 3 73 82 50 00 ▪ *fax :* +33 1 *30 74 46 48*
info@physioflow.com

EC DECLARATION OF CONFORMITY - DIRECTIVE 93/42/EEC

We, MANATEC 21 RUE DU STADE PETIT-EBERSVILLER 57 730 FOLSCHVILLER FRANCE, hereby certify that the PhysioFlow devices (devices of class IIa according to the rule 10 of the appendix IX of the EC Directive 93/42/EEC, devices whose list can be found in the attached appendix), non-invasive medical devices for hemodynamic measurements by transthoracic impedance signal analysis, are in conformity with the book Vbis L665-4 of the French Public Health Code and the decree 95-292 of March 16th 1995 which concerns those products (decree transposing the EC Directive 93/42/EEC into the French national legislation).

This declaration is based on the following elements:

- Technical files, established in accordance with the appendix II section 3.2(c) and demonstrating the conformity of the product to the standards of the Directive 93/42/EEC and in particular to the Essential Requirements (see reference for each product in attached appendix)
- EC certificate 11826 rev. 5 of approval of the full Quality Assurance System in accordance with the appendix II section 3 of the Directive 93/42/EEC and granted on December 23rd 2016 by the notified body LNE/G-MED, identification number N°0459 (1 rue Gaston Boissier – 75 724 PARIS cedex 15, FRANCE)

In Petit-Ebersviller, December 23rd 2016



Benoît Bosquet – Co-Manager

Manatec Biomedical
Headquarters :
21, rue du stade Petit Ebersviller ▪ 57730 *FOLSCHVILLER* ▪ France
tel : +33 3 73 82 50 00 ▪ *fax :* +33 1 *30 74 46 48*
info@physioflow.com

EC DECLARATION OF CONFORMITY - DIRECTIVE 93/42/EEC

APPENDIX : LIST OF THE PHYSIOFLOW PRODUCTS concerned by the EC Declaration of Conformity

Device designation	Device commercial name or article code	Technical file reference
PhysioFlow PF07 Enduro	PhysioFlow Enduro	PF07 Enduro
PhysioFlow PF07 Q-Link	PhysioFlow Q-Link	PF07 Q-Link

In Petit-Ebersviller, December 23rd 2016

Benoît Bosquet – Co-Manager

APPENDIX 19 – Copyright permissions

Caption /Page No.	Type of work	Name of work	Source of work	Copyright holder	Permission request date	Permission note/ authorisation
Figure 1.1 Page 25	Table	Table 3.4 – Sub-classification of cardiac deaths for whom information was available for an in-depth review, UK and Ireland, 2015-17	Knight M, Bunch K, Tuffnell D, *et al.* MBRRACE-UK- Saving Lives, Improving Mothers' Care - Lessons learned to inform maternity care from the UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2015–17. *Oxford: National Perinatal Epidemiology Unit, University of Oxford*. 2019.	© 2019 Healthcare Quality Improvement Partnership and National Perinatal Epidemiology Unit, University of Oxford	NA	Public domain
Figure 1.2 Page 28	Figure	Figure 3 – Compiled weighted mean and 95% CIs derived from meta-analyses for cardiac output, heart rate (HR), stroke volume (SV), mean arterial pressure (MAP), systemic vascular resistance (SVR) and left ventricular (LV) mass at each gestational age. Coloured bars represent the first, second and third trimester of gestation. NP, non-pregnant; T1, trimester one; T2, trimester two; T3, trimester three; PP, postpartum.	Meah VL, Cockcroft JR, Backx K, Shave R, Stöhr EJ. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. *Heart.* 2016;102(7):518-526.	© 2016 BMJ Publishing Group Ltd and the British Cardiovascular Society	08 Aug 2019	4644231399737
Figure 1.3 Page 31 **Figure 5.1** Page 170	Table	Table 1 – Consensus-based definitions for early and late FGR	Gordijn SJ, Beune IM, Ganzevoort W. Building consensus and standards in fetal growth restriction studies. *Best Pract Res Clin Obstet Gynaecol*. 2018;49:117-126.	© 2018 Elsevier Ltd.	14 Aug 2019	4647720503956

Caption /Page No.	Type of work	Name of work	Source of work	Copyright holder	Permission request date	Permission note/ authorisation
Figure 1.4 Page 41	Figure	Figure 2 – A photograph of the tape-on electrodes in place. It is important to maintain good separation between electrodes 1 and 2. This electrode configuration is used for obtaining 1) cardiac function data and 2) changes in total fluid in the chest by observing Z, (the total impedance between electrodes 2 and 3). The Z, measurement has been found to be a very sensitive indicator of the development or reversal of such conditions as pulmonary edema, pulmonary congestion, and pleural effusion.	Kubicek WG, From AH, Patterson RP, *et al.* Impedance cardiography as a noninvasive means to monitor cardiac function. *J Assoc Adv Med Instrum.* 1970;4(2):79-84.	© 2006 John Wiley and Sons	08 Mar 2020	4784350899257
Figure 1.5 Page 43	Figure	Figure 2 – Impedance cardiography trace with a simultaneously ECG. At level (A) the constitution of the bioimpedance signal ΔZ is shown. These comprise a resistive and reactance component and the geometric sum of both make up the total impedance. At level (B) a standard ECG trace is shown, synchronized to the thoracic impedance dZ (C) and the impedance cardiography signal, dZ/dt (D). CAV: Closure of aortic valve; LVET: Left ventricular ejection time; OAV: Opening of aortic valve; OMV: Opening mitral valve; PEP: Pre-ejection period.	Staelens A, Tomsin K, Grieten L, *et al.* Non-invasive assessment of gestational hemodynamics: benefits and limitations of impedance cardiography versus other techniques. *Expert Rev Med Devices.* 2013;10(6):765-779.	© 2013 Taylor & Francis	18 Aug 2019	Not required for thesis
Figure 1.6 Page 44	Figure	Fig.1 – Waveforms obtained with PhysioFlow®. The *upper trace* is the electrocardiogram (ECG). The *middle* and *bottom* traces are impedance (ΔZ) and its first derivative, dZ/dt, respectively. Thoracic fluid inversion time (TFIT) and dZ/dt_{max} are indicated on the first derivative waveform.	Charloux A, Lonsdorfer-Wolf E, Richard R, *et al.* A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method. *Eur J Appl Physiol.* 2000;82(4):313-320.	© 2000 Springer-Verlag Berlin Heidelberg	14 Aug 2019	4647730261607

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Figure 2.2 Page 71	Figure	Standard electrode position (rest and exercise).	PhysioFlow® Hemodynamics Redefined™ v5.1 19December2017, Manatech Biomedical, France.	©2017 Manatech Biomedical	04 Jul 2019	Authorised by email: 08Jul2019
Figure 2.3 Page 72	Figure	Electrode placement with a pacemaker implanted.	PhysioFlow® Hemodynamics Redefined™ v5.1 19December2017, Manatech Biomedical, France.	©2017 Manatech Biomedical	04 Jul 2019	Authorised by email: 08Jul2019
Figure 2.8 Page 89	Figure	Figure 1. Nomogram for boys. Figure 2 Nomogram for girls.	Altman, DJ & Coles EC. Nomograms for precise determination of birth weight for dates. *Br J Obstet Gynaeco.*1980; 87(2):81-86.	©2005 John Wiley and Sons	15 May 2020	4830410498207