THE UNIVERSITY *of* EDINBURGH

Adjunctive technologies for intrapartum fetal monitoring: current perspectives and proof of concept for a novel approach

Andrew Patrick Brown

Thesis for the degree of Doctor of Medicine

The University of Edinburgh

2022

Declaration

I declare that this thesis is a presentation of my own original research. Where others have contributed to the work presented here, specifically data collection and analysis, I have made every effort to indicate this clearly. I have not submitted this work in candidature for any other degree or professional qualification.

Andrew Patrick Brown

December 2022

Acknowledgements

This thesis is dedicated to my primary supervisor and mentor, Professor Fiona Denison, who sadly passed away in January 2022. Ever patient, Fiona continued to support and encourage me in completing this research throughout her long battle with ill health. She was an inspiration to me, and so many others, in both her academic and clinical roles, with a passion for translational obstetrics and inter-disciplinary collaboration which was truly unique. It is my hope that this thesis is true to that passion and the work presented here may contribute some small part to her far-reaching legacy in improving maternal and fetal health.

I would like to thank my other supervisors for their tireless support through this project: Dr Michael Crichton and Professor Marc Desmulliez of Heriot-Watt University, for welcoming me into their engineering world and their practical guidance from conception to thesis submission; Professor Julia Lawton, for her wisdom and patience at every stage of the qualitative study; and Professor Rebecca Reynolds, for her invaluable support during the latter stages of this research and thesis.

I am forever indebted to Professor Ed Clutton and the team at the Large Animal Research and Imaging Facility (previously WTCCLLA), who made so much of this research a reality through their dedication, knowledge and skills. And to the piglets, without whom there would be no data.

The qualitative study would not have been possible without the enthusiasm of so many colleagues and members of the British Intrapartum Care Society and UK-ARCOG, who willingly gave their valuable time to be interviewed. Likewise, my heartfelt thanks to Tommy's Charity for funding much of the work presented in this thesis.

A special thanks to Dr Sarah Caughey, for her companionship and incredibly hard work during the piglet studies and processing of microdialysis data, as well as patient instruction in all things laboratory related. And to the rest of my fellow students and wonderful colleagues at the QMRI and Simpsons – Lizzie, Emma, Alex, Elly, Sara VB, Sarah M – all of whom made this journey worthwhile.

I am incredibly grateful to my father-in-law, Jim, for providing a home from home in Edinburgh when needed, and to my parents, Alan and Janette, for believing in me even when I didn’t.

Lastly, thank you to Katie, Isobel and Charlie – for everything that matters most.

Abstract

Fetal monitoring is a recurring theme in perinatal morbidity and mortality reports, highlighting the limitations of cardiotocography and current adjunctive technologies, such as fetal blood sampling (FBS). There is an unmet need for more robust methods of intrapartum fetal assessment. Microdialysis may help to detect babies at risk of hypoxia by monitoring trends in lactate and related metabolites from fetal scalp interstitial fluid in a minimally invasive manner. However, its clinical value remains unproven because there is limited evidence on the relationship between interstitial and arterial lactate. Translating advances in fetal monitoring technology into improved clinical outcomes also depends on how obstetricians use such technology in their practice, which few past studies have explored in depth.

This research comprised two components. The first part aimed (1) to develop a neonatal piglet model of hyperlactataemia; and, using this model, (2) to investigate the relationship between interstitial and arterial lactate; and (3) to explore the feasibility of using subcutaneous microdialysis to monitor the metabolic response to hypoxia *in vivo.* Eight neonatal piglets were monitored under non-recovery general anaesthesia. Hyperlactataemia was achieved by means of alveolar hypoxia and/or intravenous sodium L-lactate infusion, with target lactate concentrations above 12 mmol/L. Microdialysate from two subcutaneous microdialysis catheters inserted into the scalp of each piglet was analysed for interstitial lactate, pyruvate, glucose and glutamate concentrations, which were compared to arterial blood gas measurements. A subset of dialysate samples underwent secondary analyses with the StatStrip Xpress® point-of-care lactate meter to assess its performance.

In total, 432 dialysate samples were collected from seven piglets. There was variation in the piglets' response to hypoxia therefore two piglets received lactate infusions, with four overall achieving target hyperlactataemia. Interstitial lactate, pyruvate and glucose concentrations were not affected by microdialysis catheter insertion. There was a strong positive correlation between arterial lactate and interstitial lactate, and weaker positive correlations with interstitial lactate-to-pyruvate and lactate-to-glucose ratios. Interstitial lactate mirrored trends in arterial lactate with an approximate time lag of 10

to 20 min, although the closeness of agreement varied between piglets. StatStrip Xpress® lactate values showed a proportional negative bias relative to the reference microdialysis analyser, but trend data and assay precision were comparable.

The second part of this research sought to understand how UK obstetricians use adjunctive fetal monitoring technologies and what factors influence their practice, as well as exploring attitudes towards new technology and other areas for improving practice. Data were collected through semi-structured telephone interviews with 16 obstetricians of varying career grade from nine maternity units across the UK, prior to thematic analysis. Most obstetricians reported performing FBS but attitudes towards it varied. The use of fetal monitoring technology was influenced by obstetricians' individual clinical autonomy, the socio-cultural norms of their unit, and wider external factors, such as guidelines. Obstetricians recognised the limitations of current methods of monitoring, but enthusiasm towards new technology was checked by a scepticism of 'computerisation' and perceived barriers to changing practice; hence, better staff training was seen as the immediate priority for improving outcomes.

In summary, the work presented in this thesis provides new insight into the current role of adjunctive technologies in UK obstetric practice and demonstrates proof of concept for subcutaneous microdialysis as a novel approach to monitoring metabolic wellbeing in the fetus and neonate. Although interstitial lactate reflected trends in arterial lactate in response to hypoxia and lactate infusion in neonatal piglets, further research is required to fully characterise this relationship, including standardisation of the hyperlactataemia model described here.

This research has also identified a range of individual and contextual factors that influence how obstetricians use fetal monitoring technology and highlights the urgent need for future qualitative studies to improve understanding of this complex process, alongside efforts to develop new technology.

Lay summary

Babies' heart rates are monitored during labour to check their wellbeing. An additional test, known as fetal blood sampling, is used in some babies. This involves taking blood from the scalp to check for high lactate or low pH levels, which indicate that the baby is not getting enough oxygen. Unfortunately, problems with these tests are common and can lead to babies being harmed or dying during labour.

Checking lactate levels from the interstitial fluid layer just underneath the skin, rather than blood, may allow doctors to detect babies becoming short of oxygen earlier and deliver them before harm occurs. This could be done by inserting a small flexible 'microdialysis' probe into a baby's scalp during labour. However, it is not known if lactate levels in the interstitial fluid respond to low oxygen supplies in the same way as blood lactate levels. It is also important to understand how doctors use different tests for checking babies' wellbeing in their everyday practice, and to identify potential barriers to introducing a new test.

The first part of this research investigated the relationship between lactate levels in the interstitial fluid and blood, and whether it may be possible to use microdialysis to check babies' wellbeing during labour. In newborn piglets, which are similar in development to human babies, high blood lactate levels were achieved by reducing their oxygen supply and/or giving concentrated lactate infusions. Levels of lactate and three other substances (glutamate, pyruvate, and glucose) in the interstitial fluid were measured every 10 minutes from two microdialysis probes in the scalp of each piglet.

Results for seven piglets were included. Inserting the probes did not appear to affect the levels of lactate, pyruvate or glucose in the interstitial fluid. It was difficult to predict the response of piglets to low oxygen, so lactate infusions were given in two piglets. Four of seven piglets achieved very high blood lactate levels as aimed. Overall, there was strong agreement between lactate levels in the interstitial fluid and blood. Interstitial lactate tracked trends in blood lactate with a time lag of around 10 to 20 minutes, but the closeness of this agreement varied between piglets. Combining interstitial lactate with other measurements did not improve agreement with blood lactate. Some microdialysis samples were also tested on a handheld lactate meter, the

StatStrip Xpress®, to check its performance. These measurements were lower than those seen on the reference analyser, but information on interstitial lactate trends was similar. This may offer a low-cost approach to using microdialysis in future studies.

The second part of this research was based on interviews with 16 doctors from maternity units across the UK. The interviews explored how and why doctors use existing tests for checking babies' wellbeing during labour, including fetal blood sampling, as well as their views on the development of new technologies. Most doctors described performing fetal blood sampling but their attitudes varied, and many had conflicted feelings about its value. The way in which doctors used fetal blood sampling was influenced by their own clinical experience and independence, as well as how the tests were typically used in each unit, and various outside factors, such as guidelines. Doctors saw staff training as the main priority for improving outcomes in the future. Although they recognised a need for better methods to check babies' wellbeing during labour, some raised concerns about the role of complex technology and the difficulties of changing practice in the NHS.

The findings of this research show that microdialysis of the scalp may offer a promising new approach to checking babies' wellbeing around the time of birth. In newborn piglets, lactate levels in the interstitial fluid followed trends in blood lactate when they were deprived of oxygen or given lactate infusions. However, further studies are needed to better understand this relationship, including any time lag between changes in the interstitial fluid and blood, and to design a microdialysis device which could be used in babies during labour. Research into developing and evaluating a new device should also consider the views of doctors and the environment in which they practice, as these factors may influence how such tests are used in real life and whether they lead to better outcomes.

Table of contents

List of figures

List of tables

List of abbreviations

Abbreviation	Full definition
ACCORD	Academic and Clinical Central Office for Research and Development
ACOG	American College of Obstetricians and Gynecologists
ACT	activated clotting time
ADP	adenosine diphosphate
ANOVA	analysis of variance
ARRIVE	Animal Research: Reporting *In Vivo* Experiments
ART	arterial
ATP	adenosine triphosphate
BD	base deficit
BE	base excess
c (prefix)	calculated
CaO_2	arterial oxygen content
CEFM	continuous electronic fetal monitoring
CGM	continuous glucose monitoring
CO_2	carbon dioxide
CoA	coenzyme A
COREQ	consolidated criteria for reporting qualitative research
CP	cerebral palsy
CS	caesarean section
CTG	cardiotocography
CV	coefficient of variation
DoH	Department of Health
ECF	extracellular fluid
ECG	electrocardiogram
ETI	endo-tracheal intubation
FBS	fetal blood sampling
FDA	Food and Drug Administration
FHR	fetal heart rate

FIGO	International Federation of Gynecology and Obstetrics
FiO_2	fraction of inspired oxygen
FSE	fetal scalp electrode
FSL	fetal scalp lactate
LDH	lactate dehydrogenase
L/G	lactate:glucose
L/P	lactate:pyruvate
H^+	hydrogen
Hb	haemoglobin
HCO_3-	bicarbonate
HI	hypoxia-ischaemia
HIE	hypoxic-ischaemic encephalopathy
HR	heart rate
IA	intermittent auscultation
ISF	interstitial fluid
IQR	interquartile range
MA	metabolic acidosis
MAP	mean arterial pressure
MBRRACE-UK	Mothers and Babies: Reducing Risk through Audit and Confidential Enquiries across the UK
MD	microdialysis
MDT	multidisciplinary
NAD(+/H)	nicotinamide adenine dinucleotide
NE	neonatal encephalopathy
NHS	National Health Service
NHSLA	National Health Service Litigation Authority
NICE	National Institute for Health and Care Excellence
NPV	negative predictive value
O_2	oxygen
P	partial pressure (*P*a = arterial partial pressure)
PIS	participant information sheet
POC	point-of-care
PPV	positive predictive value

PREPARE	Planning Research and Experimental Procedures on Animals: Recommendations for Excellence
PROMPT	PRactical Obstetric Multi-Professional Training
RCT	randomised controlled trial
RCM	Royal College of Midwives
RCOG	Royal College of Obstetricians and Gynaecologists
RR	relative recovery
SAS	specialty and associate specialist
SAT	subcutaneous adipose tissue
SD	standard deviation
SO_2	oxygen saturation
ST	specialty trainee
STAN	ST waveform analysis
tc (prefix)	temperature-corrected
UCBGA	umbilical cord blood gas analysis
UK	United Kingdom of Great Britain and Northern Ireland
UK-ARCOG	UK Audit and Research Collaborative in Obstetrics and Gynaecology
USA	United States of America

Chapter 1 Introduction

1.1 Monitoring fetal wellbeing during labour

1.1.1 Clinical burden of intrapartum hypoxia-ischaemia

Every year in the UK, over 1100 term babies die or are left severely disabled because of incidents that occur around the time of birth (Royal College of Obstetricians and Gynaecologists [RCOG], 2018). The causal pathways leading to such tragedies are complex and multifactorial. Predisposing intrauterine factors and events in the neonatal period may play an important role, but labour and birth are times of particular risk due to the metabolic demands placed on the fetus (Yli and Kjellmer, 2016). Intrapartum hypoxia-ischaemia (HI) represents a failure of the fetus to meet those demands through an inadequate supply of oxygen (hypoxia) and/or blood (ischaemia) to critical organs. Despite major advances in obstetric and perinatal care in the past half century, it remains an important global cause of morbidity and mortality (Lee et al., 2013).

The consequences of intrapartum HI range from transient depression of the newborn at birth, indicated by low Apgar scores (American College of Obstetricians and Gynecologists [ACOG], 2015); to disturbances of neurological function in the first days of life, known as neonatal encephalopathy; to long-term cognitive, behavioural and developmental problems, such as cerebral palsy (ACOG, 2014). Measuring these outcomes and attributing causation to intrapartum events is a complicated task, especially in the presence of prematurity or other comorbidities. The terms ‘birth asphyxia’ and ‘perinatal asphyxia’ reflected the historical assumption that most neonatal encephalopathy was intrapartum in origin (Nelson and Leviton, 1991). However, it is now estimated that only 30% of cases in developed countries are associated with evidence of intrapartum HI. Clinically, this is termed hypoxic-ischaemic encephalopathy (HIE), the incidence of which is approximately 2 per 1000 live births (Graham et al., 2008, Kurinczuk et al., 2010). Similarly, the majority of cerebral palsy cannot be attributed to intrapartum hypoxia (Blair and Stanley, 1988, Graham et al.,

2008, Himmelmann and Uvebrant, 2018). Intrapartum-related cerebral palsy, nevertheless, comes at enormous human and financial costs. Cerebral palsy and neonatal brain damage claims, totalling £1.9 billion, accounted for almost half the value of all clinical negligence claims received by NHS Resolution in 2016-2017 (Magro, 2017). Individual claims can exceed £20 million, and this figure is only expected to rise in the future. Reviews of obstetric litigation spanning three decades have identified errors in monitoring fetal wellbeing as a contributing factor in around two thirds of these claims (Symonds and Senior, 1991, NHS Litigation Authority [NHSLA], 2012), a finding echoed in recent perinatal Confidential Enquiries (Draper et al., 2017) and the RCOG's Each Baby Counts reports (RCOG, 2017).

As a result, intrapartum fetal monitoring has been a focus of the national maternity and neonatal health strategy for many years (King's Fund, 2008). This was brought to the fore by the UK Government's mandate to NHS England to halve the number of intrapartum-related stillbirths, neonatal deaths and brain injuries by 2025 (Department of Health [DoH], 2017). Unfortunately, substandard fetal monitoring remains one of the major contributors to potentially avoidable harm and death during labour in the UK (RCOG, 2017). If anything, the limitations of existing fetal monitoring technology in the prevention of intrapartum HI have become clearer than ever (Clark et al., 2017).

This chapter begins by addressing key concepts of fetal physiology and metabolism relevant to the pathophysiology of intrapartum HI injury and outlining the main goals and principles of intrapartum fetal monitoring.

1.1.2 Physiology of fetal oxygenation

All human cells require oxygen and glucose for aerobic metabolism, their main source of energy production. Glucose can be stored as glycogen and therefore mobilised when needed, however the fetal oxygen supply is dependent on dynamic interactions between maternal respiration and circulation, placental perfusion, gas exchange across the placenta, and the umbilical and fetal circulations (Ayres-de-Campos et al., 2015a). Disruption to any of these processes may result in a reduced oxygen concentration in the fetal blood (hypoxaemia) and ultimately in the tissues (hypoxia). Hypoxia may also result from an inadequate supply of blood to the tissues, known as

ischaemia. When hypoxia occurs, energy production can be maintained for a limited time by anaerobic metabolism before the onset of cellular damage and its sequelae.

1.1.2.1 Fetal metabolic pathways

Glycolysis is the initial step in fetal metabolism and involves the conversion of glucose into pyruvate and adenosine triphosphate (ATP), the principal unit of intracellular energy transfer. If oxygen (O_2) is readily available, pyruvate is converted to acetyl coenzyme A (acetyl CoA), which enters the citric acid cycle to complete the oxidation of glucose. This process produces large amounts of energy, mainly through oxidative phosphorylation, in addition to waste carbon dioxide (CO_2) and water. Aerobic metabolism theoretically yields 38 ATP molecules for the oxidation of one glucose molecule in this manner (Yli and Kjellmer, 2016).

When oxygen supplies are insufficient, the fetus must switch from aerobic to anaerobic metabolism. Under such conditions, pyruvate is reduced to lactate via lactate dehydrogenase (LDH). This reaction regenerates the oxidised form of nicotinamide adenine dinucleotide (NAD^+), which is essential for glycolysis to continue:

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \leftrightarrow \text{lactate} + \text{NAD}^+$$

Anaerobic glycolysis is capable of making ATP faster than oxidative phosphorylation but is highly inefficient, yielding only two ATP per molecule of glucose. Unless oxygen supplies are restored, this quickly leads to the depletion of glycogen stores and accumulation of intracellular lactate, which disperses into the extracellular fluid and fetal circulation. The blood lactate-to-pyruvate (L/P) ratio is normally 10-16:1 but rises as the ratio of NADH:NAD^+ (redox state) increases under anaerobic conditions (Kraut and Madias, 2014, Yli and Kjellmer, 2016).

Lactate produced through anaerobic glycolysis is accompanied by a net release of hydrogen ions (H^+), hence the association between elevated blood lactates levels (hyperlactataemia) and metabolic acidosis (MA), which describes increased H^+ concentrations in the tissues (Kraut and Madias, 2004). Excess H^+ are neutralised by circulating bases, including bicarbonate (HCO_3-), haemoglobin (Hb) and plasma

proteins, until the fetal buffering capacity is exhausted, as reflected by an increasing base deficit (BD) and decreasing pH (Blechner, 1993). Continued production of H^+ in these circumstances will impair cellular functioning and lead to tissue injury, as discussed below. The aerobic and anaerobic pathways for ATP production are shown in Figure 1.1.

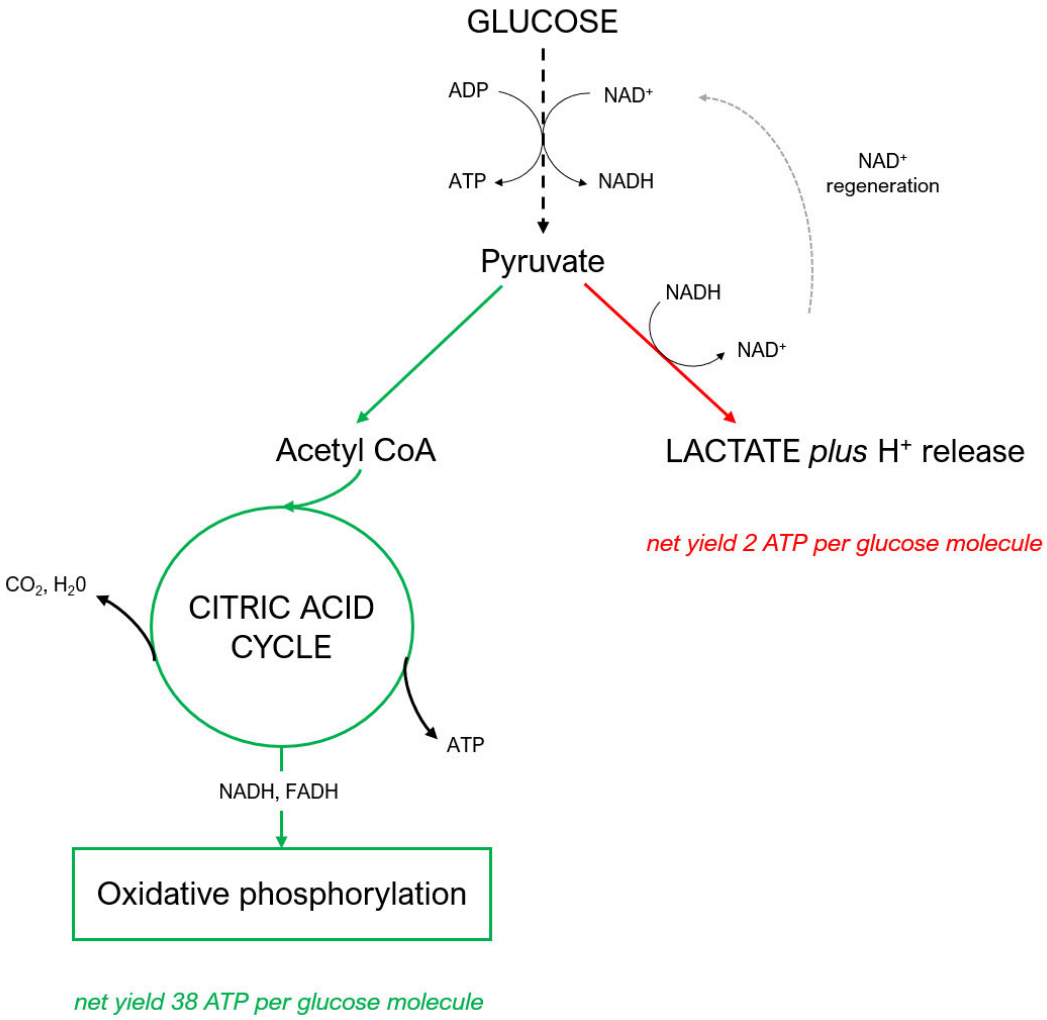

Figure 1.1 Fetal metabolic pathways. *Aerobic (green) and anaerobic (red) pathways for energy production in the fetus.*

1.1.2.2 Intrapartum physiology

In the healthy fetus under normal conditions, the feto-placental circulation ensures adequate oxygen supplies to maintain aerobic metabolism and balance lactate production and consumption. Diffusion of oxygen and CO_2 between maternal and fetal

blood in the intervillous space of the placenta occurs quickly. Oxygenated blood travels to the fetus via the umbilical vein and is preferentially delivered to the fetal myocardium and brain through several circulatory adaptations, including the presence of intra- and extra-cardiac shunts (Baschat, 2006). The two umbilical arteries then return de-oxygenated blood and waste products from the fetus to the placenta (Yli and Kjellmer, 2016). Umbilical arterial blood therefore most accurately reflects the fetal or neonatal condition, whereas umbilical venous blood also depends on maternal acid-base status and placental function.

During labour, uterine contractions reduce uteroplacental perfusion and maternal-fetal gas exchange. Compression of the umbilical cord between fetal parts and/or the uterine wall can also temporarily interrupt the fetal circulation. The frequency, duration and intensity of these contractions, as well as the interval between them, largely determine the fetus' ability to withstand the effects of labour (Ayres-de-Campos et al., 2015a). The majority of appropriately grown, healthy, term fetuses can cope with a reduction in arterial oxygen levels of up to 50% because of their myocardial glycogen stores and other protective adaptations (Lear at el., 2018; Turner et al., 2020). These ensure that short periods of hypoxia and anaerobic metabolism, as occurs during normal labour, are relatively well tolerated. As gas exchange is restored between uterine contractions and oxygen becomes available again, lactate can be metabolised efficiently with rapid reversal of any acidosis that occurred during the preceding contraction. Nevertheless, this repeated impairment of gas exchange results in a small decrease in arterial oxygen levels and pH, and an increase in arterial CO_2 and lactate, even in uncomplicated labours (Blechner, 1993, Yli and Kjellmer, 2016).

1.1.2.3 Pathogenesis of hypoxic-ischaemic injury

When there is not enough time between contractions for reperfusion of critical organs, or when poor placental function precludes adequate gas exchange during this interval, the fetus will eventually decompensate as anaerobic reserves are exhausted (Turner et al., 2020). This results in impaired myocardial contractility and a failure to maintain cardiac output in response to further episodes of hypoxia. Progressive intermittent hypotension and cerebral hypoperfusion ensues, which represent the hallmark of evolving intrapartum fetal compromise (Lear et al., 2018). The fetal brain is particularly

sensitive to hypoxic injury and oxidative stress because of its high metabolic rate and lack of glycogen stores. Extensive research in human infants and animal models has sought to characterise the neuropathophysiology of intrapartum hypoxic-ischaemic injury, but a discussion of these mechanisms is beyond the scope of this thesis. Clinically, this cascade of events manifests in characteristic patterns of short-term neurological dysfunction, or HIE. Although there is no universally accepted definition, the diagnosis of HIE generally requires confirmation of MA in umbilical arterial cord blood, low Apgar scores, early imaging evidence of cerebral oedema, and the appearance of abnormal muscular tone or sucking movements, seizures and/or coma in the first 48 hours of life (Low, 1997, ACOG, 2014).

1.1.3 Goals of intrapartum fetal monitoring

The main goal of intrapartum fetal monitoring is to detect potential fetal compromise thereby allowing timely and effective action to prevent perinatal morbidity or mortality. Such action may range from a simple change of maternal position, to steps aimed at reducing uterine contractions, to urgent delivery of the fetus by emergency caesarean section (CS). It is important to note that the above discussion on the pathophysiology of HIE has focused on repeated periods of intermittent hypoxia leading to a gradual decompensation of the fetus, which often may be corrected by conservative measures. However, intrapartum monitoring must also detect sentinel hypoxic or ischaemic events that can rapidly compromise fetal wellbeing and would typically merit immediate operative delivery, such as uterine rupture, placental abruption, and umbilical cord prolapse (Ayres-de-Campos et al., 2015a). At the same time, monitoring should provide reassurance in fetuses where oxygenation is adequate to avoid unnecessary obstetric intervention. This is more important than ever as rates of CS continue to rise worldwide (Vogel et al., 2015).

1.1.3.1 Markers for detecting fetal hypoxia

Although the fetal brain is the primary organ of interest in the prevention of HI injury, direct measurement of cerebral perfusion and oxygenation is currently not feasible. Indeed, the only way of objectively quantifying the presence of hypoxia and/or acidosis

affecting critical organs immediately prior to birth is through blood gas and lactate analysis from the umbilical cord or neonatal circulation within the first few minutes of life. Umbilical cord blood gas analysis therefore remains the gold standard for diagnosing intrapartum fetal hypoxia and represents a crucial outcome measure in obstetrics (Thorp et al., 1996, Jonsson et al., 2009, Kitlinski et al., 2003, Armstrong and Stenson, 2007). Ideally, this should be undertaken as soon as possible after delivery by drawing blood from the umbilical artery and vein into two different pre-heparinised syringes (Ayres de Campos et al., 2015a, Armstrong and Stenson, 2007). Umbilical arterial blood best reflects the fetal acid-base status, as previously discussed, however sampling from both vessels ensures that a valid arterial sample has been obtained; very low arterio-venous differences in pH and CO_2 partial pressure (PCO_2) most likely indicate mixed or erroneous sampling (Wiberg et al., 2010, Skiöld et al., 2017). In addition to pH and PCO_2, umbilical cord blood gas analysis yields derived values for HCO_3- and BD. Lactate measurement may also be provided by some blood gas analysers or undertaken separately with a handheld, point-of-care (POC) meter. These parameters are used, as follows, to evaluate the presence of fetal hypoxia and MA.

1.1.3.1.1 pH

The normal arterial pH of a healthy fetus is about 7.35, however there is a physiological decrease during labour such that the mean pH in singleton, term infants following uncomplicated delivery is 7.25 (Skiöld et al., 2015). This mild respiratory or mixed acidaemia primarily reflects hypercapnia from impaired placental gas exchange during uterine contractions and is not linked to adverse neonatal outcomes (Wiberg et al., 2006a). As pH progressively falls, the acidaemia is more likely to be metabolic in origin, with thresholds of 7.00 or 7.05 being commonly used to define pathological acidaemia (Low et al., 1997, ACOG, 2014, Ayres de Campos et al., 2015a).

1.1.3.1.2 Base deficit

The use of base excess (BE) was originally proposed by Siggaard-Andersen (1971) as a measure of the metabolic component of acid-base status. At birth, most BE values are negative therefore the term base deficit is used synonymously. BD is an artificial measure calculated either in the extracellular fluid (ECF) or blood. As H^+ ions are

produced in the tissues and the fetus has a relatively large ECF compartment, BD [ecf] is preferred for assessing fetal acid-base status (Olofsson, 2015). Values ≥ 12 mmol/L are generally accepted to reflect severe MA (Low et al., 1997, ACOG, 2014). However, it is important to note that BD is not an independent parameter, but calculated from pH and $PaCO_2$, and that blood gas analysers may use different algorithms for this calculation, so affecting the apparent prevalence of MA (Mokarami et al., 2012).

1.1.3.1.3 Lactate

Lactate, the major end-product of anaerobic metabolism, offers an alternative, theoretically independent measure of oxygen debt to pH or BD. One important advantage of using umbilical arterial lactate to evaluate the metabolic component of acidosis is that it can be directly measured, unlike BD. Several independent studies have investigated its utility in recent decades and have repeatedly shown it to correlate with pH and BD, as summarised in a recent systematic review (Allanson et al., 2017). However, reported mean values for umbilical arterial lactate vary widely in the literature, even within study populations limited to normal deliveries (Wiberg et al., 2008). Establishing a reference range and optimal cut-off for diagnosing MA is further complicated by the use of different lactate assays, which is addressed in Section 1.2.1.

1.1.3.2 Umbilical cord values in relation to neonatal outcomes

In addition to providing information on the fetal metabolic condition immediately prior to birth, blood gas and lactate analysis from the umbilical cord helps clinicians to identify those newborns at risk of short and long-term morbidity and mortality. Several large observational studies have shown that the risk of adverse neurological outcomes rises with progressive levels of acidaemia (Victory et al., 2004, Wiberg et al., 2010, Yeh et al., 2012, Tuuli et al., 2014). In a cohort of 51 159 umbilical cord blood samples, newborns with an arterial pH below 7.00 carried an 18-fold increased risk of encephalopathy with seizures and/or death compared to those with an ‘ideal’ pH of 7.26-7.30 (Yeh et al., 2012). The risk of an Apgar score below 7 at 5 min was increased nearly 50-fold in the severely acidaemic group. Georgieva et al. (2013) used a subset of the same data to generate ‘Event Rate Estimate’ plots, which provide a clear picture

of absolute neonatal risks across the distribution of pH values. The risk of severe complications, such as seizures and/or death, only increased significantly at pH values below 7.00 and the event rate remained under 2% for all but the most acidaemic newborns. However, milder degrees of acidaemia were associated with higher rates of low Apgar scores and neonatal resuscitation, as has been reported elsewhere (Bailey et al., 2019).

Understanding this continuum is important for clinicians who must balance the risks of neonatal injury against those of obstetric intervention. However, the challenge remains to define thresholds for clinical practice which are both sensitive and specific for the outcomes of interest. The majority of newborns with MA, based on the commonly accepted definition of an umbilical arterial pH below 7.00 and BD above 12.0 mmol/L (ACOG, 2014), will be neurologically normal. Similarly, most infants with adverse short- or long-term outcomes are not born acidotic.

The performance of umbilical arterial lactate in predicting low Apgar scores and neurological morbidity, such as HIE, is comparable to pH and BD. A meta-analysis including over 38 000 participants showed that, using a threshold of > 3.21 mmol/L, lactate had a sensitivity of 0.697 and specificity of 0.93 for the combined neurological adverse outcome (Allanson et al., 2017). The strong correlation between lactate and pH or BD suggests it may be used as a supplement at umbilical cord blood gas analysis or as the primary acid-base parameter. This may be particularly appealing in resource-constrained settings where lactate can be measured using a handheld meter, which requires less blood and is cheaper to maintain than a blood gas analyser.

In summary, umbilical cord blood gas analysis is the gold standard for diagnosing intrapartum fetal hypoxia based on the measurement of umbilical arterial pH, BD and/or lactate. However, at present this diagnosis can only be applied retrospectively after birth and, even in this context, is of limited value in the prediction of hard clinical endpoints. Umbilical cord blood gas analysis nevertheless serves as an important outcome measure and a reference against which different methods of intrapartum fetal monitoring may be compared.

1.1.4 Fetal heart rate monitoring

Without the ability to evaluate umbilical arterial acid-base status during labour, surrogate markers of hypoxia must be used to assess fetal wellbeing. Continuous electronic fetal monitoring (CEFM) refers to the interpretation of fetal heart rate patterns acquired through cardiotocography (CTG) and is the mainstay of current practice. CTG graphically records the fetal heart rate and uterine contractions (*kardia* meaning heart, *tokos* meaning labour) by means of ultrasound transducers placed on the maternal abdomen (Ayres-de-Campos et al., 2015b). Although the terms CEFM and CTG are often used interchangeably, CTG will be used for clarity in this thesis.

The fetal heart rate is controlled through the autonomic and somatic components of the central nervous system, hence a normal CTG should reflect a healthy, well-oxygenated fetal brain. Conversely, changes in key features of the fetal heart rate – baseline rate, baseline variability, accelerations and decelerations – during labour may indicate a fetus that is decompensating and at risk of hypoxic injury without timely intervention (Gracia-Perez-Bonfils and Chandraharan, 2017). While a comprehensive review of the interpretation and classification of CTG tracings is not presented here, examples of normal and abnormal CTGs are shown in Figure 1.2.

CTG was introduced widely into clinical practice in the 1970s and its use, both in developed and many developing countries (Housseine et al., 2018), has risen steadily since despite a lack of evidence from randomised controlled trials (RCTs). It is currently recommended in all major guidelines for women at high risk of labour complications (ACOG, 2009, Ayres-de-Campos et al., 2015b, National Institute for Health and Care Excellence [NICE], 2017). In the UK and similar populations, where maternal comorbidities, induction of labour and regional analgesia are increasingly common, continuous CTG is therefore offered to a large proportion of women planning vaginal birth.

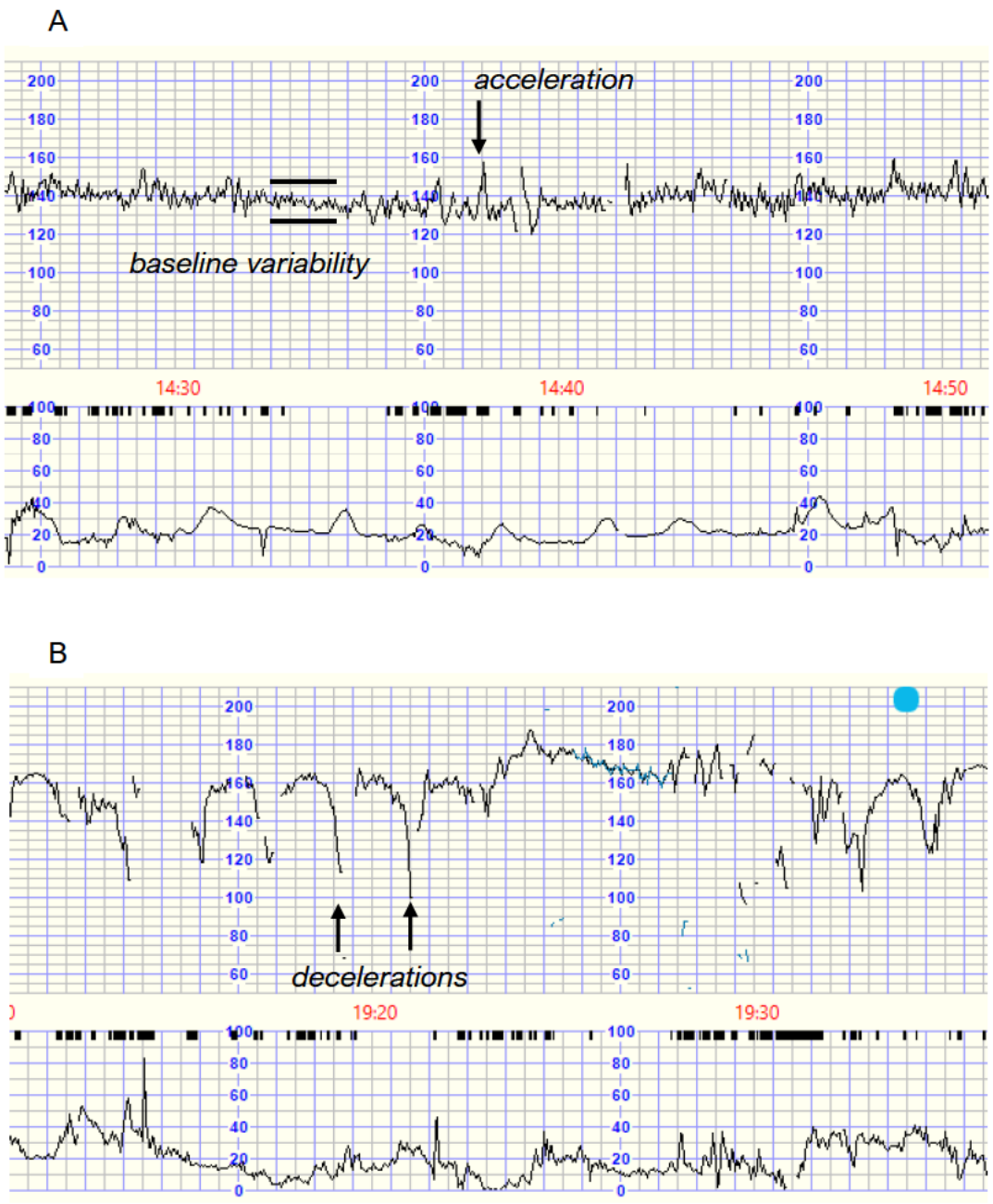

Figure 1.2 CTG examples. *(A) Normal CTG with stable baseline fetal heart rate, good baseline variability and accelerations. (B) Abnormal CTG showing features of hypoxic stress, with rising baseline rate, reduced baseline variability, and repetitive decelerations.*

1.1.4.1 Limitations

Despite its almost ubiquitous use, CTG has well-documented limitations. It has a low positive predictive value (PPV) of approximately 30% (Pinas and Chandraharan, 2016), meaning that many fetuses with non-reassuring heart rate patterns will not have clinically important acidosis (Ayres-de-Campos et al., 2015b). The predictive value for

serious adverse outcomes, such as HIE, is even lower given the rarity of these events. No single feature, category, or multivariate model of fetal heart rate patterns has been demonstrated to achieve both high sensitivity and specificity (Reynolds et al., 2022). Consequently, false positive cases of suspected fetal distress contributed to an exponential rise in operative vaginal deliveries and emergency CS when CTG was first introduced into practice. CTG is also subject to considerable inter- and intra-observer variability, particularly in the classification of decelerations, baseline variability and non-reassuring tracings (Rhöse et al., 2014, Ayres-de-Campos et al., 2011). Several guidelines have been published with the aim of standardising interpretation, most notably those of the NICE, ACOG and the International Federation of Gynecology and Obstetrics (FIGO). These are all based on a three-tiered classification system ranging from normal (category I) tracings, where no action is suggested, to suspicious (category II) and pathological (III) tracings, the latter requiring urgent intervention. However, the guidelines themselves present a source of disagreement and debate. The distribution and reliability of CTG classifications, as well as the sensitivity and specificity of pathological/category III tracings for predicting acidosis, vary significantly according to the guideline used (Santo et al., 2017). As a result, several authors argue for a more physiological approach to CTG interpretation, with less focus placed on the morphological appearance of decelerations and normal reference ranges for fetal heart baseline rate and variability (Ugwumadu, 2014, Chandraharan, 2017). A number of NHS trusts have now adopted peer-reviewed guidelines on physiological CTG interpretation (personal communication; Physiological CTG Interpretation, 2018), however, high-quality, prospective data in support of this approach is currently lacking.

In the UK, development and revision of the NICE intrapartum care guidelines has been complemented by concerted efforts to standardise staff training and competency assessment in CTG interpretation (Royal College of Midwives [RCM], 2017, NHS England, 2019). While there is some evidence that such training improves inter-observer agreement and intrapartum CTG management, the clinical impact remains unclear (Pehrson et al., 2011, Kelly et al., 2021). At a national level, substandard care related to CTG monitoring is a theme that persists in the most recent perinatal Confidential Enquiries (Draper et al., 2017) and Each Baby Counts reports (RCOG, 2018). The subjectivity of CTG interpretation and its low specificity for predicting fetal

acidosis must therefore be borne in mind when applying this technology to monitor fetal wellbeing during labour.

1.1.4.2 Fetal scalp electrodes

Monitoring the fetal heart rate through an external transducer can be difficult, for example in cases of maternal obesity or multiple pregnancy. A fetal scalp electrode (FSE) can be applied directly to the fetal scalp in such instances to ensure a reliable fetal heart rate signal. To permit FSE application during digital vaginal examination, the membranes must be ruptured and the cervix sufficiently dilated; typically, this can be achieved at 1-2 cm dilated. Figure 1.3 shows an FSE commonly used in UK practice.

Figure 1.3 Fetal scalp electrode design *(Rocket Medical plc, Watford, UK). Zoomed inset shows solid curved needle, which penetrates and securely attaches to the scalp via spring-loaded rotation.*

FSEs are used in 13-22% of women monitored by CTG because the transabdominal route is unsatisfactory (Redshaw and Henderson, 2015, Kawakita et al., 2016). They are a valuable tool for clinicians in the assessment of intrapartum fetal hypoxia by enabling the acquisition of good-quality, continuous CTG tracings in these women. FSEs are also a prerequisite for ST waveform analysis (STAN), discussed below. However, they generally do not provide additional information to that obtained through external transducers and therefore do not improve the performance of CTG as a screening test for intrapartum hypoxia.

The next section discusses the use of additional tests to assess fetal oxygenation during labour as an adjunct to fetal heart rate monitoring.

1.2 Adjunctive technologies for intrapartum monitoring

To reduce the false positive rate of fetal heart rate monitoring and so avoid unnecessary obstetric interventions, additional tests to assess fetal oxygenation have been proposed in the context of a non-reassuring CTG. Several such adjunctive technologies have been developed, of which fetal blood sampling (FBS) is the most common (Visser et al., 2015). This section discusses the role of fetal blood sampling and other adjunctive technologies in past and present obstetric practice.

1.2.1 Fetal blood sampling

FBS involves sampling capillary blood from the presenting part of the fetus during labour for measurement of biochemical parameters of acidosis, such as pH, BD, and lactate, which is considered separately below. It was first described in the 1960s (Saling, 1962), preceding the introduction of CTG into practice, but its use as an adjunctive technology has only been established in recent decades as continuous CTG has become more routine. Today, FBS is mainly used in central and northern Europe, where it may be undertaken to evaluate fetal acid-base status in the presence of a suspicious or pathological CTG (Visser et al., 2015). It is not advised if an acute or severe intrapartum event is suspected, as this would delay urgent intervention (NICE, 2017). Although technically feasible, it is also not recommended in breech presentation - therefore FBS generally refers to sampling from the fetal scalp.

The procedure requires the membranes to be ruptured and the cervix dilated to 3 cm or more for adequate visualisation of the scalp using a modified speculum, or amnioscope, which is inserted vaginally. The amnioscope is held tightly in place while the fetal scalp is dried with swabs. A local anaesthetic spray, ethyl chloride, may be used to promote vasodilatation and arterialisation of the capillary blood. A thin layer of paraffin is applied so that the blood will form in a large droplet and a small incision then made on the scalp. Approximately 30-50 µL of blood is collected into a heparinised

capillary tube for analysis on a benchtop blood gas analyser, usually maintained centrally on the labour ward. Results may not be obtained in some cases due to an insufficient blood sample, the presence of air bubbles or blood clots in the capillary tube, or because the unit is calibrating at the time the sample needs to be analysed, with failures rates for pH measurement above 20% seen in some series (Westgren et al., 1998). Figure 1.4 shows a disposal FBS kit (Rocket Medical plc, Watford, UK) which is commonly available in UK maternity units.

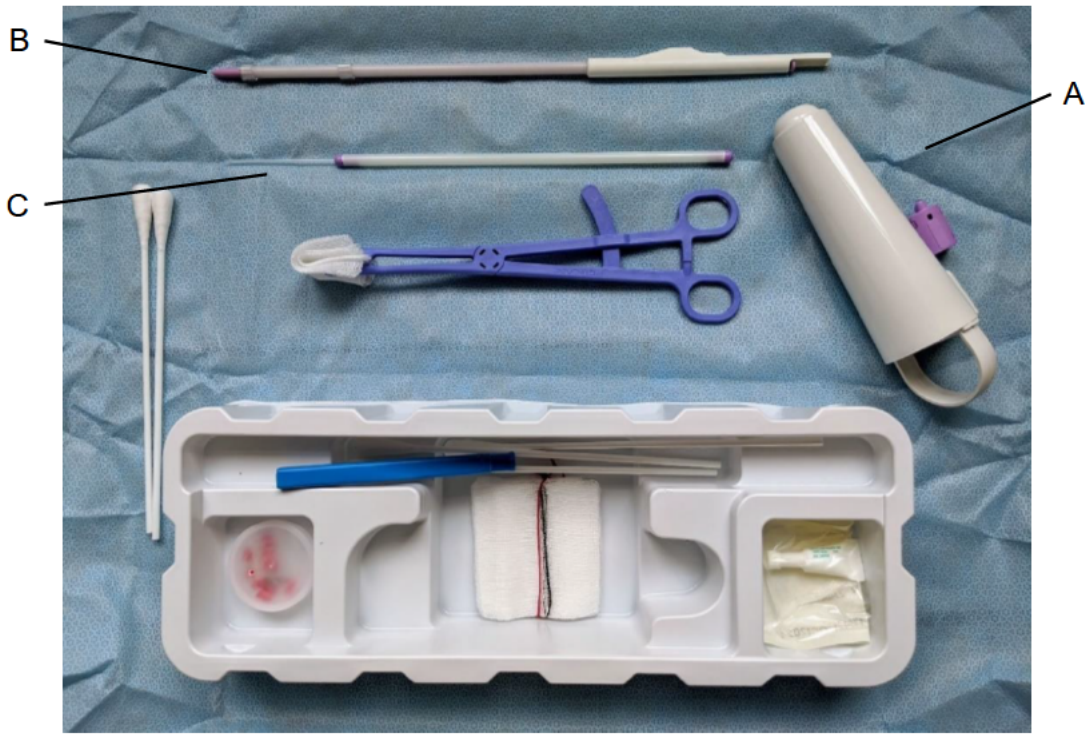

Figure 1.4 Disposal FBS kit. *(A) Amnioscope; (B) sampling 'wand' with blade; and (C) heparinised capillary tube (own image).*

1.2.1.1 Interpretation of results

Reference fetal scalp pH values were established in the 1960s from small observational studies (Beard et al., 1967, Bretscher and Saling, 1967). pH values below 7.20 were considered abnormal, with those between 7.20 and 7.24 indicating a pre-pathological state for which FBS should be repeated and delivery expedited if the pH fell further. Lending support to these findings, Adamsons et al. (1970) demonstrated a good correlation between acid-base measurements obtained simultaneously from the fetal scalp, carotid artery and jugular vein of rhesus monkeys during labour. Human data

have shown similar correlations between pH and lactate measurements from FBS performed shortly before birth with umbilical cord blood gas values (Teramo, 1969, Kruger et al., 1998). As a result, these thresholds and recommendations were widely adopted and remain essentially unchanged in the current NICE guidelines half a century later (Table 1.1; NICE, 2017).

Table 1.1 FBS interpretation. *Recommended thresholds for interpreting pH and lactate results from FBS (NICE, 2017).*

pH	Interpretation	Lactate (mmol/L)
≥ 7.25	**Normal** – consider repeating no more than 1 h later if no accelerations in response to FBS and still indicated by CTG	≤ 4.1
7.21–7.24	**Borderline** – consider repeating no more than 30 min later if there are no accelerations in response to FBS and still indicated by CTG	4.2–4.8
≤ 7.20	**Abnormal** – expedite birth	≥ 4.9
Interpret results taking into account any previous measurements and the clinical features of the woman and baby, such as rate of progress in labour.		

1.2.1.2 Evidence base

Like CTG, FBS has never been evaluated in large scale RCTs and much of the available evidence is conflicting. A subgroup analysis in the Cochrane review of continuous CTG versus intermittent auscultation found that access to FBS during labour increased operative vaginal delivery but reduced the risk of CS and cord blood acidosis compared to CTG alone (Alfirevic et al., 2017). The only prospective RCT included in the Cochrane review randomised 690 high-risk women to intermittent auscultation, CTG alone, or CTG plus FBS (Haverkamp et al., 1979). There was a non-statistically significant reduction in caesarean delivery when FBS was used in addition to CTG (11% vs 18%), however women in both groups had a markedly increased risk of CS compared to those auscultated (6%) and neonatal outcomes in all three groups were

similar. The relevance of these results today, when CS rates exceed 30% in most developed countries, is also unclear. A second RCT from a single centre in Australia recently reported on the impact of FBS for lactate measurement (see also next section). There was no difference in CS rates for all indications between women monitored with CTG + FBS (41%) versus CTG without FBS (45%), nor any difference between groups in assisted vaginal birth. Neonatal outcomes were also generally similar, however these findings must be interpreted with caution as the trial only enrolled 20% of the proposed sample size due to difficulties recruiting (East et al., 2021).

Despite the absence of high-quality evidence from RCTs, there is data from retrospective observational studies supporting the use of FBS. Most studies were conducted many years ago and their interpretation is undermined by risk of bias and considerable heterogeneity in study design. However, Jørgensen and Weber (2014) showed that increasing FBS use in Denmark between 2005 and 2011 was associated with a reduction in operative delivery. Other authors have shown no increase in CS or adverse neonatal outcomes following the elimination of FBS from clinical practice (Goodwin et al., 1994, Clark and Paul, 1985). In the USA, this has led to FBS being virtually abandoned. In countries where FBS remains in everyday use, its value as an adjunct for intrapartum fetal monitoring has also been questioned (Steer, 1987, Mahendru and Lees, 2011, Chandraharan and Wiberg, 2014).

The diagnostic accuracy of FBS was recently evaluated in a multicentre observational study in 44 UK maternity units (Al Wattar et al., 2019). The authors found that a suboptimal FBS result (pH < 7.25) had a poor sensitivity (22%) and PPV (4.9%) to predict neonatal acidaemia, with similar performance for predicting Apgar scores < 7 at 1 and 5 minutes. In summary, despite its widespread use, there is limited evidence to support the role of FBS as an adjunct to continuous CTG for improving the prediction of neonatal acidaemia and adverse outcomes or for reducing rates of operative birth.

1.2.1.3 Fetal scalp lactate

The use of fetal scalp lactate (FSL) as an alternative to pH measurement gained prominence in the 1990s with the advent of lactate test strip devices. These allowed lactate estimation at the bedside in much smaller samples (5 µL) than those required

for traditional blood gas analysis. FSL using POC devices thereby aims to reduce the failure rate of FBS for pH measurement, whilst offering a cheaper, low maintenance adjunct to continuous CTG. As previously discussed, lactate is also considered a better reflection of the metabolic component of fetal hypoxia-acidosis because low pH values may signify the development of a physiological respiratory acidosis in an uncompromised fetus during labour.

Cut-off limits for FSL were first established from a retrospective study by Kruger et al. (1999) involving pH and lactate measurements on 1221 and 814 patients, respectively. Lactate was more sensitive and specific than scalp pH for predicting a range of outcome variables including umbilical artery pH < 7.0, low Apgar scores, and HIE. The optimal lactate value for predicting moderate to severe HIE was 6.5 mmol/L (sensitivity 67% and specificity 93%, compared to 49% and 76% for pH value of 7.20), therefore a lower threshold for intervention of 4.8 mmol/L was suggested in order to prevent, rather than predict, adverse outcomes. This value corresponded to the 75th centile in the study's high-risk population; notably, the equivalent pH cut-off (representing the 25th centile) was 7.21, which closely aligned with previously accepted limits for FBS interpretation. Two randomised trials have directly compared FBS for lactate and pH determination in the management of intrapartum fetal distress (Westgren et al., 1998, Wiberg-Itzel et al., 2008). There were no significant differences in metabolic acidosis, operative deliveries for fetal distress, or low Apgar scores between the groups in either study. However, Wiberg-Itzel et al. (2008) noted that six babies with a normal scalp pH > 7.20 within 60 minutes of delivery had an umbilical arterial pH < 7.00, while there were no such 'false negatives' in babies with a normal FSL < 4.8 mmol/L within 60 minutes of delivery. FSL was also more likely to be successful than pH estimation in both RCTs, consistent with reported failure rates of 1-2% in the literature (Westgren et al., 1998, Ramanah et al., 2010). These findings were echoed in a Cochrane review of FSL (East et al., 2015) and have encouraged the uptake of FSL in several northern European countries and Australia. However, direct evidence for FSL an adjunctive test to continuous CTG is limited to the aforementioned RCT by East et al. (2021), which did not demonstrate clear maternal or neonatal benefits over CTG alone.

One important consideration is that lactate values vary depending on the analyser used, due to different test characteristics and the properties of the POC devices

themselves. The recommended cut-offs shown in Table 1.1 (Kruger et al., 1999), which have since been evaluated in an RCT (Wiberg-Itzel et al., 2008) and endorsed by guidelines (Visser et al., 2015, NICE, 2017), relate only to the now discontinued Lactate Pro™ (Arkray, Kyoto, Japan). Thresholds for newer devices, such as the Lactate Pro 2™ (Arkray) and StatStrip® Lactate (Nova Biomedical, MA, USA) meters, are yet to be agreed but differ considerably from those of Lactate Pro™ (Table 1.2; Birgisdottir et al., 2017, Heinis et al., 2017, Iorizzo et al., 2019a). Any new device and the thresholds used for clinical decision-making ideally should be evaluated prospectively with respect to intervention rates and neonatal outcomes. Clinicians must also take into account the degree of error in accuracy of lactate measurement and within-brand variation, i.e. agreement between devices (Wang et al., 2018).

Table 1.2 FSL interpretation. *Suggested thresholds for interpreting FSL results in different POC devices.*

	POC device lactate (mmol/L)			
FSL interpretation	Lactate Pro™	Lactate Pro 2™	Lactate Pro 2™	StatStrip® Lactate
Normal	≤ 4.1	< 6.4	< 6.3	< 5.7
Borderline	4.2 – 4.8	6.4 – 7.3	6.3 – 7.1	5.7 – 7.0
Abnormal	≥ 4.9	> 7.3	> 7.1	> 7.0
Reference	Kruger et al., 1999; NICE, 2017	Birgisdottir et al. 2017	Iorizzo et al. 2019a	Heinis et al., 2017

1.2.1.4 Limitations

FBS, whether for pH or lactate measurement, has important limitations. From a pathophysiological perspective, the hypoxic fetus releases a surge of catecholamines which results in peripheral vasoconstriction and the centralisation of blood flow to critical organs. Chandraharan (Chandraharan and Wiberg, 2014) argues that this redistribution undermines the validity of capillary scalp blood as a test of central fetal

acid-base status. External compression of the superficial vessels supplying the scalp during uterine contractions and the formation of caput succedaneum may also affect FBS results, although there is little evidence to support or refute these hypotheses.

The traditional view of FBS as a diagnostic test, rather than a second-line screening tool, has also been challenged (Mahendru and Lees, 2011, Chandraharan, 2014). Fetal scalp pH and lactate both have low PPV for diagnosing acidosis based on umbilical cord blood gas analysis, similar to CTG (Al Wattar et al., 2019, Tsikouras et al., 2018, Bowler and Beckmann, 2014). This means the potential for unnecessary obstetric intervention in false positive cases, particularly where FBS is undertaken for suspicious rather than pathological CTGs. FBS may be better viewed as a 'rule out' test based on its high negative predictive value (Steer, 1987, Bowler and Beckmann, 2014); but even in this context, it provides only a snapshot assessment of the fetal metabolic status. Repetitive sampling is often required for persisting or evolving CTG concerns in labour. In a prospective observational study of 1070 labouring women at a university hospital in Sweden, 52% underwent two or more FBS procedures (Holzmann et al., 2015).

The clinical use of stationary cut-offs for interpreting FBS results, as shown in Table 1.1 and 1.2, may also contribute to false positives. Fetal acid-base balance changes with advancing gestational age, characterised by the development of a physiological mixed metabolic and respiratory acidaemia (Wiberg et al., 2006a). Kitlinski et al. (2003) found that the mean umbilical arterial pH decreased from 7.26 at 37 weeks to 7.22 at 42 weeks. The odds ratio for an umbilical arterial pH < 7.10, defining acidosis at birth, steadily increased throughout the term period as a result. Using stationary cut-offs, the authors concluded that a quarter of all term infants would be diagnosed as acidotic despite having a pH within the gestational age-adjusted reference range (mean ± 2 standard deviations). Accompanying changes in BD have been shown (Wiberg et al., 2006b), whilst the effect of gestational age on lactate may be even greater. In a population of over 10 000 vigorous newborns with validated cord blood gases, the median umbilical arterial lactate level increased from 3.9 to 4.9 between 37 and 42 weeks' completed gestation (Wiberg et al., 2008). Hence, physiological lactate values in uncompromised term infants may encompass the full range of normal to abnormal thresholds proposed for interpreting FSL. Together, these data appear to support the

adoption of gestational age-adjusted limits for interpreting umbilical cord blood gases and FBS, however the impact of this on clinical outcomes is yet to be evaluated.

FBS also has practical drawbacks. In addition to the aforementioned failure rates, it is a time-consuming procedure which can be technically difficult. In previous UK series (Tuffnell et al., 2006, Annappa et al., 2008), the median time intervals between the decision to perform FBS and the results were 17–18 minutes. Although the use of FSL reduces sampling and analysis time (Westgren et al., 1998), these delays must considered carefully when planning delivery or undertaking repetitive sampling in the interest of fetal wellbeing, given the dynamic nature of labour.

Finally, FBS is an invasive procedure for both mother and baby. A survey of women's experiences found that FBS was well tolerated in women with epidural analgesia, with a median pain score of 3.5 on a 10-point scale. However, those with no epidural analgesia and less cervical dilatation experienced FBS as more painful and complicated (Liljeström et al., 2014). Pain scores correlated with obstetricians' perceived difficulty in performing FBS and the duration of the procedure. Moreover, the invasive nature of FBS means it carries a small risk of infection and bleeding and is therefore contraindicated where there is concern about vertical transmission of active maternal infection (e.g. hepatitis) or fetal bleeding disorders. Severe complications, although reported, are rare (Jørgensen and Weber, 2014).

1.2.2 ST waveform analysis

Several other adjunctive technologies have been developed in recent decades, ST waveform analysis (STAN) being the most widely used of these after FBS and FSL. STAN refers to the addition of fetal electrocardiographic (ECG) assessment to conventional CTG monitoring. STAN software continuously evaluates the fetal ECG complex for specific changes in the ST interval, which are then indicated to the clinician as warnings called "ST events". ST interval changes have been one of the cornerstones of diagnosis of myocardial ischaemia for many decades, and animal experiments have shown that these changes precede signs of cardiovascular decompensation during hypoxia (Rosén and Kjellmer, 1975, Rosén et al., 1984, Lindecrantz et al., 1988). Compared to FBS, STAN provides continuous information on

oxygenation of central organs and is less invasive, albeit it requires the application of an FSE in order to acquire the FHR and ECG signals. Importantly, because it relies on detecting changes in the ST interval from baseline, STAN must be commenced when there is certainty that the fetus is not already hypoxic (Sacco et al., 2015). Likewise, it is not appropriate in response to acute intrapartum events and should be used with caution if there are other clinical factors which may increase the risk of hypoxic injury, such as meconium-stained liquor or intrauterine infection.

STAN was commercialised in its currently available form in 2000 (Neoventa, Gothenburg, Sweden), and has undergone extensive evaluation since the first phase 3 RCT of its use in 1993 (Westgate et al., 1993). This trial showed a decrease in total operative delivery for fetal distress in the CTG plus STAN group (OR 0.51, 95% CI 0.37-0.70), as well as a trend towards reduced MA. However, four subsequent RCTs in Europe between 2001 and 2010 reported conflicting results (Amer-Wåhlin et al., 2001, Ojala et al., 2006, Vayssière et al., 2007, Westerhuis et al., 2010), with only the Swedish RCT showing a decrease in MA and operative delivery for fetal distress (Amer-Wåhlin et al., 2001). The largest multi-centre RCT, which randomised 11 108 women across 23 centres in the United States, did not demonstrate any difference in a primary composite neonatal outcome which included MA, nor any difference in operative delivery rate between the CTG plus STAN and CTG alone groups (Belfort et al., 2015). The available data from these RCTs has been subjected to repeated meta-analyses and commentary, with diverging opinions (Bhide et al. 2016, Bloom et al., 2016, Amer-Wåhlin et al., 2019). Three meta-analyses have included all six RCTs with data from over 26 000 women, together concluding that STAN did not improve perinatal outcomes (e.g. occurrence of Apgar score < 7 at 5 min, neonatal encephalopathy or seizures, admission to neonatal intensive care units), nor did it decrease CS rates; however, assisted vaginal delivery rates were lower in women allocated to CTG plus STAN (Neilson et al., 2015, Saccone et al., 2016, Blix et al., 2016).

In the midst of such conflicting evidence, several centres have also published data on local outcomes following the introduction of STAN technology (Doria et al., 2007, Timonen and Holberg, 2018, Landman et al., 2019). In the UK, Doria et al. (2007) found that the STAN did not reduce emergency operative deliveries or HIE in its first 3.5 years of use (1502 cases) at St. George's Hospital, London. They recommended "better

training, assessment and supervision of users" to improve outcomes. Intensive training was subsequently introduced in 2007, followed by a mandatory competency assessment test for staff in 2010, and outcomes were re-evaluated over a 5-year period from 2008 to 2012 (Chandraharan et al., 2013). This showed a significant reduction in emergency CS, with a trend towards reduced HIE and early neonatal death. Timonen and Holmberg (2018) also highlighted the importance of the 'learning curve' when introducing STAN in a single tertiary unit in Finland. The authors argued that improvements in clinical outcomes may not be seen in the early stages of adoption, for example in an RCT with a relatively short follow-up period, because this curve is gradual and 'quite long'. In line with the findings of Amer-Wåhlin et al. (2005), the implementation of STAN was facilitated by a comprehensive training and educational programme and reinforced through regular audit.

1.2.3 Computerised interpretation of fetal heart rate monitoring

One of the criticisms of STAN is that relies heavily on accurate CTG interpretation and is therefore subject to the same limitations of fetal heart rate monitoring outlined in the preceding section of this chapter. Computerised analysis of CTGs was developed to overcome these limitations by reducing inter-observer variability in interpretation and providing a more objective assessment of certain CTG features (e.g. baseline variability) than is offered by visual analysis alone. Several proprietary systems have been developed, all of which provide real-time visual and sound alerts through central monitoring stations on labour ward to inform clinicians when CTG features associated with hypoxia are detected. The first RCT (Ignatov and Lutomski, 2016), from a single tertiary centre in Bulgaria, showed a reduced risk of acidosis, CS and neonatal intensive care unit admission in women monitored with the NEXUS/OBSTETRICS system (Nexus GMT, Frankfurt, Germany). However, these findings were not replicated in two larger multi-centre RCTs in the UK. The FM-ALERT study (Nunes et al., 2016) evaluated the Omniview-SisPorto program (Speculum, Lisbon, Portugal) in 7730 women from five centres, showing no difference in rates of MA or obstetric intervention between the groups. The INFANT trial (INFANT Collaborative Group, 2017), which randomised over 46 000 women from 24 UK centres to CTG with or without the INFANT decision support system (K2 Medical Systems, Plymouth, UK), also noted no difference

in the incidence of their composite primary neonatal outcome. There were no differences in any component of the composite outcome, nor in any secondary neonatal or maternal outcomes between groups except for use of FBS, which increased in women monitored decision support. A subsequent meta-analysis of all three RCTs concluded that computerised analysis did not reduce MA nor obstetric intervention (Campanile et al., 2020) compared to standard visual analysis.

In both the FM-ALERT and INFANT trials, investigators highlighted that the incidence of the primary outcome was unexpectedly low, as was also the case in the largest trial of STAN (Belfort et al., 2015). This means these studies may have been underpowered to detect pre-defined differences in outcomes, as well as raising the possibility of the Hawthorne effect (Gittelsohn et al., 1997), in which involvement in the trial itself improves outcomes across all intervention groups; in the case of fetal monitoring, this may have been mediated through staff training related to the trial and improved CTG interpretation skills (Amer-Wåhlin et al., 2019, Georgieva et al., 2019). Concerningly, detailed analysis of infants with adverse outcomes in the INFANT study found that, whilst FHR abnormalities were reliably recognised in both the computerised and clinician interpretation groups, a failure to interpret the CTG in the wider clinical context contributed to substandard care in a high proportion of cases (Steer et al., 2019).

As with STAN, observational data on the potential benefits of computerised CTG analysis is more optimistic. A retrospective cohort study from a tertiary centre in Portugal showed significant reductions in rates of HIE, overall CS, and emergency CS following the introduction of the Omniview-SisPorto system for computerised CTG analysis plus STAN (Lopes-Pereira et al., 2019). Moreover, rapid advances in artificial intelligence and data analytics in recent years may yet help to create new computerised systems for intrapartum fetal monitoring which translate into better outcomes (Ogasawara et al., 2021, Georgieva et al., 2019).

1.2.4 Other adjunctive technologies

In addition to STAN and computerised CTG interpretation, the only other adjunctive technology to be evaluated in a large clinical trial is fetal pulse oximetry. Fetal pulse oximetry was designed to continuously monitor fetal oxygen saturation in the presence

of a non-reassuring CTG. The fetal pulse oximetry system, conditionally approved in the US Food and Drug Administration (FDA) in 2000, involved a specialised sensor inserted through the dilated cervix (after rupture of membranes) and applied to the fetal face. Observational studies in both animals and humans showed a correlation between MA and increasing duration of fetal pulse oximetry saturations below 30%. While the first randomised trial by Garite et al. (2000) demonstrated a reduction in the primary outcome of CS for non-reassuring fetal status in the fetal pulse oximetry arm, there were no significant differences in the overall caesarean rate due to an increase in CS for labour dystocia. Rates of overall operative vaginal delivery and operative vaginal delivery for non-reassuring fetal status were also unchanged. Similar findings were reported in the FOREMOST trial (East et al., 2006), conducted across four Australian centres, with a reduction in operative delivery for non-reassuring fetal status but no difference in overall operative births amongst women monitored by CTG plus fetal pulse oximetry.

These results were not easily explained, hence a larger RCT, enrolling over 5000 women across 13 centres, was undertaken by the US National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network (Bloom et al., 2006). All women were monitored by both CTG and fetal pulse oximetry before randomisation into two groups in which fetal oxygen saturation data were either available or masked to managing clinicians. There were no differences in rates of CS overall nor CS for non-reassuring fetal status between the open and masked groups, and this was true for the subgroup of women with a non-reassuring CTG detected prior to randomisation. Neonatal outcomes in the two groups were also similar, including the incidence of MA. A subsequent Cochrane meta-analysis of seven published trials (East et al., 2014) concluded that the addition of fetal pulse oximetry to CTG does not reduce CS rates and “a better method than pulse oximetry is required to enhance the overall evaluation of fetal wellbeing in labour.”

Alternative adjuncts to fetal heart rate monitoring have been proposed, including continuous tissue pH monitoring and fetal scalp stimulation. Continuous pH monitoring by means of glass and fibreoptic tissue pH electrodes was reported by several authors in the 1980s (Kellner et al., 1980, Weber, 1982, Chatterjee et al., 1984), however, technical limitations in the development and refinement of these systems prevented

them from reaching clinical practice or being formally evaluated in clinical trials. Fetal scalp stimulation during digital vaginal examination to elicit an acceleration in the fetal heart rate may also provide reassurance of fetal wellbeing in CTGs with reduced baseline variability, but it is considered of limited value in the assessment of other CTG patterns (Visser et al., 2015) and therefore not discussed further here.

1.2.5 Adjunctive technologies in current practice

The lack of high-quality evidence supporting the adjunctive technologies described above has contributed to variation in fetal monitoring practices amongst developed countries. For example, FBS is commonly used in much of Europe and Australasia but never became popular in the USA. Several reasons for this have been proposed, including clinician experience and availability, technical difficulties with the procedure, and concerns about invasiveness (ACOG, 2009, Parer, 2003). Influential papers by Clark and Paul (1985) and Goodwin et al. (1994) further de-emphasised the value of FBS in clinical practice. Current North American guidelines therefore promote fetal scalp stimulation as a less invasive alternative to FBS, which is capable of providing similar information about the likelihood of fetal acidosis (ACOG, 2009, Liston et al., 2018). Likewise, STAN is not recommended in either guideline.

By contrast, recent literature from northern Europe suggests the widespread use of FBS – with lactate generally preferred over pH – and STAN. A 2008 survey (Holzmann and Nordström, 2010) showed that FBS was available in all 46 labour wards in Sweden, with 61% of those analysing lactate alone. FBS was used in 3-14% of deliveries in the nine units that provided such information. STAN was also available in 48% of units, representing more than half of all Swedish births. A Dutch survey (Bullens et al., 2016) reported similar findings, with 98% of units using FBS, albeit most analysed scalp pH. STAN was available in 23% of units in accordance with national guidelines endorsing its use (Nederlandse Vereniging voor Obstetrie en Gynaecologie, 2019). In Denmark, the use of FBS increased from 3.8 to 6% of all term deliveries between 2005 and 2011 (Jørgensen and Weber, 2014). And a recent survey by Kaasen et al. (2019) found that FBS was used in 48% of Norwegian units, with 83% analysing lactate alone. In addition, STAN was used in all but two of the country's tertiary obstetric units. Guidelines in other European countries and Australasia also recommend access to FBS for

intrapartum fetal surveillance (Nederlandse Vereniging voor Obstetrie en Gynaecologie, 2019, German Society of Gynecology and Obstetrics, 2014, Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2019). In a large tertiary centre in Australia, FSL was performed in 1.7-3.5% of all deliveries from 2011-2013 (Lowe and Beckmann, 2016).

In the UK, contemporary data regarding the use of FBS and other adjuncts is more limited. The last comprehensive survey of practice was undertaken in the 1980s and showed that 44% of units used FBS in the first stage of labour, with 11% also reporting use in the second stage (Wheble et al., 1989). Recent studies suggest it continues to play an important role in intrapartum fetal monitoring. FBS was performed in around 10% of women in the INFANT trial, although higher incidences of nulliparity and labour induction in the study population are likely to have increased the rate of FBS and other interventions (INFANT Collaborative Group, 2017). Estimates of 4-5% of all deliveries may be more representative, based on retrospective studies in the northwest of England (Heazell et al., 2011) and Ireland (Murphy and Macdonald, 1990). Compared to the Nordic nations, pH analysis remains more common than FSL in the UK. Lactate was available in only 13% of cases in a recent retrospective study of 1422 FBS samples taken in 44 units between 2016 and 2018 (Al Wattar et al., 2019). Data on the frequency of FBS was not reported, although the authors commented that several units withdrew participation due to the low numbers of FBS tests performed. Meanwhile, STAN use appears limited to a small number of UK units (Doria et al., 2007, Sacco et al., 2015).

1.2.6 The unmet clinical need

Despite the widespread availability of FBS, FSL and STAN, it is clear that the use of adjunctive technologies has evolved differently across maternity care settings in developed countries and that much uncertainty remains about how best to monitor fetal wellbeing during labour. Crucially, these technologies all rely on the acquisition and interpretation of CTGs, with their own inherent limitations. At the same time, national initiatives to improve the standard of intrapartum fetal monitoring, such as the Saving Babies' Lives Care Bundle, continue to focus on CTG training (NHS England, 2019). While this has encouraged the introduction of more formalised and mandatory

training, including requirements for individual competency assessments, there is still no validated, evidence-based training programme for CTG interpretation.

Rather, there is increasing awareness of the limitations of fetal heart rate monitoring. The RCOG and MBRRACE-UK have both highlighted the inadequacy of fetal heart rate monitoring, even when perfectly applied, to avoid adverse maternal and neonatal outcomes in often complex, high-risk situations (RCOG, 2017, Draper et al., 2017). These bodies have called on future research to explore alternative methods of fetal monitoring. If we are to realise the Government's ambitions to reduce intrapartum-related morbidity and mortality by 2030, there is therefore an urgent need for a more effective and reliable means to detect babies at risk of hypoxia during labour.

1.3 Continuous monitoring of fetal metabolism

One potential solution is to monitor concentrations of lactate or other markers of fetal metabolism in the interstitial fluid (ISF) of the fetal scalp, rather than capillary blood. This is similar in principle to continuous glucose monitoring, which uses subcutaneous needle-type electrodes to measure interstitial glucose levels. Continuous glucose monitoring (CGM) devices have revolutionised care for people with diabetes, who have historically relied on capillary blood glucose measurements taken several times per day with painful finger-prick lancets. However, the success of such an approach demands a thorough understanding of the relationship between concentrations of the chosen analyte (e.g. glucose, lactate) in the interstitial fluid and blood compartments.

1.3.1 Interstitial fluid

In the term fetus, approximately 30% of body weight is intracellular fluid and 45% extracellular fluid (Friis-Hansen, 1961, Toro-Ramos et al., 2015). Extracellular fluid is subdivided functionally into interstitial fluid (ISF) and plasma, the latter contained within the intravascular space. ISF is the much larger of these compartments, particularly during the fetal and neonatal period, with the ratio of interstitial:plasma volume estimated at about 8:1 (Simpson and Stephenson, 1993). In basic terms, ISF is a salt solution derived from the ultrafiltration of plasma. Extensive research on ISF

composition over several decades has led to the following general observations (Aukland and Reed, 1993, Fogh-Andersen et al., 1995, Wiig and Swartz, 2012):

- ISF contains about one-third as much protein as plasma.
- The electrolyte content of ISF is similar to that of plasma, although cations like sodium and potassium may be slightly more abundant in plasma as a result of its higher protein content and relative negativity.
- Small water-soluble molecules, such as glucose and lactate, appear to circulate relatively freely between (and within) these two compartments so their interstitial and plasma concentrations are similar.

In addition to ISF, the interstitium contains the structural elements of the extracellular matrix: a predominantly collagen fibre framework, a gel phase of glycosaminoglycans, and plasma proteins. While all tissues have an interstitium, its abundance and composition varies. For example, ISF accounts for approximately 50% of wet tissue weight in skin but only 10% in skeletal muscle (Aukland and Reed, 1993).

1.3.1.1 Blood-interstitial dynamics

The interstitium represents the immediate environment, or 'milieu intérieur', of all cells (Holmes, 1986) and therefore plays a central role in maintaining tissue homeostasis. Oxygen and nutrients being transported to cells must traverse this space, as well as metabolites and waste products returning via the bloodstream to excretory organs. This blood-interstitial exchange occurs at the level of capillaries, the direction and magnitude being governed by various factors which are collectively known as Starling's forces (Levick and Michel, 2010). In steady states, these are in equilibrium and there is no net fluid gain by the interstitial tissue; plasma filtered into the interstitial compartment is removed in equal measure by lymph flow. However, this underplays the dynamic nature of blood-interstitial communication and flow within the interstitial compartment itself. Calnan et al. (1972), who described ISF as "a river, not a pool", first demonstrated the rapid movement of radioactive sodium from the intravascular to interstitial compartments, and vice versa. In the case of glucose and lactate, transfer across the capillary endothelium to the ISF occurs by simple diffusion down a concentration gradient without the need for active transporters. Interstitial

concentrations therefore depend on blood flow to the area, the rate of diffusion from blood to ISF, and the rate of uptake by tissue cells (Cengiz and Tamborlane, 2009).

In recent decades, interest in blood-interstitial glucose kinetics has been driven by the need for improved glucose monitoring techniques in the management of diabetes. Estimation of any blood-to-ISF delay and quantitative assessment of the relationship between glucose concentrations in these two compartments is critical to evaluating the accuracy of implantable CGM sensors. Most authors have reported a time lag of approximately 5 minutes from intravenous infusion of tracer molecules to their detection in the ISF (Smith et al., 1999, Basu et al., 2013). In steady state conditions, Schiavon et al. (2015) reported an equilibration time of 10 minutes between these compartments. However, when plasma glucose is rapidly rising or falling this delay is likely to be affected due to magnitude and slope of the concentration gradient between compartments (Cengiz and Tamborlane, 2009).

Much less is known about the relationship between lactate in the intravascular and interstitial compartments. At a molecular level, lactate ($C_3H_5O_3$-, molecular weight 89.07 g/mol) shares several properties with glucose ($C_6H_{12}O_6$, 180.16 g/mol), and they are closely related metabolically, as discussed in the previous section. However, detailed studies of lactate blood-interstitial kinetics are lacking.

1.3.1.2 Sampling interstitial fluid

Historically, one of the challenges for those investigating the interstitial compartment has been the complexity of sampling methods. Methods of directly extracting ISF, such as wick sampling and suction blisters (Heltne et al., 1998), have been largely abandoned because of their invasiveness, meaning that indirect methods of assessing ISF have predominated. Recent technological advances in microneedles (micron-scale needles) have brought a less invasive means of accessing the epidermal or dermal interstitium, however, providing opportunities both to extract ISF, through hollow needles (Miller et al., 2018), and to measure analytes *in situ* when combined with biosensors. Miller et al. (2012) and Bollella et al. (2019) both described the use of microneedle biosensor arrays for the detection of lactate *in vitro* and *ex vivo*, and the first in-human data has recently been published by Ming et al. (2022). The authors

reported on real-time continuous measurement of subcutaneous lactate in five adult humans through a low-cost, solid microneedle biosensor patch during rest and exercise. In all participants, microneedle biosensor current followed venous lactate concentrations and trends, with an estimated time lag of 5 min.

Modern CGM devices are not microneedles by definition, typically consisting of a small filament (less than 13 mm length) inserted into the subcutaneous tissue via an introducer needle. However, they share many of the same technological principles with the biosensors described above (Vaddiraju et al., 2010) and the wealth of data related to their development has largely informed advances in microneedle-based systems. However, any form of implantable biosensor is likely to present additional challenges in the development of a fetal monitoring device for intrapartum use, some of the requirements for which are outlined below (Cummins et al., 2018):

- easily applicable (and removable), ideally at digital vaginal examination;
- attach securely to the fetal scalp to avoid displacement during contractions, fetal and/or maternal movement; and
- maintain accuracy and functionality when exposed to genital tract secretions, amniotic fluid and blood.

These parameters do not reflect the environment in which CGM or existing microneedle-based biosensors have been designed to work, which may limit the application of implantable biosensors in this setting.

In spite of the aforementioned advances in implantable biosensors, indirect methods of monitoring ISF remain commonly used, including those which are entirely non-invasive. Reverse iontophoresis refers to the reverse extraction of glucose and other molecules through intact skin by electro-osmotic flow upon the application of a low-level electrical current. The GlucoWatch® biographer paired reverse iontophoresis with an amperometric biosensor to enable continuous non-invasive monitoring of interstitial glucose over up to 13 hours via a wrist-worn device (Potts et al., 2002). Although approved by the FDA in 2001, the GlucoWatch® failed to gain widespread adoption and was withdrawn from the market in 2007 (McCormick et al., 2012). Reverse iontophoresis of lactate in humans has also been demonstrated, although the

limited data available have shown poor correlations between extracted lactate levels and blood lactate (Nixon et al., 2007, Ching and Connolly, 2008).

Capillary ultrafiltration is another means of indirect sampling, which requires the insertion of semi-permeable, hollow fibre probes into a tissue of interest; negative pressure is applied to induce, by convection, the extraction of fluid and molecules from ISF into the ultrafiltrate. This approach has been used to monitor subcutaneous glucose (Ash et al., 1993) and lactate (Tiessen et al., 1999) levels in humans, as reviewed by Leegsma-Vogt et al. (2003). Ultrafiltration shares several characteristics with microdialysis, discussed in detail below, with the notable advantage that analyte recovery approximates 100%, i.e. ultrafiltrate concentrations directly reflect tissue concentrations. However, it is more invasive than microdialysis and, overall, there is considerably less experience in the use of ultrafiltration for sampling ISF in humans (Leegsma-Vogt et al., 2003).

1.3.2 Principles of microdialysis

Like ultrafiltration, microdialysis (MD) relies on the movement of molecules across a semi-permeable membrane. In microdialysis, however, this separation occurs exclusively by the diffusion of analytes down their concentration gradient. Since its original description for measuring neurotransmitter concentrations in rat brain (Ungerstedt and Pycock, 1974), microdialysis has been used to sample ISF in a variety of tissues and species and is a widely used technique for monitoring biological events *in vivo* (reviewed in Saylor and Lunte, 2015, Shippenberg and Thompson, 2001, Plock and Kloft, 2005). A basic system consists of a syringe pump, a microdialysis probe or catheter with inlet and outlet tubing, and a microvial for collecting samples (Figure 1.5). Physiological perfusion fluid (perfusate) is pumped through the system at a constant flow rate and molecules diffuse from the region of interest down their concentration gradient, through a semi-permeable membrane located at the tip of the catheter, into the dialysate stream. Catheters differ in their size, shape and material depending on the intended application. For neuroscience applications, spatial resolution is enhanced by small, concentric catheters with a rigid, pin-style cannula. In more homogeneous tissues, such as muscle or skin, flexible concentric or linear designs may be used to

maximise analyte recovery and minimise tissue trauma, particularly in freely-moving subjects (Davies et al., 2000).

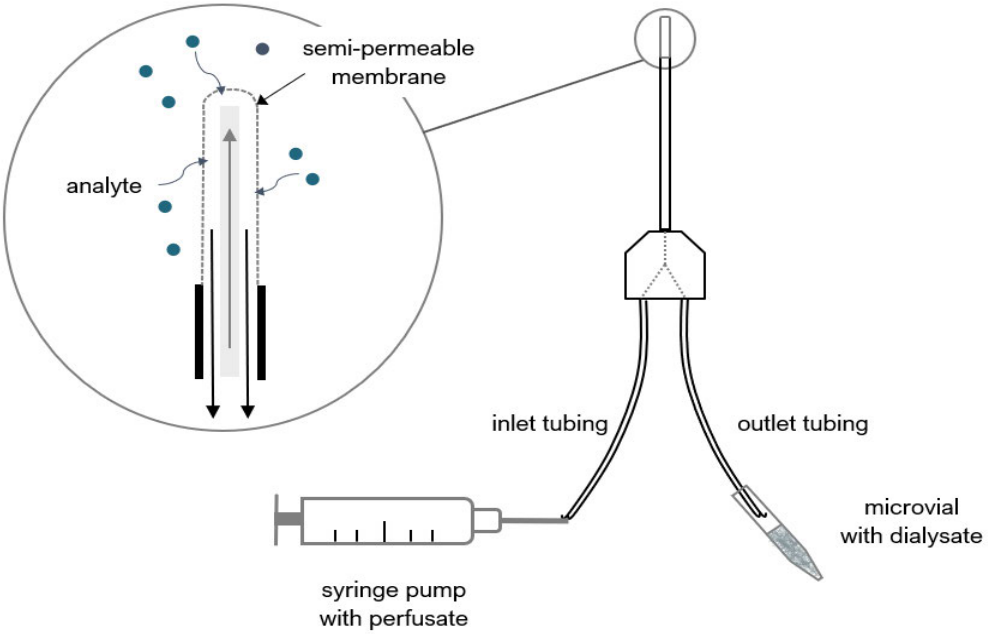

Figure 1.5 Schematic drawing of basic microdialysis system. *Perfusate enters the catheter (zoomed inset of concentric catheter design) through the inlet tubing and analytes diffuse across the membrane into the dialysate stream, which is transported via the outlet tubing for collection in microvials and subsequent analysis.*

1.3.2.1 Relative recovery and calibration

Recovery describes the relation between concentrations of the analyte in the tissue or fluid surrounding the catheter and those in the dialysate, and is usually expressed in relative terms as the ratio of these concentrations (Shippenberg and Thompson, 2001, de Lange, 2013):

$$\text{relative recovery, RR (\%)} = \frac{C_{dial}}{C_{tissue}} \times 100$$

Unlike ultrafiltration, concentrations in these compartments always differ because the constant flow of perfusate prevents an equilibrium from being established. A number of parameters influence analyte recovery. The velocity of the diffusion process itself is dependent on temperature, concentration gradient (and therefore composition of the perfusate), and the molecular weight cut-off and surface area of the catheter

membrane. A molecular weight cut-off at least four-fold higher than the molecular mass of the analyte of interest is recommend (Plock and Kloft, 2005). Commercially available catheters have typical cut-offs above 5 kDa, hence small molecules, like glucose and lactate (89.07 g/mol or Da), are expected to diffuse freely and rapidly across. Perfusate flow rate is the other major factor affecting relative recovery, with low flow rates resulting in higher recoveries, and vice versa. However, the use of lower flow rates is limited by the small sample volumes obtained, as discussed below.

In vitro, relative recovery can be measured directly as the concentrations of analytes in the fluid around the catheter are assumed to be known. Hence, these parameters may be modified to maximise relative recovery for a given analyte of interest. *In vivo*, however, diffusion of analytes is further reduced by the tortuosity of the sample matrix and recovery may also be influenced by physiological processes, including extra-intracellular and capillary exchange (Plock and Kloft, 2005; de Lange, 2013). Moreover, true tissue concentrations of analytes are not known but must be determined indirectly by one of several methods of calibration (reviewed in de Lange, 2013).

1.3.2.2 Analytical methods

In the schematic shown in Figure 1.5, discrete sample volumes of dialysate are collected in microvials for subsequent analysis, known as offline analysis. The need to manually manipulate small sample volumes for offline analysis limits the temporal resolution, or response time, of the microdialysis system, which is determined mainly by the perfusate flow rate (and resulting sample volume), analyte recovery, and sensitivity of the analytical method (Davies et al., 2000). Typical flow rates used *in vivo* (1-2 µL/min) result in temporal resolutions from 5 to 10 min for most offline microdialysis studies (Nandi and Lunte, 2009), which does not take into account any delay resulting from blood-interstitial kinetics.

Online microdialysis, in which dialysate collection is seamlessly integrated with flow injection systems and automated analysis on a planar device, negates the need handling and storage of samples and enhances temporal resolution by enabling sub-microlitre samples to be processed (Saylor and Lunte, 2015, Jin et al., 2008). In recent years, several online microdialysis systems for continuous, real-time or near real-time lactate monitoring have been developed and used in preliminary human studies

(Poscia et al., 2005, Rogers et al., 2013, Schierenbeck et al., 2014, Rogers et al., 2017). In both offline and online analyses, separation methods can also be introduced to enable monitoring of multiple analytes simultaneously. For example, Rogers et al. (2017) monitored intracerebral potassium, glucose and lactate in patients requiring emergency brain surgery using online microfluidic analysis, which incorporated an auto-calibration system for sensors. The ability to monitor multiple analytes and externally calibrate coupled analytical devices are two key advantages of microdialysis over implantable biosensors.

1.3.3 Microdialysis for monitoring interstitial lactate

The potential value of monitoring interstitial lactate and related metabolites *in vivo* depends on the extent to which interstitial concentrations of these analytes reflect changes in blood concentrations or a related reference standard. In the case of intrapartum monitoring, arterial lactate and/or pH sampled from the umbilical cord at delivery provides this reference. Animal and human studies which provide insight into the relationship between interstitial and blood lactate are summarised below, focusing on those which have used subcutaneous microdialysis.

1.3.3.1 Animal studies

A large proportion of microdialysis research in animals has been in the field of neurosciences, with very few studies using microdialysis to report on the agreement between blood and interstitial lactate concentrations. In adult rats, skeletal muscle interstitial lactate concentrations have been shown to increase several-fold in response to hypoxia and quickly return to baseline with reoxygenation; however, no reference data on blood lactate concentrations were reported in this study (Zoremba et al., 2014). Kastellorizios and Burgess (2015) also showed an increase in subcutaneous adipose tissue (SAT) interstitial lactate in rats exercised to exhaustion, with general agreement between interstitial lactate and venous lactate profiles. In rabbits, Poscia et al. (2005) modified an online microdialysis system for continuous glucose assessment (GlucoDay®, Menarini Diagnostics, Florence, Italy) to monitor lactate by substituting the glucose biosensor with an enzymatic lactate oxidase biosensor. Interstitial lactate

profiles closely matched reference venous samples following infusion of either sodium L-lactate or a physiological buffer solution. Wolf et al. (2018) also administered an intravenous sodium L-lactate challenge in an adult pig model to demonstrate the potential of a subcutaneously implanted lactate biosensor. Interstitial lactate profiles closely matched those of arterial blood, albeit agreement reduced over time with repeated lactate challenges. Notably, only one study (Tigchelaar et al., 2020) has investigated subcutaneous microdialysis for the proposed application of intrapartum fetal monitoring and this is discussed in detail in the introduction to Chapter 3.

1.3.3.2 Human studies

Data from human studies on the relationship between interstitial and arterial lactate is also limited, and in some cases conflicting. Rosdahl et al. (1993) first described monitoring interstitial lactate from the SAT of the abdominal wall in exercising adults and several early studies followed in this vein, related mainly to interstitial lactate concentrations in the skin (Petersen, 1999) and SAT (Jansson et al., 1990, de Boer et al., 1994, Ellmerer et al., 1998) both at rest and following exercise. Collectively, these studies indicated that interstitial lactate concentrations under steady-state conditions exceeded those of blood; at other times, however, the degree to which interstitial lactate mirrored blood lactate varied. In response to exercise, interstitial lactate was shown to increase more gradually than blood lactate, after a time delay of 4 to 10 min, and to a lower peak concentration (de Boer et al., 1994, Ellmerer et al., 1998). Furthermore, the correlation between interstitial and blood lactate concentrations ranged widely between individual experiments in these studies.

In the past two decades, numerous clinical microdialysis studies have also been published, the majority in post-operative or critical care patients in whom hyperlactataemia has been shown to predict outcomes (Bakker et al., 1991, Hatherill et al., 2000) and to help guide therapy (Evans et al., 2021). Overall, similar findings have emerged from these studies regarding variation in the relationship between interstitial and blood lactate concentrations. In their study of 40 patients after major cardiac surgery, Ellmerer et al. (2009) pre-defined zones indicating acceptable and unacceptable (first, second and third order) interstitial lactate measurements from abdominal SAT based on widely recognised clinical thresholds for arterial lactate.

Although a moderate positive correlation between interstitial and arterial lactate (r^2 = 0.71, p < 0.001) was seen, a large proportion of interstitial lactate measurements (24%) and trends (35%) were not comparable to arterial blood. However, as arterial lactate increased, the proportion of unacceptable interstitial lactate measurements decreased, such that over 95% of readings were deemed acceptable when arterial lactate exceeded 5 mmol/L. The authors also highlighted inter-individual variation in the relationship between interstitial and arterial lactate, with a reliable relationship between the compartments found in approximately half of all patients.

Van den Heuvel et al. (2009) described similar variation in agreement between interstitial lactate (abdominal SAT) and arterial lactate in 15 children following congenital heart surgery: interstitial lactate profiles followed arterial lactate in only 10 of 15 children. The incidence of hyperlactataemia > 5 mmol/L in their population appears to have been very low. The generalisability of these findings to interstitial lactate monitoring in the fetus and neonate is questionable, not only because the pathophysiology of hypoxia (and hyperlactataemia) is likely to differ, but because the range of normal lactate concentrations is much higher around the time of birth. And whilst there have been no studies of interstitial lactate in human infants, the feasibility of long-term subcutaneous microdialysis in the neonatal population has been demonstrated with respect to glucose monitoring (Holzinger et al., 2006).

1.3.3.3 Multi-analyte monitoring

One of the advantages of microdialysis over implantable biosensors is its potential for monitoring multiple analytes simultaneously. As well as providing valuable information on inter-dependent metabolic pathways, multi-analyte ratios offer a unique advantage in microdialysis studies because they are independent of relative recovery, assuming the analytes are of similar molecular weight (Hutchinson et al., 2015). There has been particular interest in the prognostic value of the L/P ratio, which reflects the cellular redox state. In adult rats, previous research has demonstrated that interstitial L/P ratios measured by microdialysis of abdominal SAT increase in response to hypoxia (Klaus et al., 2003) and exsanguination (Ohashi et al., 2009). Relatedly, Nikitas et al. (2013) showed an association between interstitial L/P ratio and mortality in critically ill patients with septic shock who were monitored by microdialysis of upper thigh SAT. More

recent research has suggested that interstitial lactate/glucose (L/G) ratios may be more sensitive to metabolic changes than blood parameters in a rat model of intense exercise leading to exhaustion (Kastellorizios and Burgess, 2015).

In the intrapartum setting, there is a paucity of data on the use of these ratios, partly due to technical difficulties in measuring blood pyruvate (Chuang et al., 2006). In a study of 56 women with intrapartum risk factors, Nordström et al. (1998) found a median umbilical artery L/P ratio of 13.7 but neonatal outcomes were not reported. Chou et al. (1998) showed a clearer association between L/P ratio and adverse outcomes in a prospective study of 126 infants. High-risk preterm neonates (with abnormal FHR patterns, meconium aspiration, or low Apgar scores) had a significantly higher umbilical artery L/P ratios at birth compared to healthy preterm controls. The combination of an elevated umbilical artery lactate and L/P ratio predicted neonatal encephalopathy with 100% sensitivity in their cohort. Although more contemporary data is lacking, these findings suggest that interstitial multi-analyte monitoring may provide valuable information on the metabolic response to perinatal hypoxia.

1.3.4 Potential application to intrapartum fetal monitoring

In summary, current understanding of the relationship between blood lactate and interstitial concentrations of lactate and related metabolites remains limited. Nevertheless, available evidence on the use of microdialysis for monitoring interstitial lactate provides an important foundation upon which to base further enquiries. Compared to other techniques for sampling ISF, microdialysis offers several advantages which are deemed pertinent to the present research and, more generally, to its application for intrapartum fetal monitoring:

- Microdialysis is an established technique with commercially available and affordable catheters licensed for *in vivo* studies in animals and humans. The use of these catheters was deemed appropriate for a proof-of-concept study on the relationship between arterial and interstitial lactate, as it minimised the risk of analytical problems, enhanced scope for repeatability and reproducibility, and removed the need to develop and fabricate a custom lactate biosensor.

- Dialysate collection for offline analysis provides greater flexibility with respect to the separation, storage and subsequent analysis of samples. This enables the investigation of multiple analytes in an exploratory manner, whereas customisation of biosensors typically requires primacy of a single analyte of interest. It is also possible to analyse samples across different platforms and, in doing so, validate new analytical devices, such as POC lactate meters. In online microdialysis systems, dialysate may also be collected to provide a means of externally calibrating coupled biosensors, which is not possible for devices monitoring *in situ*.
- Finally, subcutaneous microdialysis is similar in terms of its invasiveness to existing intrapartum procedures, such as FSE application and FBS, and presents minimal biocompatibility issues because fetal tissue is exposed only to inert catheter material, with no risk of chemical leaching and no net exchange of fluid. These considerations are relevant to the longer-term device development pathway, including safety testing and regulatory approval (Cummins et al., 2018).

1.4 Aims, hypotheses and thesis outline

Based on growing evidence of the limitations of fetal heart rate monitoring, there is a clear and urgent need for alternative methods of fetal assessment during labour. A microdialysis-based device which continuously monitors interstitial lactate and related metabolites may improve the detection of hypoxia and evolving acidosis, thereby preventing hypoxic birth injury and avoiding unnecessary obstetric intervention. However, the potential value of this approach remains unclear because current understanding of the relationship between these markers and reference parameters of fetal acid-base status, such as arterial lactate, is limited. Furthermore, recent experience relating to the implementation and evaluation of STAN and other adjunctive technologies has highlighted how difficult the path to clinical translation may be for new fetal monitoring technology.

This proof-of-concept research aimed to explore the potential of monitoring interstitial lactate as a novel approach to intrapartum fetal assessment, and so inform the future development of a microdialysis-based fetal monitoring device. Specifically, the aims of this work were:

1. To investigate the relationship between interstitial and arterial lactate in animal model of perinatal hypoxia.
2. To explore the feasibility of subcutaneous microdialysis for monitoring fetal wellbeing during labour.
3. To validate a POC meter for analysis of microdialysis lactate.
4. To explore the use of adjunctive fetal monitoring technologies in current UK practice, including obstetricians' views towards a new device.

These questions were addressed through two parallel streams of work: *in vivo* animal studies and a qualitative interview study. This work is presented across three chapters in this thesis, with methods and results considered separately for each section.

Chapter 2 describes the development of a neonatal piglet model of hyperlactataemia. Informed by published animal models of perinatal HI, protocols for achieving hyperlactataemia by means of alveolar hypoxia and/or intravenous sodium L-lactate infusion were evaluated and refined during *in vivo* studies in neonatal piglets. A related

aim of this work was to assess skin thickness at potential sites of microdialysis sampling in neonatal piglets.

In **Chapter 3**, the above model was used to investigate the relationship between interstitial and arterial lactate and to assess the potential application of subcutaneous microdialysis for intrapartum fetal monitoring. It was hypothesised that interstitial lactate would correlate positively with arterial lactate and accurately reflect blood lactate trends at rest and in response to hypoxia and re-oxygenation. Secondary objectives and hypotheses related to the feasibility of microdialysis are detailed at the start of this chapter.

A method comparison study of the StatStrip Xpress® lactate meter and reference ISCUS*flex* microdialysis analyser is also presented in Chapter 3. It was hypothesised that the performance of the StatStrip Xpress® for measuring dialysate lactate would be comparable to the ISCUS*flex.*

Chapter 4 presents a qualitative interview study, which aimed to explore how and why obstetricians use adjunctive technologies to monitor fetal wellbeing during labour. Semi-structured interviews were undertaken with obstetricians from across the UK to address five key research questions relating to: their experiences of using adjunctive technologies; factors that influence their attitudes and practice; the development of new fetal monitoring technology; perceived barriers and facilitators to implementing new technology; and priorities for future research.

In **Chapter 5**, the main findings of this thesis are summarised, with specific consideration to the proposed design of a microdialysis-based device for intrapartum fetal monitoring. The implications of this research for current clinical practice are discussed, as well as areas for future research.

Chapter 2 Development of a neonatal piglet model of hyperlactataemia

2.1 Introduction

Current evidence regarding the relationship between interstitial and arterial lactate comes mainly from microdialysis studies in adult humans, discussed in Chapter 1. Several studies have reported good agreement between interstitial and arterial lactate trends in some subjects, but poor agreement in others (Ellmerer et al., 2009, van den Heuvel et al., 2009, Dimopoulou et al., 2011). It is unclear if such conflicting results are due to sampling and analytical methodology, physiological factors (e.g. biological variation between subjects), or some combination thereof. Considerable heterogeneity in the data reported further limits our ability to draw conclusions about the arterio-interstitial relationship in humans. Several microdialysis studies have also been undertaken in experimental animal models of hypoxia (Engidawork et al., 1997, Kusaka et al., 2004, Klaus et al., 2003, Tigchelaar et al., 2020), however, most available data on interstitial lactate is of limited relevance to the proposed application of intrapartum fetal monitoring. Thus, the potential value of measuring interstitial lactate from the fetal scalp during labour as a means of monitoring fetal wellbeing remains unknown.

To answer this question, a clinically relevant animal model is needed to assess the dynamic relationship between interstitial and arterial lactate. An *in vivo* model also allows one to address important questions related to the microdialysis technique itself: for example, how does microdialysis catheter insertion affect the local tissue environment; and what proportion of lactate is recovered in the dialysate? Recovery *in vivo* is not equal to that seen *in vitro*, as the diffusion of analytes depends on several tissue-specific factors, including the tortuosity of the interstitial fluid matrix, cellular uptake and metabolism, and blood flow. Therefore, *in vivo* models are required to estimate true interstitial metabolite concentrations for comparison with reference arterial measurements. Finally, a robust and reproducible animal model provides a valuable platform for researchers designing and evaluating new medical devices

because of the time scale and complexity of the device development pathway.

This chapter discusses the development of a neonatal piglet model of hyperlactataemia and serves as a background to the microdialysis studies described in Chapter 3.

2.1.1 Animal models of perinatal hypoxic-ischaemic injury

The model developed for this study was informed by existing animal models of perinatal hypoxia-ischaemia (HI), which are summarised below.

Rodent HI models are the most widely used due to their cost-effectiveness and convenience, and have contributed greatly to the current understanding of perinatal brain injury (Hamdy et al., 2020). The most common of these is the Rice-Vannucci model (Rice et al., 1981), which involves a combination of transient systemic hypoxia with unilateral ligation of the common carotid artery in postnatal rat pups. Although adapted extensively since its first description, this model does not mimic the insult encountered by the human fetus during labour nor the type of injury produced (Rumajogee et al., 2016). Recent studies have therefore favoured hypoxia-only rodent models to create a more clinically relevant model of perinatal HI injury (Takada et al., 2011, Zhang et al., 2013). One of the major limitations of rodent models, however, is that the structural maturation of the developing brain is markedly different in rodents and humans. The time scale for brain development in large animals, such as sheep, pigs, and non-human primates, is much closer to that of humans (Huang et al., 2017), prompting extensive research into HI models in these species.

Of the large animals, sheep and pigs have been the most widely used for the study of perinatal HI. Brain development in sheep is more advanced at birth compared to humans (Koehler et al., 2018) but they benefit from a long gestation period, allowing investigators to select the timing of the insult and its evaluation flexibly in order to address the research questions. Compared to rodent models, their size also facilitates instrumentation and chronic monitoring of different organ systems and biological events (Huang et al., 2017). For these reasons, the late-gestation ovine fetus (135 days or 0.9 gestation) presents the most established model of *in utero* fetal hypoxia, achieved through intermittent occlusion of the umbilical cord (Gunn et al., 1992, Gardner et al., 2002, Castillo-Melendez et al., 2013). This mechanism not only induces

tissue hypoxia and acidosis, but also results in episodes of ischaemia-reperfusion, which promote the generation of damaging reactive oxygen species (Derks et al., 2010). The ovine fetus is also the model which has been most frequently used to assess intrapartum fetal monitoring technology, such as fetal pulse oximetry (Nijland et al., 1996) and STAN (Blad et al., 2008, Andriessen et al., 2018). While this makes it an attractive option for evaluating microdialysis-based monitoring, an *in utero* large animal model was not considered feasible given the time and resource constraints of this exploratory study. Experimental and animal care costs, as well ethical considerations in the use of a higher order primate species, have similarly restricted the application of non-human primate models of hypoxia-ischaemia, which are usually regarded as the ideal animal model due to their physiological similarities to humans and longer survival (Inder et al., 2004).

The neonatal piglet is an established HI model and offered several advantages for this translational research project. Firstly, research has demonstrated that the relative maturity of the lungs, cardiovascular system, and brain of piglets (Dobbing and Sands, 1979, Pond et al., 2000, Eiby et al., 2013) is similar to that of early term human infants. Secondly, like other large animals, their size at birth (1-2 kg) allows for easier instrumentation and detailed physiological monitoring. In practical terms, piglets are also relatively inexpensive and their large litter size provides an opportunity for the study of outcomes across litter-matched pairs, or in spontaneously growth restricted offspring, which are a common occurrence (Eiby et al., 2013). Finally, porcine skin is considered the optimal model for human skin in terms of its anatomy and physiology (Sullivan et al., 2001, Debeer et al., 2013), immunology (Summerfield et al., 2015), and mechanical properties (Ranamukhaarachchi et al., 2016). The skin of rodents and other small animals, by contrast, is thinner and loosely attached to the underlying connective tissue (Debeer et al., 2013, Zomer and Trentin, 2018). These factors make pigs an ideal candidate model for developing and evaluating the performance of a subcutaneously implanted device.

Regardless of the species used, the vast majority of research conducted to date in animal models of perinatal HI has been directed towards investigating the pathophysiological mechanisms of hypoxic brain injury and/or developing neuro-protective interventions, such as therapeutic hypothermia. Notably, very few studies

have measured or reported blood lactate levels in their subjects. It is therefore difficult to ascertain the appropriateness of existing models to address the key research questions in this study.

2.1.2 Existing neonatal piglet models

Having identified the pig as a pragmatic and clinically relevant choice of species, previously published studies of neonatal piglet HI models formed the basis for developing the current model of hyperlactataemia. Models of alveolar hypoxia were selected, to avoid the additional complexity of protocols combining hypoxia with either complete airway occlusion (Martin et al., 1997) or surgical ligation of the carotid arteries (Thoresen et al., 1995). In alveolar hypoxia, hypoxaemia is achieved by reducing the fraction of inspired oxygen (FiO_2) and tissue ischaemia results from the subsequent failure of compensatory adaptations. However, there is considerable variation amongst published models in the severity and duration of the hypoxic insult employed. To date, only one study has systematically reported blood lactate levels pre- and post-hypoxia (Garberg et al., 2016), so providing a direct frame of reference for the current protocol. Mean arterial lactate levels rose from 1.6-2.2 mmol/L at baseline to 14.0-16.4 mmol/L following approximately 40 min of hypercapnic hypoxia (FiO_2 8%) in their study of 55 newborn piglets. Kyng et al. (2015) reported similar results (peak lactate of 19 mmol/L based on representative data) following exposure to a variable hypoxic insult, in which FiO_2 was adjusted to achieve low amplitude electroencephalography activity in each piglet. Several other studies have used variable FiO_2, titrated to the physiological responses of individual animals, with the aim of more consistent neuropathological damage and improved piglet survival (Björkman et al., 2006, Chakkarapani and Thoresen, 2015). The aim of the present model was to achieve elevated arterial lactate levels in as many piglets as possible, therefore a relatively cautious, flexible approach to hypoxia was initially planned (Richards et al., 2006, Cheung et al., 2011), reducing FiO_2 to 10-14% with subsequent titration to achieve pre-defined target endpoints for arterial lactate concentrations.

Alternative methods of achieving hyperlactataemia were also considered, in the event that lactate targets could not be reliably achieved through alveolar hypoxia. Previous studies in adult pigs have administered concentrated lactate solutions intravenously to

investigate lactate metabolism (Barthelmes et al., 2010) and the performance of subcutaneous lactate sensors (Wolf et al., 2018). Historically, sodium lactate challenges have also been widely used as a provoking agent for the investigation of panic disorders in both animal and human subjects (Rifkin and Siris, 1984, Olsson et al., 2002). Whilst such an approach does not reflect the pathophysiology of hyperlactataemia seen in intrapartum fetal hypoxia, it was nevertheless seen as a potentially valuable means of exploring the relationship between interstitial and arterial lactate.

2.2 Aims

The principal aim of this pilot study was to develop a perinatal animal model of hyperlactataemia. This model would be used to assess the relationship between interstitial and arterial lactate and serve as a platform for developing and evaluating a microdialysis-based monitoring device in future, larger-scale studies. The specific objectives were:

- To investigate skin thickness in neonatal piglets at potential sites of microdialysis sampling.
- To describe a protocol for achieving elevated arterial lactate concentrations (target peak lactate $\geq$ 12 mmol/L) in neonatal piglets, by means of alveolar hypoxia and/or sodium L-lactate infusion.

2.3 Methods

All piglet studies were planned and undertaken in close collaboration with researchers at the University of Edinburgh's Wellcome Trust Critical Care Laboratory for Large Animals (WTCCLLA), led by Professor Ed Clutton. They were responsible for transport, anaesthetic induction and surgical instrumentation of the animals, and arterial sampling. I undertook all microdialysis catheter insertions, whilst retaining general oversight of the experiments. The study protocol and amendments were approved by the University of Edinburgh's Animal Welfare and Ethical Review Body and the Home Office. This was a non-recovery study conducted under general anaesthesia and live

animals were killed by a Schedule 1 method, in line with the Animals (Scientific Procedures) Act 1986. The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines were used to formulate the study protocol and were referred to regularly when conducting experiments (Smith et al., 2018).

2.3.1 Experimental design

The experimental design chosen for the study reflected its exploratory nature and the salience of the primary aim of this research: *to investigate the relationship between interstitial and arterial lactate concentrations in an animal model of perinatal hypoxia*. Specifically, a single experimental protocol was used for all piglets during *in vivo* studies, albeit this protocol was refined as the project progressed to meet pre-defined targets for hyperlactataemia. Due to the small sample size, there was no control or sham group (in which piglets were not subjected to hypoxia or other methods to achieve hyperlactataemia) and therefore no requirement for randomisation or blinding.

2.3.1.1 Sample size

Although formal power tests were not possible, an initial sample size of eight to ten pigs was considered appropriate to obtain preliminary data on the relationship between interstitial and arterial lactate and to explore the potential value of microdialysis-based monitoring. It was acknowledged that past microdialysis studies in humans, with sample sizes typically $n > 10$, have shown marked inter-individual variability in the agreement between these compartments, which may not be reflected in smaller groups (Ellmerer et al., 2009, van den Heuvel et al., 2009, Dimopoulou et al., 2011).

This sample size was also considered realistic for developing a reliable protocol for achieving hyperlactataemia. Although the WTCCLLA research team had extensive experience in conducting studies of this nature in adult pigs, anaesthetising and instrumenting newborn piglets presented new technical challenges and uncertainties. Amongst these, tolerance to hypoxia has been shown to vary considerably between individual piglets (Björkman et al., 2006), therefore I anticipated making several adjustments to the hypoxia protocol to consistently achieve target arterial lactate levels.

Finally, as the piglets were studied in pairs in line with the farrowing schedule of the source farm, it was only possible to make protocol changes after completing each study day, with significant amendments requiring formal ethical review and approval.

To maximise data collection, two microdialysis catheters were inserted into each piglet for simultaneous sampling. This also allowed for the direct comparison of results obtained from different catheter sites and/or at different perfusate flow rates. Based on a maximum study duration of 8 h, approximately 30 dialysate samples were expected per catheter, generating 450-500 dialysate samples in total. However, problems encountered during the first study day meant that no dialysate samples were recovered from piglet A and data from piglet B were also potentially affected. Approval and funding were therefore secured for further studies in four pigs, which would have brought the sample size to 12 piglets (11 providing microdialysis data). However, it was not possible to complete these studies due to the emerging COVID-19 pandemic.

2.3.1.2 Cadaveric training and histology

Prior to *in vivo* studies, access was obtained to three fresh piglet cadavers, which were found overlaid (crushed) by the sow. Firstly, this provided an opportunity for *ex vivo* training on subcutaneous insertion of two microdialysis catheters: the 63MD catheter (M Dialysis AB, Sweden), which was chosen for subsequent *in vivo* studies, and the CMA 20 Elite catheter (CMA Microdialysis AB, Sweden). Secondly, skin biopsies were obtained from three potential sites of microdialysis sampling to assess the epidermal and dermal thickness of piglet skin. In brief, full thickness skin samples were obtained by sharp dissection from three sites on one piglet: the skin overlying the frontal bone (referred to as the frontal scalp), the skin posterior to the nuchal crest, and the post-auricular area (Figure 2.1). Final biopsy samples were cut to approximately 1 x 1 cm and fixed in 4% formaldehyde (Sigma Aldrich, UK) before being paraffin embedded, sectioned, and stained for Haematoxylin and Eosin (H&E) by personnel at the Shared University Research Facilities (Queen's Medical Research Institute, University of Edinburgh) for confocal microscopy.

2.3.2 Study protocol

On each study day, two commercial piglets (Landrace/Large White cross, male or female, 6-48 hours of age) were studied in a staggered manner, as illustrated by the study timeline in Figure 2.2. The piglets were transported individually from a local source farm to the research facility (WTCCLLA, Dryden Farm) immediately prior to experimentation. Piglets were required to have suckled before transport, as observed by the stockperson at the farm, and were transported in straw-bedded, pre-heated containers with the journey time not exceeding 20 min. On arrival, the piglets underwent mask/chamber inhalational induction with sevoflurane 1-5% in 100% oxygen, followed by endo-tracheal intubation (ETI). Central vascular access (jugular vein and carotid artery) was established by the anaesthetic team and a baseline arterial blood gas (ABG) performed. The urinary bladder was cannulated by means of an indwelling Foley catheter due to the study duration. Physiological monitoring included direct arterial blood pressure, central venous pressure, pulse oximetry, ECG, and rectal temperature, which was maintained between 37-39.5°C by means of a Bair Hugger™ warming blanket (3M, UK) and overlaid silver space blanket.

General anaesthesia was maintained via isoflurane in oxygen:air (FiO_2 45-55%) and partial intravenous anaesthesia, comprising alfaxalone, morphine, medetomidine and midazolam (overall volume 0.5 ml/kg/hour, administered via jugular venous catheter). Pigs were initially mechanically ventilated to normocapnia ($PaCO_2$ 35-45 mmHg) at a rate of 20-30 breaths/min with a tidal volume of 10-12ml/kg, inspiratory: expiratory ratio of 1:2.5, and positive end-expiratory pressure of 5 cm H_2O, using an Aestiva anaesthesia delivery machine (GE Medical Systems Ltd, UK). Two subcutaneous microdialysis catheters were inserted on contralateral sides of the frontal scalp, with sampling undertaken as detailed in Chapter 4. ABGs were obtained every 30-60 min during baseline periods of normocapnia and every 15-20 min during hyperlactataemia, adhering to recommendations on blood removal in animal research (Diehl et al., 2001). The protocol permitted up to 20 ABGs, each with a sample volume of less than 0.3 mL; this equated to approximately 10% of total blood volume (6 mL) for a piglet weighing 1.0 kg with a minimum blood volume of 60 ml/kg (Linderkamp et al., 1981). Samples were analysed with the epoc® Blood Analysis System (Siemens Healthcare Limited, UK) for lactate, glucose, temperature-corrected pH, $PaCO_2$, and PaO_2, as well as calculated values for haemoglobin (Hb) and BE [ecf].

Following an initial baseline period for equilibration and calibration of the microdialysis catheters, FiO_2 was reduced by adding nitrogen to the inhaled gas mix via a free-standing cylinder. FiO_2 was reduced to 10-14% and further adjustments made in response to serial ABG results, with the aim of achieving peak lactate concentrations $\geq$ 12 mmol/L. If signs of severe hypoxia were identified (bradycardia or refractory hypotension i.e. mean arterial blood pressure < 35 mmHg) then FiO_2 was increased and additional supportive measures were taken as appropriate, e.g. administration of an intravenous colloid bolus or noradrenaline. Adjustments to the ventilation rate and/or inspired carbon dioxide were utilised to maintain baseline normocapnia ($PaCO_2$ 35-35 mmHg) and mild hypercapnia during hypoxia ($PaCO_2$ > 45mmHg), wherever possible. Piglets were re-oxygenated at individualised FiO_2, either when target lactate levels were achieved or if the maximum study duration was reached. Animals were killed by an overdose of intravenous phenobarbital (40 mg/kg) followed by confirmation of permanent cessation of circulation.

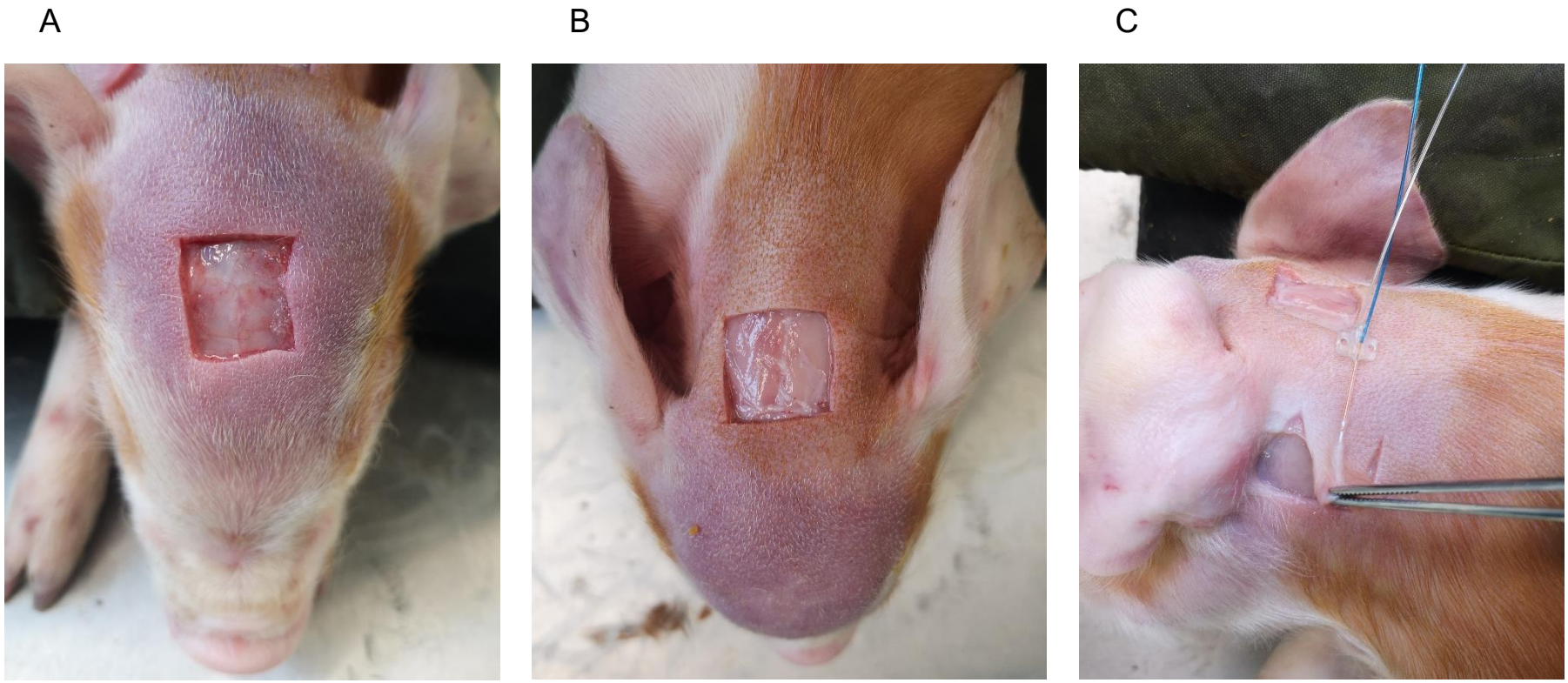

Figure 2.1 Sampling sites for skin histology in piglet cadaver. *(A) Scalp overlying frontal bone – site chosen for 63MD catheter insertion in vivo; (B) posterior to nuchal crest; and (C) post-auricular area, with CMA 20 microdialysis catheter (CMA Microdialysis AB, Sweden) shown in situ.*

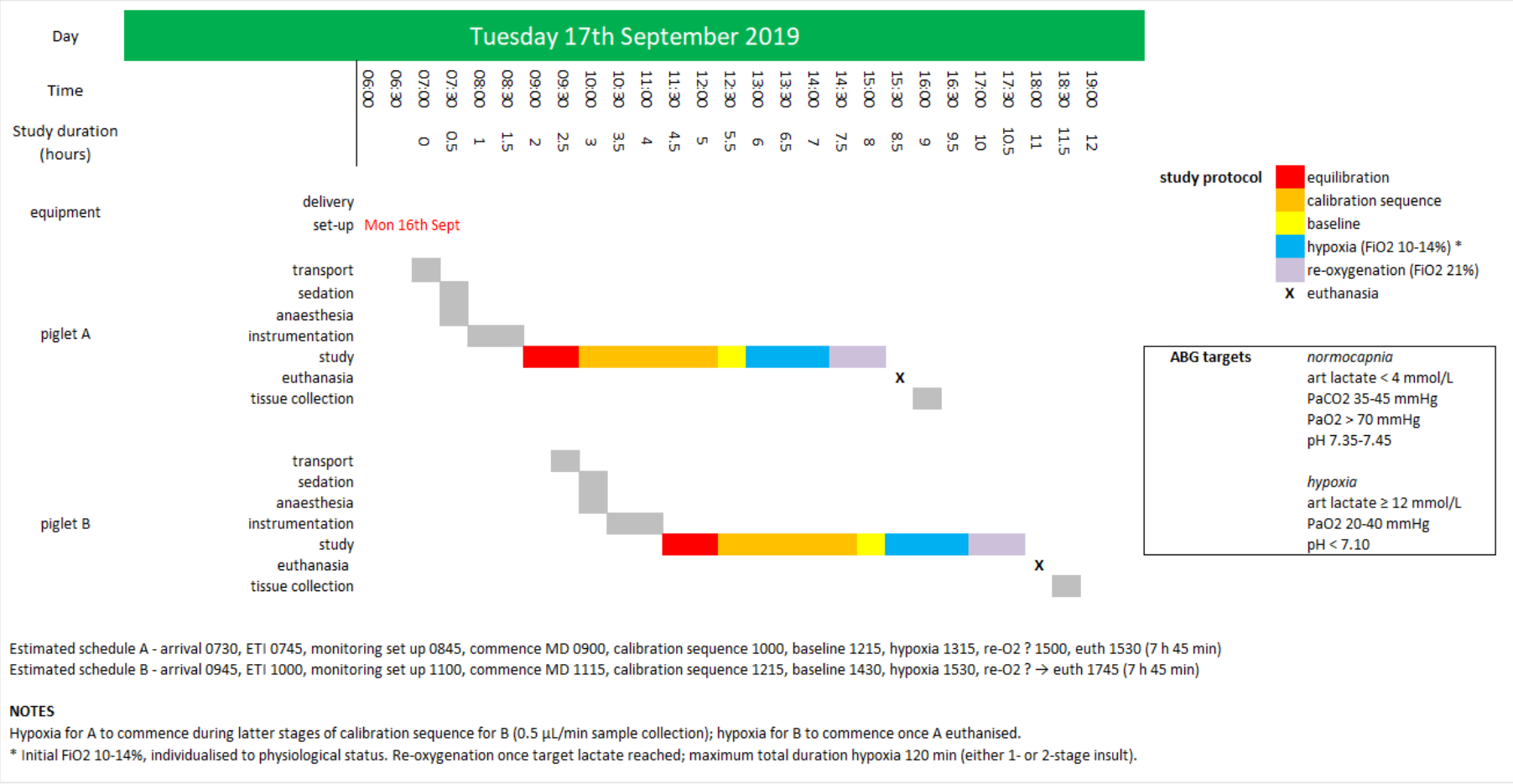

Figure 2.2 Example timeline for *in vivo* piglet studies. *Timeline shows staggered arrival and experimental protocol for two piglets from same litter. Interventions (e.g. hypoxia) were timed to optimise the flow of the study, taking into account the frequency of ABG and microdialysis sampling and personnel available for undertaking these procedures. Pre-defined ABG targets are shown in box on right.*

2.3.3 Protocol amendments

The initial protocol limited the duration of the study to 8 h from anaesthetic induction to euthanasia. However, the time taken to establish monitoring and complete both equilibration and calibration for the microdialysis system exceeded 5 h, which severely restricted the hyperlactataemia phase of the protocol during the first two study days. The study duration was therefore extended to 12 h for piglets E through H.

The other main difficulties that emerged in early studies were: (1) consistently achieving target lactate concentrations above 12 mmol/L, and (2) predicting the timing of changes in arterial lactate. This was exacerbated by the limited number of ABG samples permitted in each piglet during the 8-12h study period. These factors limited the temporal resolution of the study data, especially in relation to any time lag between the arterial and interstitial compartments. A protocol for administering an intravenous lactate challenge was therefore developed and approved for use in all studies from piglet G onwards. A 2.0 M solution of sodium L-lactate salt (Sigma Aldrich, UK) in 0.9% normal saline was prepared and stored at room temperature. This was infused via the jugular venous catheter using sterile syringe filters with 0.2 μm pore size (Cole-Parmer, UK) at an initial rate of 0.5 ml/kg/min. After commencing the infusion, arterial lactate was measured every 10 min using a StatStrip Xpress® POC lactate meter (Nova Biomedical, Waltham, MA USA). The smaller sample volume meant sampling could be undertaken more frequently during the lactate challenge, with adjustments made to the infusion rate accordingly. ABGs were also undertaken every 20 min for reference arterial lactate measurements on the previously described epoc® system.

2.3.4 Statistical analysis

Descriptive data are presented for individual piglets due to the small sample size, unless otherwise stated – for example, median values and interquartile range (IQR) for weight, haemoglobin, and hypoxia duration. Accordingly, no formal statistical analyses were undertaken in this chapter.

2.4 Results

The results of the cadaveric and *in vivo* piglet studies are reported below, following the recommendations of the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines 2.0 (Percie du Sert et al., 2020). Expectedly, significant adjustments were required after every study to refine the anaesthetic and hypoxia protocols, which limited direct comparisons between the animals. The absence of a control group and small sample size also meant it was preferable to present physiological and ABG data for individual piglets. These data have been grouped, where appropriate, by the date of experimentation and further reflections are made on the key findings from each of the four study days, which informed ongoing development of the model.

2.4.1 Catheter site selection (cadaveric studies)

Histology images from the piglet cadaver are presented in Figure 2.3. Dermal thickness and full skin thickness measurements from the frontal scalp (see also Figure 2.1A) were 586 and 708 μm, respectively. Although systematic comparisons were not made between the three biopsy sites, dermal and epidermal thickness were broadly similar. However, the frontal scalp was characterised by a relatively thin layer of connective tissue between the lower dermis and cranial structures (Figure 2.3B). This contrasted with a thicker, adipocyte-rich layer seen between the dermis and underlying skeletal muscle in both the posterior nuchal and post-auricular biopsies (Figure 2.3D). The potential relevance of these results to future *in vivo* studies was two-fold:

Firstly, based on the skin thickness measurements and known dimensions of the 63MD catheter (shaft outer diameter 0.9 mm), it was possible to conclude that the microdialysis membrane was sampling from the interstitial fluid of the hypodermis (or subcutaneous connective tissue layer) rather than being positioned intra-dermally. In relation to the design of a custom microdialysis catheter for fetal monitoring, this also indicates that existing fetal scalp electrodes should penetrate the full thickness of neonatal piglet skin when correctly applied. Secondly, these data supported the selection of the scalp as a potentially valuable site for microdialysis sampling, beyond the simple fact that it is the only area of the fetus accessible during labour. The scalp is known to have the richest vascular supply of any area of skin in the body (Standring et al., 2021) and, theoretically, the thinner hypodermis of the frontal scalp may improve lactate recovery due to the proximity of the microdialysis membrane to local blood

vessels and lower tissue resistance to analyte diffusion. The posterior nuchal and post-auricular skin, by comparison, may bear closer resemblance to sites chosen for sampling in previous animal and human microdialysis studies, namely the subcutaneous adipose tissue of anterior abdominal wall.

For *in vivo* studies, financial and practical constraints meant that it was only possible to use two microdialysis catheters per piglet. Based on these results, the decision was therefore made to sample simultaneously from contralateral sides of the frontal scalp, i.e. sites with identical tissue properties, rather than different anatomical areas. This also allowed the direct comparison of microdialysis data at different perfusate flow rates, as discussed in Chapter 3.

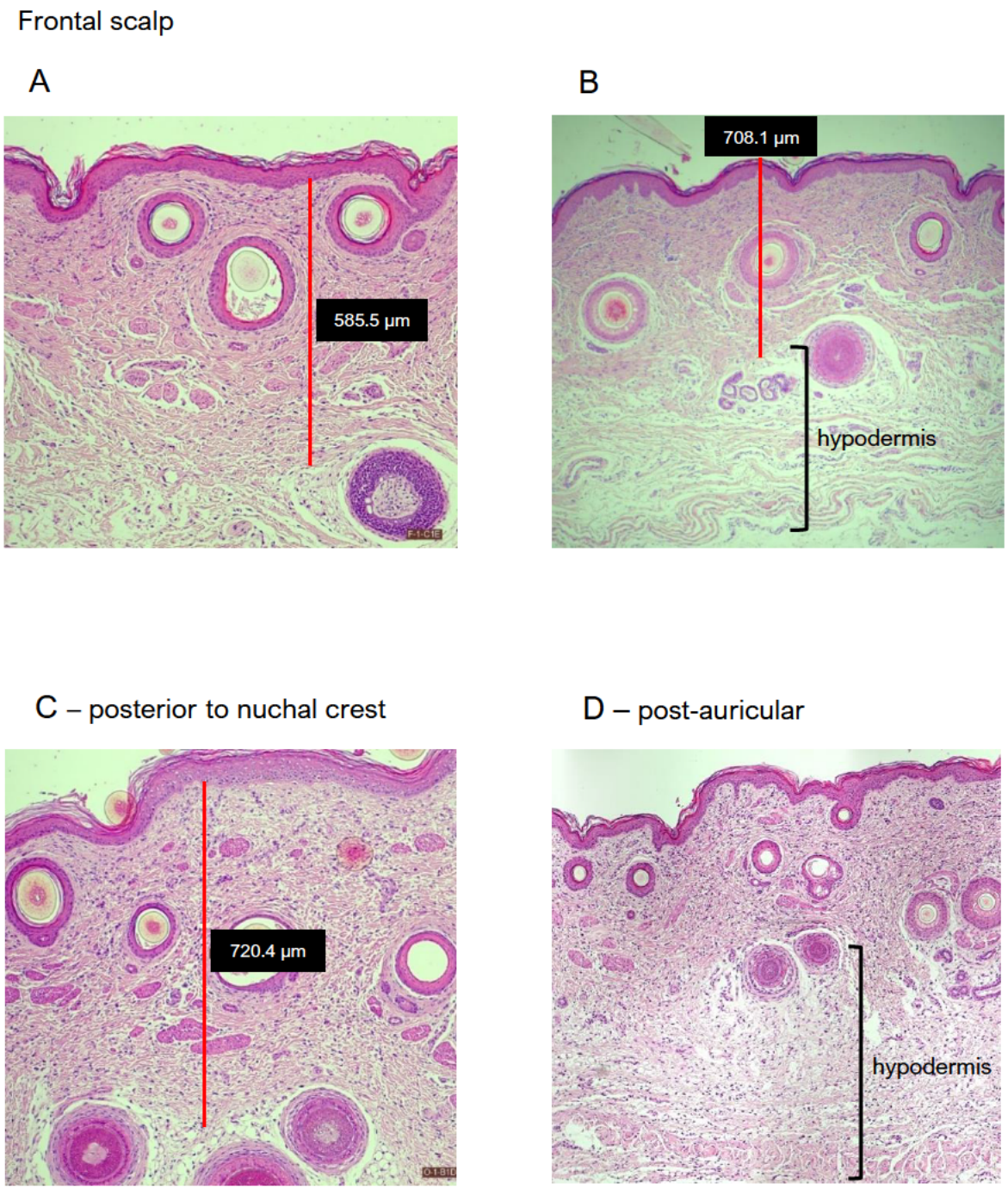

Figure 2.3 H&E-stained sections from cadaver skin biopsy sites. *Three sites were sampled in a single neonatal piglet cadaver: the scalp overlying the frontal bone (A-B), the skin posterior to the nuchal crest (C), and the post-auricular area (D). Representative measurements are shown for dermal (images A and C) and total skin thickness (B). The relatively thin hypodermis of the frontal scalp (B) contrasts with the thicker, adipocyte-rich hypodermis of the posterior nuchal and post-auricular skin (D).*

2.4.2 Animal characteristics

In vivo experiments were undertaken in eight Landrace/Large White neonatal piglets (n=8) over four study days between September 2019 and February 2020. All piglets were 12 to 48 h of age at the time of transport. Following anaesthetic induction and endotracheal intubation, there was a median interval of 104 min (IQR 97-107) prior to the start of dialysate collection, during which instrumentation was undertaken. In a small number of cases, microdialysis was briefly delayed in order to optimise the timing and flow of parallel studies.

Table 2.1 summarises the main characteristics of the animals and their initial ABG results, obtained after surgical instrumentation and immediately prior to commencing microdialysis. In keeping with past studies (Egeli et al., 1998, Eiby et al., 2013), there was considerable variation in body weight with median weight of 1.80 kg (IQR 1.28-1.95). Litter-matched pairs were generally of similar weight, excepting piglets C and D. Median calculated haemoglobin was 7.7 g/dL (IQR 7.0-8.4), which is consistent with established reference ranges in day 1 Landrace piglets (Egeli et al., 1998). Baseline ABG results prior to commencing microdialysis were broadly comparable in six of the eight piglets: arterial lactate ranged from 1.71 to 3.20 mmol/L, with pH and base excess values suggesting normal or near-normal acid-base status.

Table 2.1 Animal characteristics and baseline bloods. *ABG results shown after instrumentation and immediately prior to commencing microdialysis. pH and $PaCO_2$ values are temperature corrected; BE [ecf] and Hb are calculated.*

Piglet	Gender	Weight (kg)	Lactate (mmol/L)	pH	$PaCO_2$ (mmHg)	BE [ecf] (mmol/L)	Hb (g/dL)
A	F	1.3	7.20	6.87	100.3	-15.4	4.4
B	M	1.2	2.66	7.35	50.2	2.3	8.0
C	F	1.0	3.20	7.47	32.6	0.0	9.8
D	F	1.8	2.37	7.41	43.3	2.9	9.7
E	F	1.8	1.93	7.34	44.6	-2.1	7.3
F	M	1.9	2.18	7.35	56.1	5.1	7.9
G	F	2.3	1.71	7.40	46.9	4.4	7.5
H	F	2.1	6.14	7.47	29.6	-2.3	5.9

The two exceptions at baseline were piglets A and H, both of whom had markedly elevated arterial lactate concentrations initially. In piglet A, this was associated with severe anaemia (Hb 4.4 g/dL) and a profound metabolic acidosis (tc pH 6.87, BE [ecf] -15.4). There were no problems during induction or instrumentation to account for these findings, nor was there any identifiable source of bleeding initially. However, during the first hour of microdialysis sampling, excessive bleeding from both MD catheter insertion sites was noted, which persisted after removing the catheters and applying pressure. This was accompanied by local haematoma formation (Figure 2.4) and a further fall in Hb to 3.9 g/dL. Arterial lactate did not improve in response to supportive measures and there was a progressive deterioration in haemodynamic status. As such, it was not possible to collect microdialysis data and the decision was made to end the study prematurely and prior to any hypoxic insult (euthanasia 3 h 13 min after intubation). Piglet H had a mild anaemia (5.9 g/dL) and normal acid-base

status at baseline despite an elevated arterial lactate of 6.14 mmol/L. Lactate gradually normalised during the microdialysis equilibration and calibration periods, allowing the study to progress as per protocol.

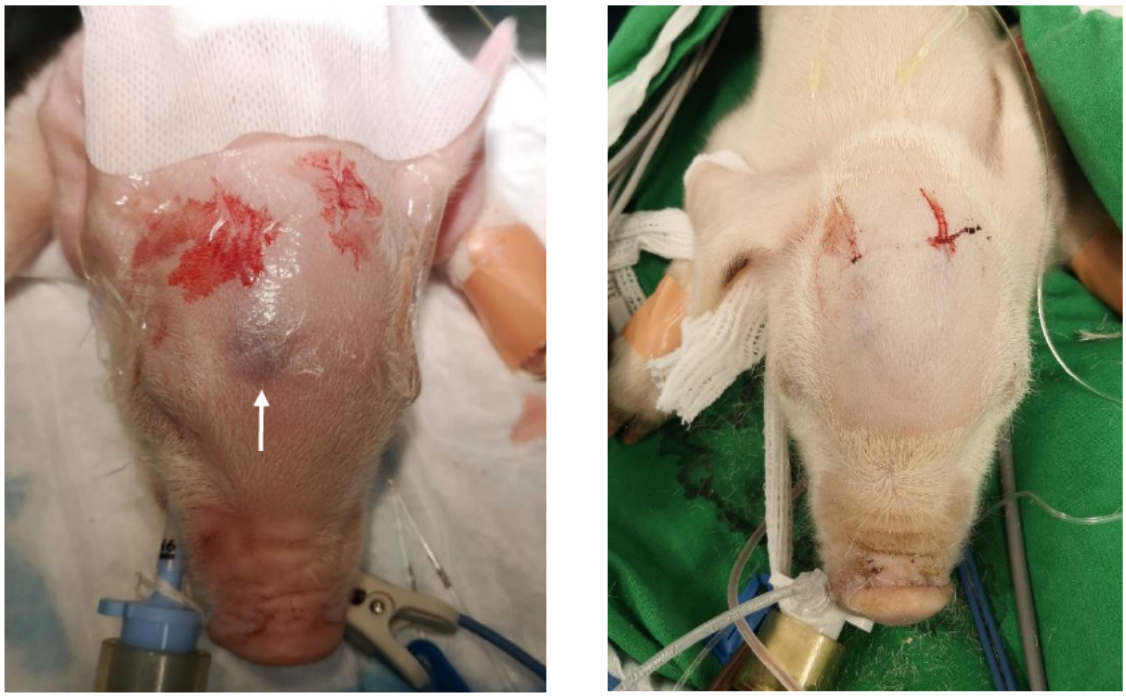

Figure 2.4 Microdialysis catheter-related trauma. *Large haematoma formation (arrow) and bleeding at the sites of microdialysis catheter insertion in piglet A (left). Bruising and local bleeding was evident, but to a lesser degree, in remaining piglets (piglet D shown on right, following euthanasia).*

2.4.3 Alveolar hypoxia

In piglets B to F, hyperlactataemia was achieved by reducing FiO_2. Minimum FiO_2 values were similar, with the total duration of hypoxia ranging from 61 to 116 min (median 81 min, IQR 80-86). The response to hypoxia varied, with pre-defined target lactate concentrations above 12 mmol/L reached in only two of five piglets. Nadir PaO_2 measurements below 40 mmHg (target 20-40 mmHg) occurred during hypoxia in all piglets, however, calculated arterial oxygen saturations (cSO_2) varied widely from 13.5% to 73%. Arterial lactate typically peaked in the early stages of re-oxygenation. Despite moderate to severe base deficits, no piglets achieved a temperature-corrected pH lower than 7.10. In several cases, this was related to $PaCO_2$ disturbances during hypoxia and the development of a respiratory alkalosis. Table 2.2 summarises key ABG and physiological parameters immediately prior to and during hypoxia. Time series graphs of arterial lactate concentration and FiO_2 for individual piglets are shown in Figure 2.5. Possible explanations for these observations are discussed further below, according to the date of experimentation. Piglets G and H are considered separately in the following section.

Table 2.2 ABG and physiological parameters in relation to hypoxia. *Nadir PaO_2 occurred during hypoxia, whilst peak lactate concentrations typically occurred in the early stages of re-oxygenation. Minimum FiO_2 values are shown; PaO_2 values are temperature corrected.*

	Prior to hypoxia			*During hypoxia and/or re-oxygenation*			
Piglet	**PaO_2 (mmHg)**	**Lactate (mmol/L)**	**BE [ecf] (mmol/L)**	**PaO_2 (mmHg)**	**Lactate (mmol/L)**	**BE [ecf] (mmol/L)**	**FiO_2 (%)**
B	160.1	3.39	-1.6	39.8	14.08	-16.0	9.4
C	198.6	1.54	-2.2	21.9	4.93	-4.0	7.7
D	354.9	1.56	4.2	13.8	6.13	-6.9	7.5
E	78.4	1.32	-1.2	24.6	10.49	-10.7	8.5
F	67.3	1.72	1.2	20.4	16.00	-13.1	8.0

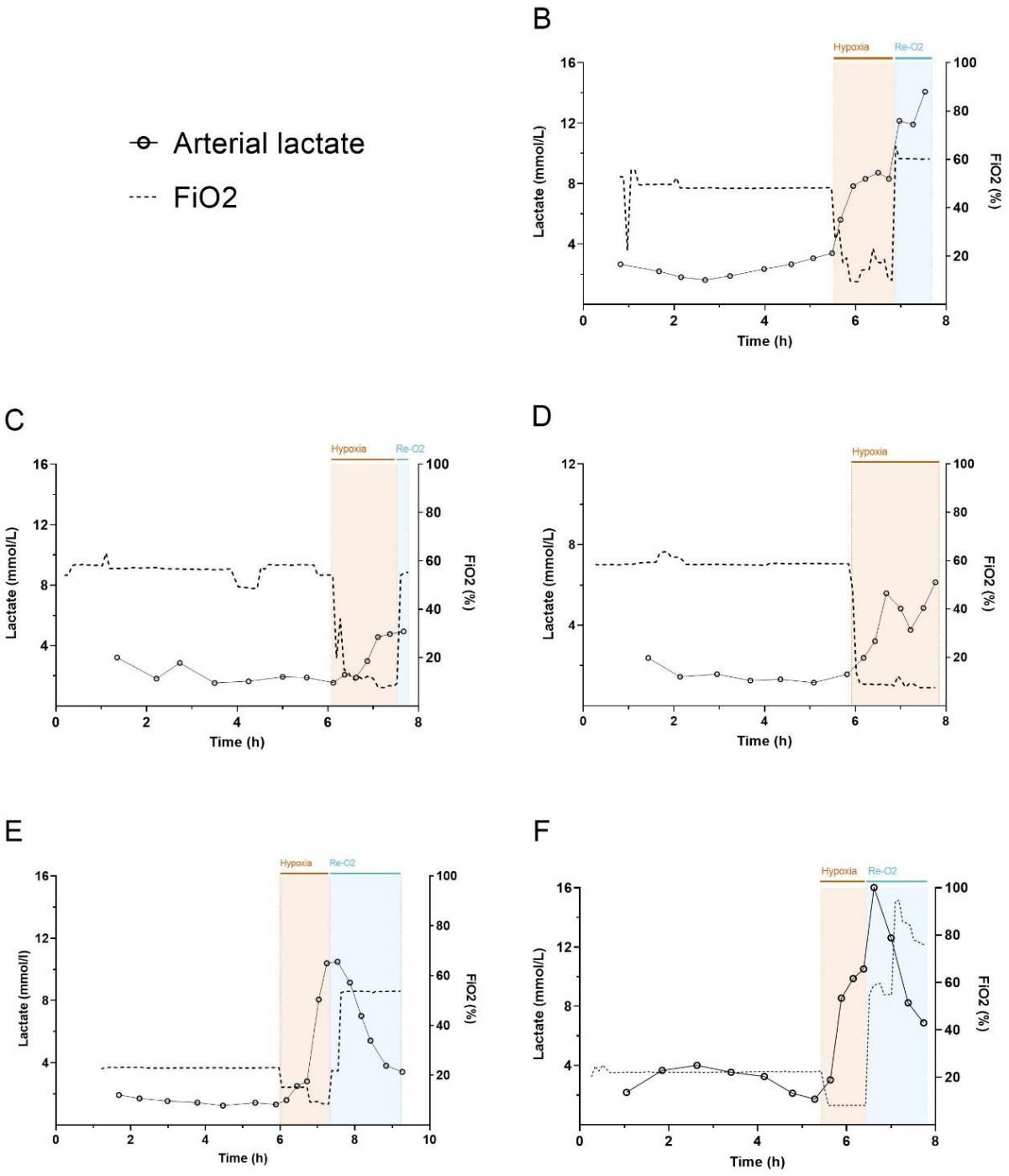

Figure 2.5 Arterial lactate trends in relation to hypoxia. *Time series graphs for piglets B to F, showing arterial lactate concentration in response to changes in FiO_2 during hypoxia and re-oxygenation ('Re-O2'). In piglets B and D, the additional of inhaled CO_2 led to a transient rise in FiO_2 during hypoxia.*

2.4.3.1 Study 1 – piglet B

Piglet B had a normal lactate, acid-base status and Hb at baseline. However, as with piglet A, bleeding was noted from both catheter insertion sites during the first hour of

microdialysis sampling. A bedside activated clotting time (ACT) test performed at this time was found to be significantly elevated at 727 seconds, compared to reported normal mean value of 107 s in porcine blood (Martini et al., 2008). This confirmed the research team's evolving suspicion that piglets A and B had become coagulopathic due to heparin administered to maintain arterial catheter patency, as is standard practice in adult pigs. The heparin infusion was stopped and visible bleeding from the MD catheter sites in piglet B subsequently resolved, allowing the study to progress. Serial ACT measurements remained persistently high for several hours but gradually decreased to 231 s by the study's completion.

During hypoxia, FiO_2 was reduced in a stepwise manner to 9.4% with a corresponding nadir PaO_2 of 39.8mmHg. Arterial lactate rose rapidly initially, then plateaued and decreased slightly in response to a brief increase in FiO_2; this was due to the addition of inhaled CO_2 via a separate anaesthetic circuit, which required a minimum volume of O_2 to be delivered as part of the gas mix. When FiO_2 was reduced again, lactate rose steeply and progressively with no sign of recovery following re-oxygenation at an FiO_2 of 60% (Figure 2.5).

Of note, piglet B was progressively anaemic during the study with Hb falling from 8.0 g/dL to 'could not calculate' (epoc® device manufacturer's stated lower limit of measurement range is 3.3 g/dL). This may have been due to concealed blood loss from the aforementioned coagulopathy, combined with an increased requirement for fluid resuscitation and resulting haemodilution. Mean arterial pressure (MAP) gradually fell and heart rate (HR) increased over the same period, as shown in Figure 2.6. These changes may help to explain the two-fold rise in arterial lactate observed in piglet B prior to the onset of hypoxia, as well as its failure to recover during re-oxygenation. In conclusion, although target lactate levels were achieved, the hyperlactataemia observed in piglet B did not appear to be solely related to experimental FiO_2 adjustments.

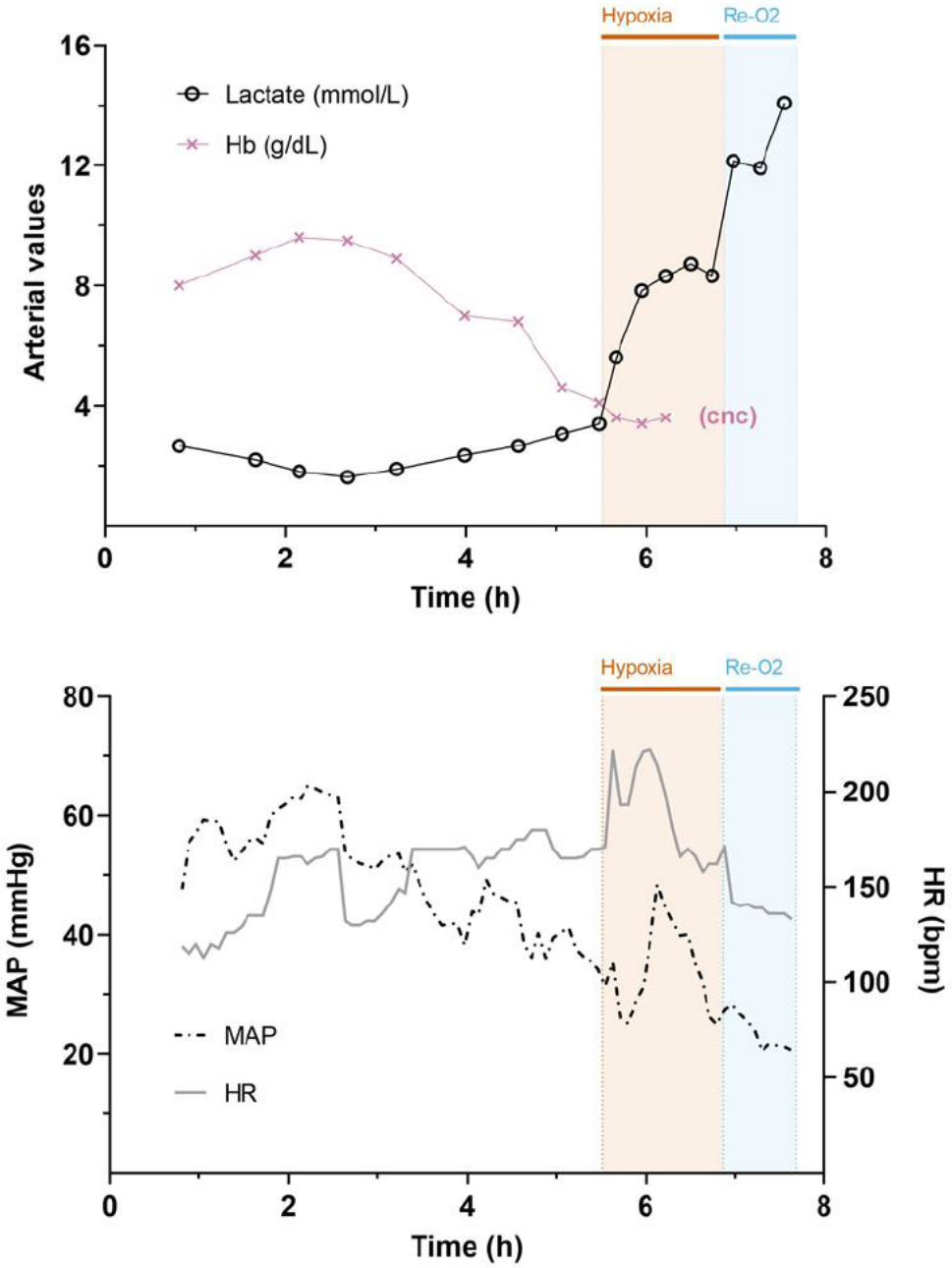

Figure 2.6 Detailed time series for piglet B. *Trends in Hb compared to arterial lactate (upper) and haemodynamic parameters (lower) are indicative of progressive anaemia and compensatory responses prior to the onset of hypoxia.*

2.4.3.2 Study 2 – piglets C and D

Refinements were made to the protocol in response to the issues encountered during the first study day. Carotid arterial catheter patency was maintained by means of an un-heparinised infusion of 0.9% saline (or Hartmann's) at a rate not exceeding 4 ml/kg/h, to minimise the risk of haemodilution. ACT measurements were performed on serial ABG samples, which demonstrated stable clotting times (126-138 s in piglet C, 138-146s in piglet D). To further limit haemodilution and avoid hyperglycaemia, intravenous glucose 5% was administered only if arterial glucose fell below 3 mmol/L. As a result of these refinements, haemoglobin concentrations were stable in all studies from piglet C onwards (range 6.9 to 8.6 g/dL prior to euthanasia).

Compared to piglet B, arterial lactate rose more slowly and to lower peak levels in piglets C and D, despite lower PaO_2 levels and longer durations of hypoxia. This supported the hypothesis that piglet B's hyperlactataemia had been due, at least partly, to evolving anaemia. In piglet C, lactate rose from 1.54 to 4.93 mmol/L following 86 min of hypoxia, with a minimum FiO_2 of 7.7% and nadir PaO_2 of 21.9 mmHg. This modest rise in arterial lactate concentration was consistent with relative haemodynamic stability throughout the protocol, as shown in Figure 2.7. During hypoxia, there was a brief spike in heart rate associated with involuntary jerking movements, which suggested an insufficient depth of anaesthesia; this resolved with an intravenous bolus of alfaxolone (2.5mg/kg) and no adjustments to FiO_2 were required. In acid-base terms, $PaCO_2$ fell to 17.2 mmHg resulting in a respiratory alkalosis (tc pH 7.60) and mild base deficit (BE [ecf] -4.4 mmol/L); a similar pattern was initially observed in piglet B. Due to time constraints, piglet C did not receive additional CO_2 and only underwent a short period of re-oxygenation at FiO_2 55%, without any corresponding decrease in arterial lactate prior to euthanasia.

The total duration of hypoxia in piglet D was 116 min, with a minimum FiO_2 of 7.5% and nadir PaO_2 of 13.8 mmHg. Arterial lactate rose rapidly from 1.56 to 5.58 mmol/L following the onset of hypoxia, accompanied by a more marked haemodynamic response than that of piglet C (Figure 2.7). The initial rise in lactate was associated with a reduction in $PaCO_2$. As with piglet B, CO_2 was therefore introduced via a second anaesthetic circuit to achieve normocapnia, which led to a transient improvement in oxygenation and reductions in both arterial lactate and HR. Thereafter FiO_2 was

reduced, generating a secondary rise in lactate to its peak concentration of 6.13 mmol/L, which resulted in a mild metabolic acidosis (pH 7.25, BE [ecf] -6.9 mmol/L) prior to euthanasia. Piglet D did not undergo re-oxygenation.

The failure of piglets C and D to achieve target lactate levels $\geq$ 12 mmol/L despite prolonged periods of severe hypoxia was unexpected in the context of previous research (Garberg et al., 2016, Kyng et al., 2015). For example, Garberg et al. (2016) reported mean peak lactate concentrations of 14.0 to 16.4 mmol/L in their study of 55 newborn piglets, despite shorter durations of hypoxia and higher minimum PaO_2 values. A key difference noted between the above data and that of reference studies was the PaO_2 prior to hypoxia. FiO_2 was maintained at 45-55% in piglets B to D during the baseline period of stabilisation, causing hyperoxaemia (PaO_2 160.1 to 354.9 mmHg), whereas previous studies have employed lower oxygen concentrations (FiO_2 21-29%) resulting in baseline PaO_2 values between 74 to 103 mmHg (Fritz et al., 2005, Domoki et al., 2006, Cheung et al., 2006, Garberg et al., 2016).

Attempts to maintain normocapnia through the addition of inhaled CO_2 in piglets B and D, which temporarily increased the minimum volume of oxygen delivered during the hypoxic insult, also appeared to have a marked impact on arterial lactate profiles (Figure 2.5) therefore this step was abandoned in the remaining studies.

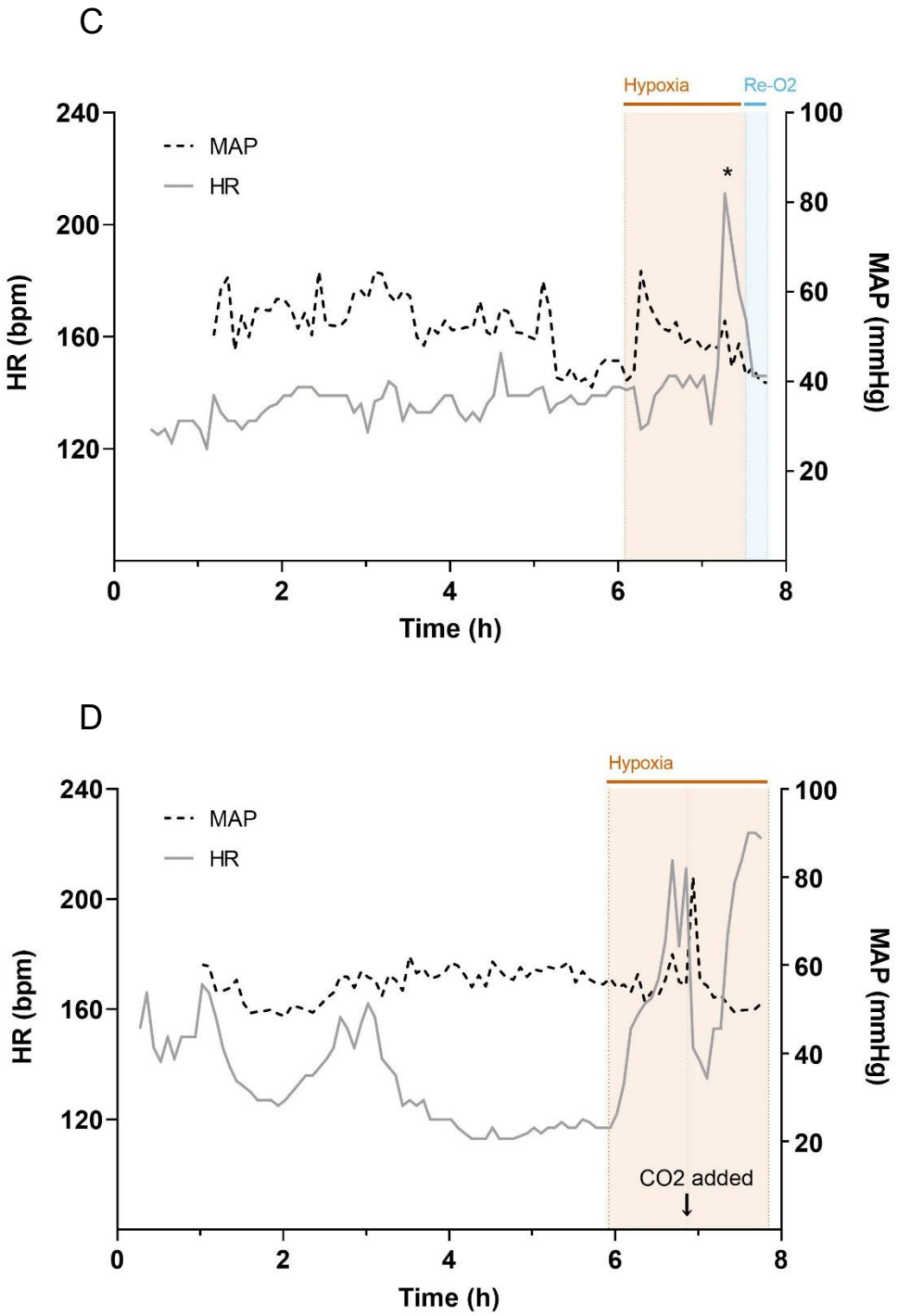

Figure 2.7 Haemodynamic response to hypoxia in piglets C and D. *In piglet C (upper), the transient rise in HR (*) during hypoxia was thought to be the result of insufficient depth of anaesthesia. In piglet D (lower), the addition of inhaled CO_2 temporarily improved oxygenation, causing a fall in HR and arterial lactate.*

2.4.3.3 Study 3 – piglets E and F

To minimise any impact of pre-oxygenation on the piglets' tolerance to hypoxia, piglets E to G were ventilated with fixed FiO_2 of 21-25% during stabilisation. *P*aO_2 values ranged from 60.7 to 74.8 mmHg immediately prior to hypoxia. Haemodynamic trends for piglets E and F are presented in Figure 2.8 and discussed below.

In piglet E, FiO_2 was reduced in a stepwise manner with a total hypoxia duration of 80 min. The first step (FiO_2 15%) was characterised by an abrupt increase in HR but with MAP stable at approximately 45 mmHg. Arterial lactate rose quickly to 2.81 mmol/L, however, the rate of increase then levelled slightly. FiO_2 was further reduced to 8.5%, after which MAP declined progressively and arterial lactate increased steeply to a maximum concentration of 10.49 mmol/L in the early re-oxygenation period, associated with a moderate metabolic acidosis (pH 7.25, BE [ecf] -10.7 mmol/L). Following 110 min of re-oxygenation, with an initial FiO_2 of 22% increasing to 54%, HR normalised, MAP improved, and arterial lactate fell to near-baseline levels.

In terms of the study protocol, piglet E demonstrated the desired physiological response to hypoxia but its peak arterial lactate remained below 12 mmol/L. For piglet F, FiO_2 was therefore reduced in single step to 8%. HR increased rapidly, as for piglet E, but this was associated with an immediate and progressive decline in MAP. Arterial lactate rose to 16.0 mmol/L with a more pronounced metabolic acidosis (pH 7.21, BE [ecf] -13.1 mmol/L). In the latter stages of hypoxia, MAP fell below 35 mmHg and HR began to fall, suggesting a failure of compensatory responses and impending cardiovascular collapse. The duration of hypoxia was shortened to 61 min followed by re-oxygenation at FiO_2 55%, increasing to a maximum FiO_2 94% to encourage normalisation of lactate levels, which fell to 6.87 mmol/L before euthanasia.

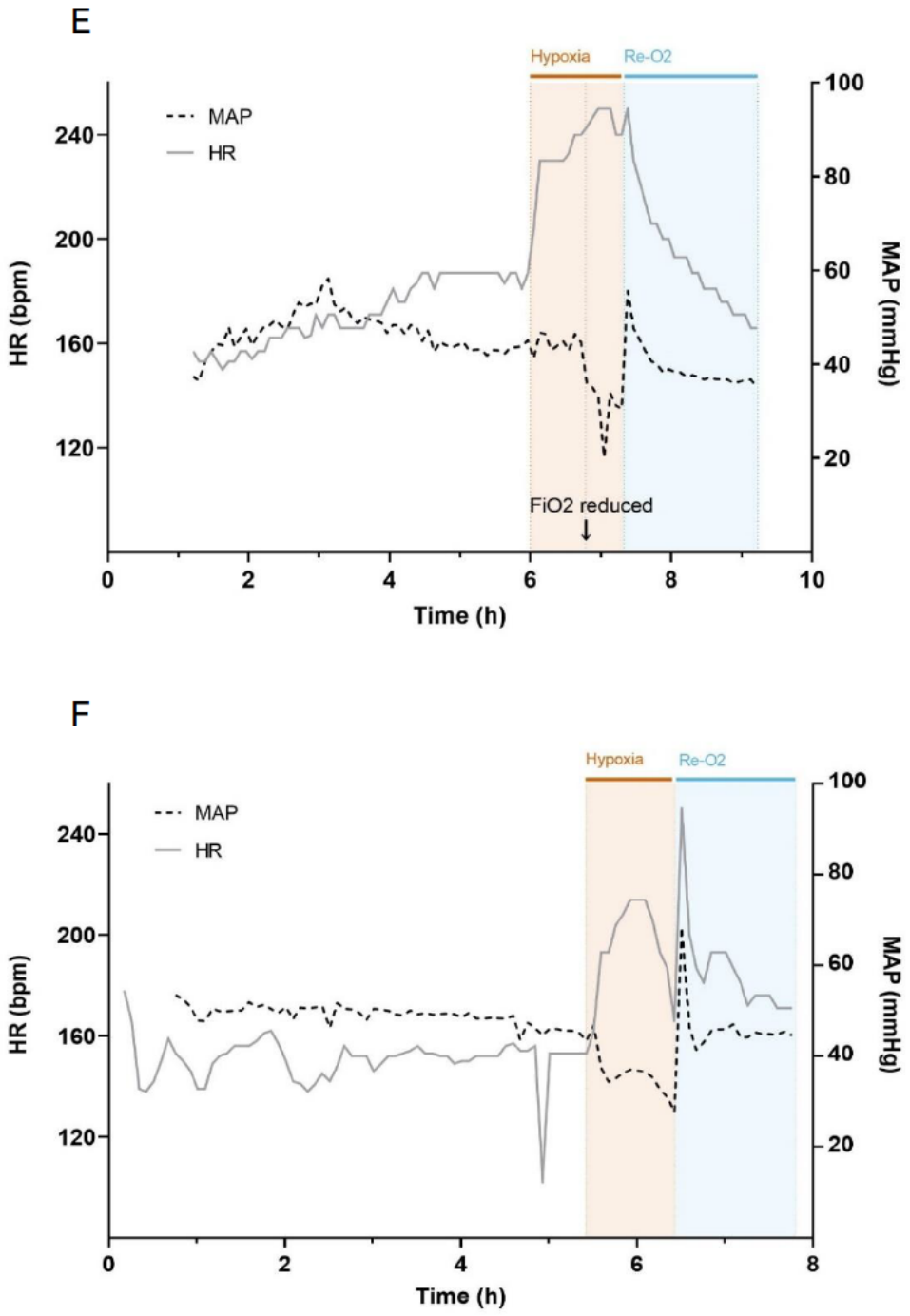

Figure 2.8 Haemodynamic response to hypoxia in piglets E and F. *In piglet E (upper), MAP was initially maintained due to the stepwise reduction in FiO_2. In piglet F (lower), FiO_2 was reduced to 8% rapidly, causing a progressive decline in MAP and subsequent fall in HR in the latter stages of hypoxia.*

2.4.4 Sodium L-lactate infusion (study 4 – piglets G and H)

For piglets G and H, a protocol amendment was approved for the intravenous administration of a lactate challenge in addition to the use of alveolar hypoxia. The rationale for this was two-fold: firstly, it would help to reliably achieve target arterial lactate concentrations $\geq$ 12 mmol/L in the remaining piglets; secondly, the timed administration of sodium L-lactate would provide a reference point to guide ABG sampling and against which microdialysis data could be directly compared. The StatStrip Xpress® point-of-care lactate meter was used to increase the frequency of arterial measurements whilst adhering to maximum sampling limits, as described above.

In piglet G, a 2.0 M sodium L-lactate infusion was commenced (t+0) at a rate of 0.5 ml/kg/min (Olsson et al., 2002). At t+11 min, arterial lactate had risen from a baseline of 1.9 mmol/L to 10.5 mmol/L (StatStrip Xpress® measurements) and the infusion rate was halved to 0.25 ml/kg/min. At t+21 min, StatStrip lactate was 14.8 mmol/L and the epoc® measurement was out of range (> 20 mmol/L) therefore the infusion was stopped. A profound metabolic alkalosis developed during and following the lactate infusion, characterised by hypernatraemia, elevated serum bicarbonate ($cHCO_3^-$ increased from 30.1 to 39.6 mmol/L) and a high (positive) base excess, as shown in Figure 2.9. Haemodynamic monitoring showed a rapid rise in HR, which plateaued following discontinuation of the infusion, as well as a gradual reduction in MAP. Ventilatory settings were unchanged and values for PaO_2 (62.6 to 74.8 mmHg) and $PaCO_2$ (32.2 to 44.7 mmHg) remained stable throughout the study.

Metabolic alkalosis is a recognised effect of sodium lactate administration, the proposed primary mechanism being the metabolism of lactate to pyruvate and subsequent generation of bicarbonate (Olsson et al., 2002, Bollmann et al., 2004, Duburcq et al., 2014). However, the magnitude and duration of the metabolic disturbance seen in piglet G was not anticipated. Arterial lactate slowly fell to 4.71 mmol/L by t+140 min with a persisting alkalosis (pH 7.69, BE [ecf] +19.8 mmol/L). Hypoxia was then induced, as per the protocol, by reducing FiO_2 to 6%. However, this was immediately followed by a terminal bradycardia, which did not respond to re-oxygenation or intravenous adrenaline (0.1 ml bolus of 1:1000 adrenaline 1mg/1ml) resulting in the study ending.

Accordingly, adjustments were made to the lactate challenge for piglet H. As previously described, piglet H had an elevated lactate following anaesthetic induction, the reasons for which were unclear. Arterial lactate gradually normalised during the stabilisation period prior to commencing the modified lactate challenge at an initial rate of 0.125 ml/kg/min (quartered relative to piglet G). At t+22 min, arterial lactate had risen from 3.37 to 10.19 mmol/L and the infusion rate was halved to 0.0675 ml/kg/min. Lactate continued to rise, albeit at a slower rate, to a maximum of 12.54 mmol/L at t+64 min, at which time the infusion was discontinued. MAP was stable with only a modest rise in HR during the lactate challenge. However, piglet H demonstrated similar acid-base derangements to piglet G, mainly a severe metabolic alkalosis with maximum BE [ecf] of +22.5 mmol/L ($cHCO_3$- concentration 43.9 mmol/L) which was delayed in timing relative to changes to arterial lactate. Arterial lactate fell slowly to a nadir of 4.01 mmol/L at t+187min, after which piglet H underwent 45 min of moderate hypoxia (nadir FiO_2 13.9, PaO_2 39.8 mmHg). There was a marked haemodynamic response to hypoxia and lactate rose to 7.31 mmol/L, with a corresponding fall in bicarbonate and BE.

Lactate, BE [ecf] and haemodynamic trends are presented for piglets G and H in Figure 2.9.

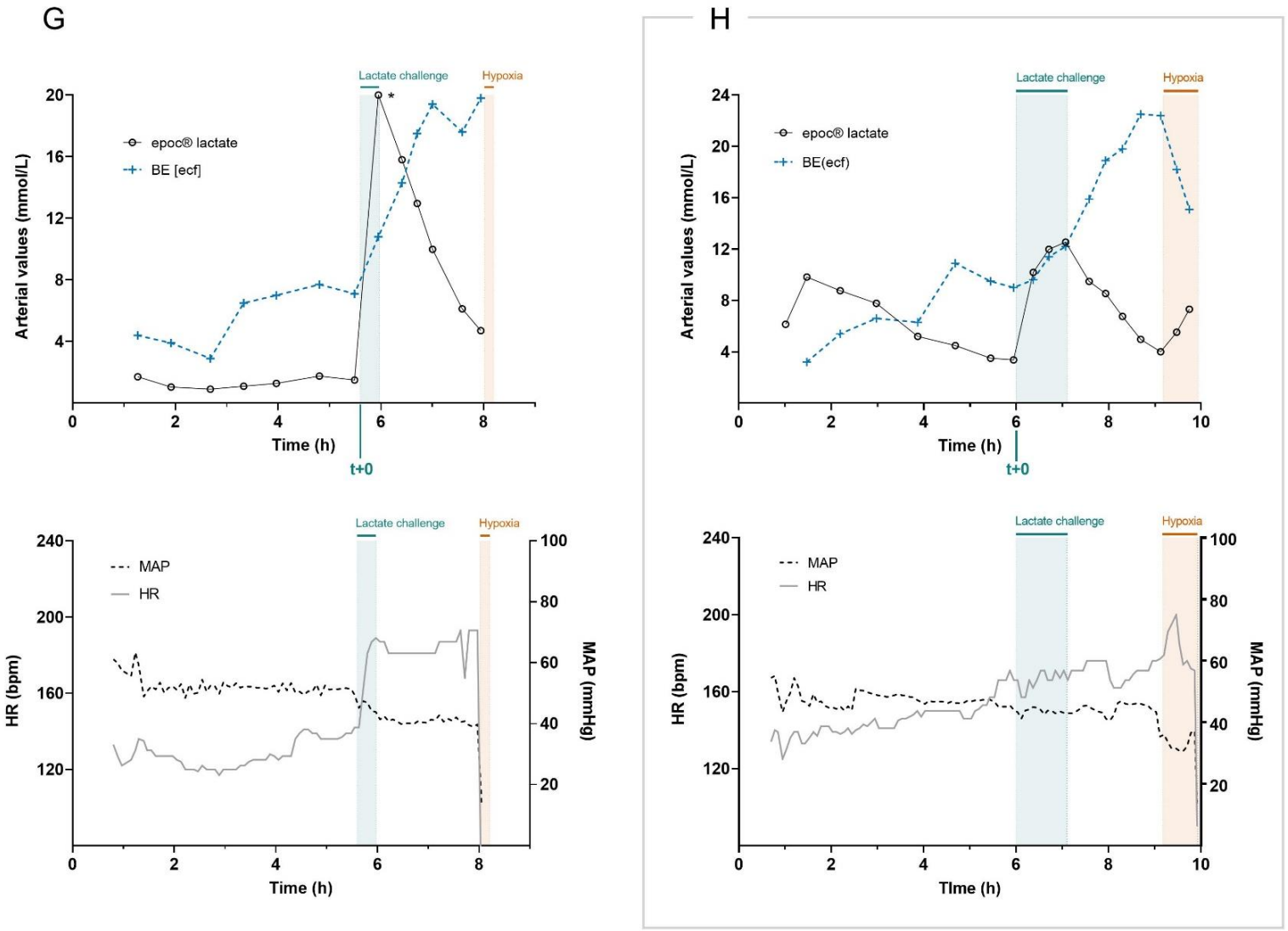

Figure 2.9 Time series in relation to sodium L-lactate infusion. *Arterial (epoc®) lactate and BE [ecf] both increased following the challenge, but BE [ecf] continued to rise after discontinuation whilst lactate gradually normalised (upper panel). Modifications to the infusion rate resulted in less pronounced haemodynamic effects in piglet H (lower panel), which tolerated a subsequent period of moderate hypoxia.*

2.5 Discussion

The aim of the work presented in this chapter was to develop a reliable model of hyperlactataemia in neonatal piglets which would, in turn, provide a suitable means for investigating the relationship between arterial lactate and interstitial lactate. Alongside pre-defined targets for lactate, PaO_2 and pH during hypoxia, the protocol was broadly designed to generate a coarse profile in arterial lactate concentrations within each piglet, characterised by the following: stable baseline concentration prior to hypoxia; clearly defined peak several-fold higher than baseline; and return to baseline levels prior to euthanasia. The presence of both upward and downward trends in arterial lactate, ideally separated by distinct change points, was considered important to facilitate comparison with interstitial lactate profiles and explore the potential application of microdialysis for monitoring the metabolic response to hypoxia. The study also sought to investigate skin thickness in neonatal piglets at the scalp and alternative sites of microdialysis sampling.

The main findings and challenges of this study are addressed in detail below, with reference to the literature on existing models of perinatal HI. Unfortunately, the COVID-19 pandemic prevented approved studies in another four piglets and so limited refinements to the protocols for alveolar hypoxia and sodium lactate infusion. In concluding, the limitations of this work are therefore considered and areas for further research suggested, which should enable standardisation of this porcine model of hyperlactataemia.

2.5.1 Alveolar hypoxia

The greatest challenge faced in this study was to achieve the desired arterial lactate profile and target concentrations solely through adjustments to FiO_2, and to do so in a reproducible manner for each animal. Some variation in the tolerance of individual piglets to hypoxia was expected at the study's outset, hence a flexible approach was taken to allow for adjustments in the severity and duration the hypoxic insult. Initial target PaO_2 values (20-40 mmHg) were based upon existing piglet models of perinatal HI, summarised at the beginning of this chapter. In all piglets, PaO_2 fell below 40 mmHg within 30 min of reducing FiO_2 and the total duration of hypoxia exceeded 60 min.

However, both arterial oxygen saturations and lactate concentrations varied widely. Peak lactate values ranged from 4.9 to 16.0 mmol/L and were generally lower than those observed by Garberg et al. (2016), the only prior study to have reported arterial lactate levels in newborn piglets following a similar hypoxic insult (FiO_2 8%, mean hypoxia duration 33 to 44 min and PaO_2 32 to 37mmHg across their treatment groups).

Piglets B and F were the only animals to reach pre-defined target lactate levels above 12 mmol/L. As previously discussed, however, the hyperlactataemia observed in piglet B most likely reflected evolving anaemia i.e. a failure of oxygen-carrying capacity, rather than hypoxaemia. Indeed, nadir values for PaO_2 (39.8 mmHg) and arterial oxygen saturation (cSO_2; 77.5%) were considerably higher in piglet B than in most other piglets. Maintenance and resuscitative intravenous fluid administration may have compounded any coagulopathy-related blood loss. For example, Pehböck et al. (2010), investigating time to critical oxygen desaturation after apnoea in a piglet model, speculated that the reduction in Hb concentrations seen in fluid-resuscitated pigs countered any benefit of higher PaO_2 levels. More generally, PaO_2 alone may not accurately reflect oxygen delivery to the tissues, nor predict the switch to anaerobic metabolism and resulting hyperlactataemia.

Tissue oxygenation depends on the arterial oxygen content of blood combined with cardiac output (Dunn et al., 2016). Arterial oxygen content (CaO_2) is the amount of oxygen in each 100 ml of arterial blood, representing the sum of the oxygen bound to haemoglobin and oxygen dissolved in plasma, and can be calculated using the following equation:

$$CaO_2 = [1.31 \text{ x Hb (g/dL) x } SO_2 \text{ (\%) x } 0.01] + [0.003 \text{ x } PaO_2 \text{ (mmHg)}]$$

where 1.31 is Hüfner's constant, the maximum oxygen-carrying capacity per gram of haemoglobin, and 0.003 is the solubility coefficient of oxygen in plasma at body temperature (Dunn et al., 2016, Pittman, 2011). In piglet B, nadir CaO_2 was 3.57 ml/100 ml blood, due primarily to a reduction in bound oxygen caused by severe anaemia. CaO_2 values during hypoxia in piglets E and F were very similar (3.48 and 3.55 ml/100 ml blood, respectively) but reflected greater severity of hypoxaemia and lower haemoglobin saturations. In piglet D, CaO_2 fell to 1.93 ml/100 ml blood during the first phase of the hypoxic insult, accompanied by a rapid rise in arterial lactate. Based on

this trajectory, it is probable that a much higher peak concentration would have been achieved but for the subsequent addition of inhaled CO_2, which inadvertently re-oxygenated piglet D (CaO_2 increasing to 6.76 ml/100 ml blood). Despite undergoing alveolar hypoxia of similar severity and duration to piglets D to F, piglet C maintained considerably higher CaO_2 levels (5.35 ml/100 ml blood) due to a relatively high haemoglobin concentration and oxygen saturation (cSO_2 50%). The latter fact may be because of the marked respiratory alkalosis (tc $PaCO_2$ 17.2 mmHg, pH 7.60) which piglet C developed; theoretically, this would have caused a leftward shift of the haemoglobin-oxygen dissociation curve, meaning a higher oxygen saturation for a given PaO_2 (Collins et al., 2015, Dunn et al., 2016). Such differences in arterial oxygen content may help to explain why peak lactate concentrations varied so widely amongst piglets.

Beyond any differences in the hypoxic insult, it is likely that individual physiological variation also contributed to the range of metabolic responses observed. Such variation has been widely reported in past studies of piglets, both controlled models of perinatal HI (Björkman et al., 2006, Aroni et al., 2012, Garberg et al., 2016) and naturalistic studies of birth asphyxia (Herpin et al., 1996, Trujillo-Ortega et al., 2007, van Dijk et al., 2008). In the current study, gender and weight were the only individual characteristics recorded prior to instrumentation and comparisons between subjects were further limited by the study design. Nevertheless, piglet C was identified as a possible outlier due to its low weight (1.0 kg), both in absolute terms and relative to its litter-mate piglet D (1.8 kg), which may have reflected underlying growth restriction. Notably, MartÍnez-RodrÍguez et al. (2011) showed significantly lower blood lactate levels immediately after birth in piglets of low birth weight (< 1000 g) compared to those of normal or high birth weight (1000-1350 g and > 1350 g, respectively). The same research group showed an inverse relationship between viability score and birthweight in piglets, speculating that larger neonates have increased oxygen requirements than growth-restricted peers (Trujillo-Ortega et al., 2007). Taken together with the above discussion on tissue oxygenation, it is plausible that piglet C's smaller size (reduced demand) and higher arterial oxygen content (greater supply) meant it did not reach a critical threshold for anaerobic metabolism during hypoxia and therefore achieved considerably lower peak arterial lactate concentrations. To formally investigate the influence of individual variation on piglets' tolerance to hypoxia, however, future studies will require larger

sample sizes, standardised experimental protocols, and to collect data on related variables which have been shown to influence acid-base disturbance in newborn piglets, such as gestational age, litter size, birth order and farrowing duration (van den Bosch et al., 2022).

Relating this study's findings to previously published reports of perinatal HI in piglets, two further areas merit specific discussion. Firstly, it was postulated that supra-physiological inspired oxygen concentrations (FiO_2 ~ 50%) during the stabilisation period in piglets B to D may have inadvertently enhanced their tolerance to hypoxia. Pre-oxygenation is a long-established anaesthetic technique that aims to replace nitrogen in the lungs' functional residual capacity with oxygen, and so delay the onset of critical hypoxia following apnoea (Sirian and Wills, 2009). In a study of piglets aged 3-4 months (Pehböck et al. 2010), pre-oxygenation with an FiO_2 of 50 or 100% resulted in a significant increase in time to critical peripheral oxygen desaturation compared to those with an FiO_2 of 21%. However, all experimental groups desaturated within 3 min of apnoea so any benefit of pre-oxygenation in piglets appears short-lived, possibly due to higher levels of oxygen consumption per unit weight than in adult humans (Hannon et al., 1990). The lengthy durations of alveolar hypoxia in the current study, corroborated by low arterial oxygen tensions and saturations in most piglets, would therefore be expected to negate any effect of higher FiO_2 during stabilisation. On reflection, it seems more likely that piglets E and F simply reached a threshold of critical hypoxia that was not achieved and/or maintained in piglets B to D. Nevertheless, maintaining atmospheric FiO_2 concentrations prior to hypoxia would ensure consistency with existing models of perinatal HI and may be especially pertinent when investigating acute metabolic responses over a shorter time-frame than the current study; for example, in models of apnoea or complete airway occlusion (Martin et al., 1997).

The final consideration is the management of CO_2 during alveolar hypoxia. Large animal and rodent models of both normocapnic (Aroni et al., 2012, Cheung et al., 2011) and hypercapnic hypoxia (Kyng et al., 2015, Garberg et al., 2016, Yang et al., 2016) have been described in the literature, with various arguments proposed for each. In the current study, ventilatory settings were set with the initial aim of maintaining normocapnia ($PaCO_2$ 35-35 mmHg), though this was achieved consistently in only half

of subjects. Settings were largely unchanged during hypoxia such that CO_2 elimination was preserved and, in fact, *P*aCO_2 fell in all piglets despite slight reductions in ventilation rate, implying a degree of mechanical hyperventilation. It is unclear if the low-to-normal *P*aCO_2 affected either the rate at which hyperlactataemia developed or its severity. However, it does explain the failure to achieve pH values < 7.10 in several piglets despite marked hyperlactataemia and moderate-to-severe base deficits: that is, the modest fall in pH observed in these piglets represents a purely metabolic acidosis, partly compensated by a respiratory alkalosis. Unfortunately, limitations of the anaesthetic equipment used in this study prevented the controlled addition of inhaled CO_2 to correct for this. Hence, some means of delivering fixed concentrations of CO_2, in combination with either variable or fixed FiO_2, should be developed for future studies to produce a more representative physiological model of perinatal hypoxia in which CO_2 elimination is also impaired. Remzső et al. (2020), for example, achieved severe mixed acidosis in piglets by administering a hypoxic-hypercapnic gas mixture (6% O_2, 20% CO_2, balance nitrogen, N_2) at a reduced ventilation rate (15 breaths/min) over a fixed 20 min insult. Such refinements would be particularly important in studies seeking to collect data on histopathological and/or clinical outcomes, given previous authors have demonstrated an association between acid-base disturbances, including arterial *P*aCO_2, and neurologic injury in both humans and animal models (Engle et al., 1999, Vannucci et al., 1995, Yang et al., 2016).

2.5.2 Lactate challenge

The use of intravenous lactate challenges successfully achieved hyperlactataemia in the final piglet studies, but with significant metabolic and haemodynamic effects. The concentration of sodium L-lactate and initial rate of infusion were based on previous animal studies in rats (Olsson et al., 2002) and adult pigs (Barthelmes et al., 2010). Compared to human studies involving lactate challenges (Pitts and McClure, 1967, Gorman et al., 1989, Peskind et al., 1998), which have typically used 0.5 M sodium lactate solution, a higher concentration was used to minimise the total volume infused and any associated risk of haemodilution. Despite this precaution, the infusion rate in piglet G proved to be too high and resulted in arterial lactate exceeding 20 mmol/L. The profound tachycardia seen during the infusion (approximately 30% rise in HR) was

accompanied by a decrease in MAP, presumably mediated directly via baroreceptors. In piglet H, the modified infusion rate (0.125 decreasing to 0.0675 ml/kg/min) increased arterial lactate to target values in a more controlled manner, as intended by the protocol. This also mitigated the haemodynamic response, with a modest rise in HR and stable MAP throughout the infusion and post-infusion periods. These findings are in keeping with the haemodynamic effects of lactate challenges reported in previous animal studies (Wikander et al., 1995, Barthelmes et al., 2010).

In both piglets, arterial lactate took longer to normalise after discontinuing the infusions than was anticipated. This unfortunately limited the time available for a secondary hypoxic insult. Moreover, the persisting severe metabolic alkalosis is likely to have impacted upon the piglets' tolerance to hypoxia, as observed in piglet G's terminal bradycardia following hypoxia. The severity and duration of alkalosis is similar to that reported by Barthelmes et al. (2010) in adult pigs infused with 2.0 M sodium L-lactate, in whom base excess continued to rise beyond 60 min post-infusion. For these reasons, future protocols should consider instigating any hypoxic insult before the lactate challenge, as well as minimising the total amount of lactate infused to avoid severe metabolic alkalosis as far as possible. It may be more prudent still to restrict the use of lactate infusions to finite boluses of radio-labelled tracer lactate for detailed modelling of arterio-interstitial kinetics, as discussed further in Chapter 3.

2.5.3 Skin thickness in relation to microdialysis sampling

This study also provides preliminary data on skin thickness in newborn piglets. The main objective of this limited enquiry was to infer the position of the microdialysis membrane relative to the dermis and hypodermis, as the different structural and morphological characteristics of these layers is likely to affect the interstitial environment, including concentrations of lactate and other metabolites. Whilst histological findings from piglet cadavers cannot be extrapolated to other species, it is possible to draw comparisons with the limited data available on skin thickness in human fetuses and infants. The only study to assess scalp thickness in newborns reported epidermal thicknesses between 25 to 81 μm and dermal thickness of 778 to 1143 μm (de Viragh and Meuli, 1995). Non-invasive imaging techniques have yielded similar results to histological section (Vitral et al., 2018, de-Souza et al., 2019). These

measurements are slightly thicker than those obtained in our piglet cadavers, but nonetheless indicate that the 63MD catheter used in this study would also sample interstitial fluid from the hypodermis of the fetal or neonatal scalp. Assessment of catheter depth by high-frequency ultrasound scanning, as has been demonstrated for intradermal microdialysis (Benfeldt et al., 2007), would help to confirm this assumption in future porcine and human studies. More broadly, further research is required to investigate the effect of skin thickness and differences in underlying tissue properties at commonly used microdialysis sampling sites on arterio-interstitial lactate dynamics.

2.5.4 Strengths and limitations

The use of newborn piglets is one of the main strengths of this study because of their developmental similarities to the term human fetus. Whilst *in utero* models remain the gold standard for research into intrapartum hypoxia, the piglets' young age at the time of study (12-48h) means their physiological response to hypoxia is less likely to have been affected by postnatal development, which may itself be influenced by a range of environmental factors. The histology findings presented also support the rationale for using piglets as a suitable model of fetal and/or neonatal human skin, whilst the decision to monitor scalp interstitial fluid maximises the relevance of this data to the proposed application of intrapartum fetal monitoring.

Despite these advantages, the choice to use neonatal animals carried a higher degree of procedural difficulty and presented unanticipated experimental issues. Two of eight piglets (A and H) were compromised after induction and surgical instrumentation, as shown by markedly elevated lactate concentrations on their initial ABGs. This was unlikely to be caused by piglet A's coagulopathy (based on the timing of ABG sampling) therefore problems during anaesthetic induction and/or surgical instrumentation are the most likely explanation, despite none being noted in either piglet at the time. Instrumentation was also time-consuming, requiring nearly 2 h of the study's maximum 8-12 h duration. Previously published protocols in newborn piglets have catheterised the umbilical vessels (Kyng et al., 2015), which may offer a more efficient means of securing central intravascular access in the future.

To some extent, however, these difficulties simply reflected the steep learning curve experienced by the research team in developing a protocol in a new experimental subject. The staggered study timeline, although advantageous in terms of planning and resources, added further complexity because piglets were at different points in a dynamic, time-restricted protocol with a high frequency of arterial and microdialysis sampling. As a result, the research team were limited in their ability to respond *ad hoc* to emerging problems. Finally, unforeseen delays in analysing microdialysis samples from the studies, which are discussed in Chapter 3, meant that microdialysis data was not available to help inform adjustments and formal protocol amendments. As a result, there was a natural focus on achieving target arterial lactate concentrations which prompted the use of lactate challenges; in hindsight, this arguably over-complicated the final studies and compromised the data collected in relation to hypoxia.

With approval and funding for four more piglet studies, it was planned to refine and standardise the protocols for hypoxia. The lactate challenge protocol used in piglet H would be used after a primary hypoxic insult, but with a modified schedule for arterial and microdialysis sampling. Unfortunately, these studies could not be undertaken due to the COVID-19 pandemic. Standardisation of the model is therefore a critical step moving forward from this proof-of-concept research, not least because it will enable formal comparisons between subjects and an evaluation of the contribution of different sources of variation (i.e. analytical and biological) to results. Further optimisation and standardisation of fluid regimens and glycaemic management, as well as introducing a fixed schedule for arterial sampling, would enhance the quality of data collected.

A related limitation of this exploratory study was the absence of a control or sham-operated group, in which piglets underwent the same procedures and monitoring but without any hypoxic insult or lactate infusion. Along with protocol standardisation, the inclusion of a sham group in future studies and randomised allocation of the piglets to different experimental groups is critical to minimise the impact of a range of known and unknown variables on outcomes (Johnson and Besselsen, 2002). In the present study, a sham group could also serve to address questions related to the microdialysis technique itself. For example, it is possible that microdialysis data may be affected over time by biofouling of the dialysis membrane, or even delayed effects of catheter-related tissue trauma, which could impact upon the clinical utility of this technique. These

observations may be more apparent in control groups where steady interstitial metabolite concentrations would be expected. Further strengths and limitations relating to the microdialysis study are considered in Chapter 3, alongside areas for future research.

2.6 Conclusions

The current study adds to the existing knowledge base on animal models of perinatal HI by providing a detailed description of the physiological and metabolic response to hypoxia in newborn piglets. There was marked variation in peak arterial lactate concentrations achieved through alveolar hypoxia, which may be explained by a combination of experimental factors and individual variation in tolerance to hypoxia. Whilst further refinement of this model is required to reliably achieve target arterial lactate levels, the following key points are suggested in relation to the hypoxia protocol:

- Minimise anaesthetic and surgical stress, e.g. through use of umbilical vessel catheters for intravascular access.
- Maintain FiO_2 at 21-25% during stabilisation, allowing for mild hypoxaemia prior to any hypoxic insult.
- Avoid hypocapnia wherever possible; if hypercapnia is required, a fixed oxygen-CO_2 mix may be preferable to titrating multiple inhaled gases.
- Arterial oxygen saturation and/or content may be more useful than PaO_2 for titrating hypoxia and the degree to which the hypoxic insult is fixed will depend on the study's objective. As previous authors have demonstrated in seeking to reproduce specific pattern of HI injury (Björkman et al., 2006, Chakkarapani and Thoresen, 2015), a variable insult is more likely to achieve pre-defined target lactate, whilst a fixed insult will facilitate direct comparisons between subjects.

In addition, this study has shown that infusion of sodium L-lactate infusion at a rate of achieves severe hyperlactataemia in newborn piglet and may serve as an alternative means of investigating blood-interstitial lactate dynamics. However, infused lactate does not replicate the lactate produced by tissues during perinatal hypoxia and the acid-base disturbances resulting from this approach must be carefully considered.

Chapter 3 Monitoring interstitial lactate and related metabolites using microdialysis

3.1 Introduction

Chapter 1 discussed the limitations of existing fetal monitoring technologies and highlighted the unmet clinical need for a means of continuously monitoring fetal metabolism to detect babies at risk of intrapartum hypoxia-ischaemia. This chapter explores the potential of subcutaneous microdialysis for this purpose, using the neonatal piglet model of hyperlactataemia described in Chapter 2.

To date, only one study has investigated microdialysis for intrapartum fetal monitoring. Tigchelaar et al. (2020) monitored subcutaneous lactate using a custom microdialysis probe inserted into the skin on the back of three adult wild-type Wistar rats. The membrane was incorporated into a spiral fetal scalp electrode design which is widely used in obstetric practice. The authors demonstrated a positive correlation (correlation coefficient 0.89) between dialysate lactate and venous lactate levels during de-oxygenation and re-oxygenation, with a blood-interstitial lag time of about 10 min. Whilst these data are promising, baseline and peak venous lactate values were considerably lower than those expected during labour in the term human fetus. The short duration and small group size of the study also limited the generalisability of these findings. Other published animal and human studies of interstitial lactate, summarised in Chapter 1, have reported conflicting results but are much less relevant to our proposed application, either because of the site chosen for microdialysis monitoring (e.g. skeletal muscle) or the study population (e.g. post-operative or septic patients). Furthermore, where there is poor agreement between interstitial and blood lactate levels, it is unclear if this is the result of physiological factors, sampling and analytical methodology, or some combination thereof (de Boer et al., 1994, Ellmerer et al., 1998, van den Heuvel et al., 2009, Ellmerer et al., 2009). Hence current understanding of the relationship between interstitial and blood lactate remains very limited.

3.2 Aims

The overarching aim of this study was to establish proof-of-concept for using subcutaneous microdialysis to monitor blood lactate and related metabolic parameters in an animal model of hyperlactataemia. The primary objective was therefore to describe the relationship between interstitial and arterial lactate, including any physiological delay between these compartments. It was hypothesised that interstitial lactate would correlate positively with arterial lactate and accurately reflect trends in blood lactate levels during steady state conditions, hypoxia, and re-oxygenation. It was hypothesised that there would be a quantifiable but clinically acceptable (under 15 min) time lag between arterial and interstitial lactate.

The study also sought to address the suitability of microdialysis as a sampling platform for an intrapartum fetal monitoring device. Specifically, the secondary objectives were:

- To assess the effect of microdialysis catheter insertion on local interstitial metabolite concentrations
- To estimate relative recovery (RR) *in vivo* using the flow rate method
- To investigate the potential of multi-analyte monitoring

It was hypothesised that interstitial concentrations of lactate and related metabolites would stabilise rapidly following microdialysis catheter insertion. It was hypothesised that ratios combining multiple analytes, e.g. lactate-to-pyruvate (L/P) or lactate-to-glucose (L/G), would also correlate positively with arterial lactate and would be independent of the perfusate flow rate used for microdialysis.

The final aim of the study was to validate the StatStrip Xpress® Lactate Meter for analysing lactate in dialysate samples by undertaking a method comparison study with the reference ISCUS*flex* microdialysis analyser.

3.3 Materials and methods

3.3.1 Microdialysis materials and equipment

Table 3.1 outlines the microdialysis and general laboratory equipment used for *in vitro* and *in vivo* microdialysis studies.

Table 3.1 Microdialysis and laboratory equipment.

Microdialysis equipment	**Manufacturer**
NE-1000 syringe pump	*New Era Pump Systems, USA*
BD 1 mL disposable Luer-Lok syringes	*Becton Dickinson, USA*
T1 perfusion fluid	*M Dialysis AB, Sweden*
63 Microdialysis catheter (63MD) 60/10	*M Dialysis AB, Sweden*
Microvials	*M Dialysis AB, Sweden*
Microvial rack	*M Dialysis AB, Sweden*
ISCUS*flex* analyser	*M Dialysis AB, Sweden*
StatStrip Xpress® Lactate Meter	*Nova Biomedical, USA*
Laboratory equipment	
Bench centrifuge	*Eppendorf, Germany*
50 mL glass beaker	*Fischer Scientific, UK*
2.0 mL graduated skirted screw cap tubes	*Starlab Ltd, UK*
Pipette tips	*Starlab Ltd, UK*
Pipettes p2, p20, p200, p1000	*Gilson, UK*
Vortex mixer	*Cole-Parmer, UK*
JB Academy unstirred water bath	*Grant Instruments, UK*

For the initial set up, T1 perfusion fluid (Na^+ 147 mmol/L, K^+ 4 mmol/L, Ca^{2+} 2.3 mmol/L, Cl^- 156 mmol/L) was withdrawn into a 1 mL syringe with a sterile, hypodermic needle. The Luer-Lok connection of the 63MD catheter was pre-filled with perfusion fluid to minimise the risk of entrapping air when connecting the syringe and catheter. The syringe pump was programmed based on the inner syringe diameter (4.699 mm) and the catheter then primed as described below. Table 3.2 details the technical properties of the 63MD catheter used for *in vivo* studies.

Table 3.2 Technical properties of 63 Microdialysis catheter.

	Material	Length (mm)	Outer diameter (mm)
Shaft	Polyurethane	60	0.9
Membrane *	Polyarylethersulphone	10	0.6
Inlet tubing	Polyurethane	600	1.0
Outlet tubing	Polyurethane	220	1.0

* Membrane cut-off 20 kDa

The ISCUS*flex* analyser (M Dialysis AB, Stockholm, Sweden) was used for the primary analysis of microdialysis samples. ISCUS*flex* is a benchtop, multi-parameter analyser optimised for the low sample volumes (0.2 to 2.0 µL per analyte) obtained through microdialysis (Figure 3.1). It is CE-approved for clinical use in Europe. Measurements are based on a two-step reaction: hydrogen peroxide is formed by the oxidation of the analyte of interest – lactate, glucose, pyruvate, and glutamate in our study – with a specific oxidase enzyme and subsequently measured by an indicator reaction involving peroxidase and a chromogenic reagent. The rate of formation of the coloured compound, whose absorbance is measured by spectrophotometry, is proportional to the concentration of the analyte.

Secondary analysis of a selection of dialysate samples was undertaken with the StatStrip Xpress® Lactate Meter (Nova Biomedical, Waltham, MA USA) to validate this instrument by comparison with the ISCUS*flex.* StatStrip Xpress® is a point-of-care device that measures lactate in whole blood samples (minimum volume 0.6 µL) using electrochemical test strips, with a turnaround time of 13 seconds.

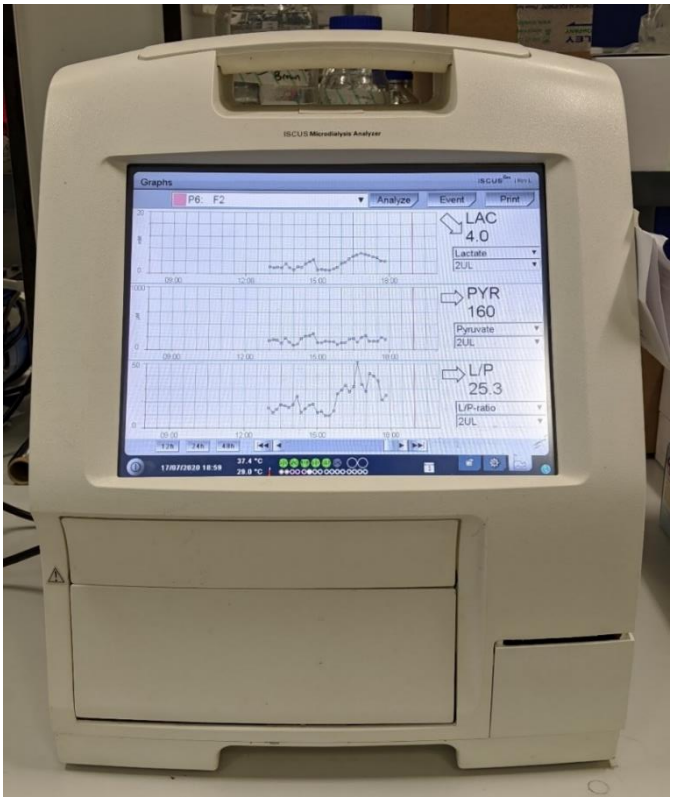

Figure 3.1 ISCUS*flex* microdialysis analyser. *Graphs screen displaying latest value and trends for lactate, pyruvate and L/P ratio.*

3.3.2 *In vitro* microdialysis

Initial experiments were conducted *in vitro* to determine RR for each analyte at flow rates of 0.3, 0.5, 1.0, 2.0, 3.0 and 5.0 µL/min. 63MD catheters were primed by immersing the tip in T1 perfusion fluid and perfusing them at 5.0 µL/min until dialysate was seen collecting at the microvial cannula. The inlet and outlet tubing were visually inspected for air bubbles and additional flush cycles (25 µL) undertaken as necessary. Primed catheters were then suspended in a 50 mL glass beaker containing stirred solutions of glucose 5.55 mmol/L, lactate 2.5 mmol/L, pyruvate 250 µmol/L, and glutamate 25 µmol/L (Calibrator A) and microdialysis performed at the stated flow rates. There were washout periods after each change of flow rate to account for dead space in the outlet tubing (6 µL) and dialysate was collected to provide a minimum sample volume of 10 µL. All *in vitro* experiments were conducted in a water bath at 37°C. Table 3.3 summarises the timings of the flow rate method used for both *in vitro* and *in vivo* studies.

Table 3.3 Flow rate method. Sequence *of perfusate flow rate changes required for calibration by the flow rate method. Shaded areas represent washout periods.*

Flow rate (µL/min)	Interval (m)	Sample volume (µL)
5.0	3	
5.0	3	15.0
3.0	3	
3.0	5	15.0
2.0	3	
2.0	5	10.0
1.0	6	
1.0	10	10.0
0.5	12	
0.5	20	10.0
0.3	20	
0.3	35	10.5
Cumulative time (m)	*125*	

3.3.3 *In vivo* microdialysis

Microdialysis was undertaken in neonatal piglets (n=8) under general anaesthesia, as described in Chapter 3. After intubation and instrumentation, piglets were placed in a prone position under a Bair Hugger™ warming blanket (3M, UK) covered with a silver space blanket. Hair was removed from the frontal scalp with clippers and the area sterilised with 0.5% chlorhexidine gluconate solution (Ecolab, UK). To maximise data, piglets were monitored with two microdialysis systems which were prepared as for *in vitro* experiments. 63MD catheters were inserted subcutaneously on each side of the frontal scalp (3-4 cm lateral separation) under aseptic conditions using a splitable introducer. The catheters were inserted sequentially within 10 minutes of each other and secured with a transparent Tegaderm™ dressing, as shown in Figure 3.2.

Catheters were randomly assigned to a perfusate flow rate of 1.0 or 2.0 µL/min, with a washout period of 6 min preceding the start of dialysate collection. Piglet A was

euthanised prematurely due to anaesthetic-related complications which were considered likely to have affected the limited data collected, therefore a total of 216 dialysate samples from seven piglets (range 27 to 40) at each flow rate were included in the final analysis. Piglet H was excluded from the equilibration and calibration analyses because arterial lactate was elevated prior to commencing microdialysis.

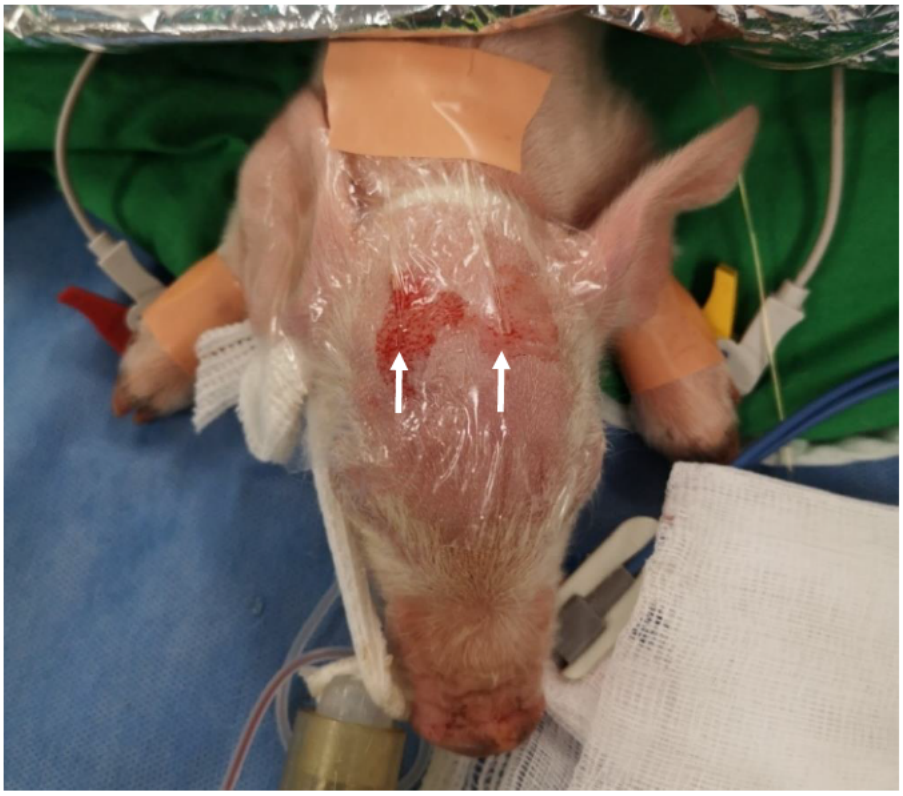

Figure 3.2 Anaesthetised piglet with 63MD catheters in situ. *Catheters (arrows) were designated by piglet (B-H) and flow rate (1.0 or 2.0 µL/min) i.e. B1, B2, C1, etc.*

For *in vivo* studies, dialysate collection and the statistical analysis of microdialysis data are considered in three distinct periods, as illustrated in the study timeline, Figure 3.3.

- *Equilibration*

  This represented the first 60 min of dialysate collection after catheter insertion and washout. Dialysate was collected at 10 min intervals during this period to assess the effect of catheter-related tissue trauma on concentrations of local metabolites.

- *Calibration*

  Following equilibration and a further washout period (6 min), dialysate was collected for each flow rate, as outlined in Table 3.3, to allow calculation of *in vivo* RR. Both catheters were perfused at identical flow rates during the calibration period.

- *Hyperlactataemia*

  After a final washout period, catheters were re-perfused at their previously allocated flow rates and dialysate was collected at 10 min intervals for the remainder of the study protocol until euthanasia.

Dialysate sampling, storage and transport during *in vivo* studies was undertaken with the assistance of Dr Sarah Caughey (Queen's Medical Research Institute, University of Edinburgh). Dr Caughey also contributed to the batch analysis of dialysate samples on the ISCUS*flex* analyser, including data input. I undertook all statistical data analysis.

3.3.4 Microdialysate analysis

Microvials were directly stored in microvial racks or screw-top microcentrifuge tubes to minimise the risk of evaporation. *In vivo* samples were stored at -80°C (duration of freezer storage ranged from 159-295 days) and thawed in an incubator for 10 min at 37°C. All analyses were performed after a single freeze-thaw cycle, with the exception of samples from one catheter (E2) which were re-frozen for pyruvate analysis due to failed calibration of the ISCUS*flex*. *In vitro* samples were analysed on the day of collection and did not undergo freeze-thaw. Immediately prior to loading into the ISCUS*flex*, samples were centrifuged for 30 s at 2000 x g, with the narrow (sampling) end of the vial facing downward in the conical bottom of the microcentrifuge tube. This prevented the small stopper from being dislodged during centrifugation.

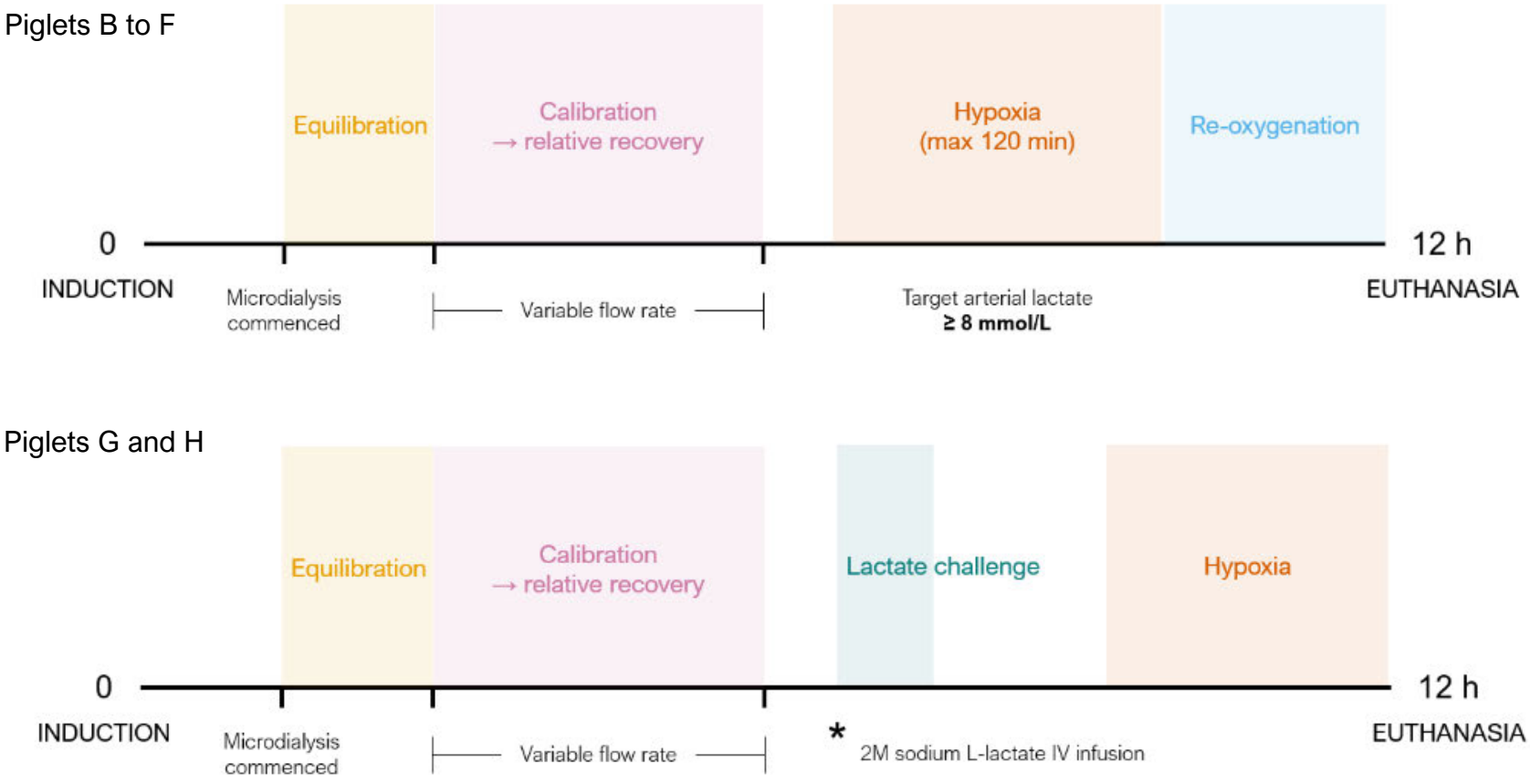

Figure 3.3 Timeline for *in vivo* studies. *After anaesthetic induction and instrumentation, microdialysis was commenced. 63MD catheters were perfused at either 1.0 or 2.0 µL/min with dialysate collected at 10 min intervals, except for during the calibration period. There was a short baseline period before hyperlactataemia was induced by reducing FiO_2 (piglets B-F, upper panel) and/or infusion of 2.0 M sodium L-lactate solution (piglets G and H, lower).*

As the ISCUS*flex* is intended for real-time clinical use, samples were analysed in chronological order for each catheter. To avoid sample evaporation, a maximum of 20 measurements were undertaken at one time (e.g. 5 samples x 4 analytes per sample). The throughput was approximately 30-35 measurements per hour in this manner, consistent with the manufacturer's stated performance. Control samples with elevated and low concentrations of lactate, which were provided with the ISCUS*flex* reagent kit (M Dialysis AB, Sweden), were analysed manually at the beginning of each day and every 8 hours during extended sessions. The procedure followed the manufacturer's instructions; in brief, 50 µL of control solution was pipetted into a microvial, which was incubated at 37°C for 10 min prior to centrifugation. Satisfactory performance was indicated if the analyte values fell within the "Acceptable Control Range".

3.3.5 Evaluation of the StatStrip Xpress® lactate meter

For the method comparison study, the StatStrip Xpress® lactate meter was prepared in accordance with the manufacturer's instructions and quality control solutions provided with the meter were run at the beginning of each day. Samples from one of the microdialysis catheters for each of four piglets (E2, F1, G2 and H1) were tested on the StatStrip immediately following analysis by the ISCUS*flex*. Microvials were removed from the ISCUS*flex,* visually inspected for air bubbles and re-centrifuged as required. The stopper was then removed from the narrow end of the microvial and a pipette used to withdraw 2 µL of dialysate, adding it to the StatStrip test strip.

Intra-assay variation, or within-run precision, of the StatStrip was determined by performing six consecutive measurements on five samples: three samples each from piglet F (catheter F2) with low, medium and high lactate concentrations, as well as the aforementioned elevated and low controls provided with the ISCUS*flex*. Inter-assay precision (between-run) was also evaluated by testing the elevated and low controls three times per day over seven days (n=21). Due to small sample volumes, it was not possible to analyse experimental dialysate samples over multiple days in this manner. The precision of the ISCUS*flex* lactate assay was evaluated following similar procedures: for intra-assay precision, samples from catheter G2 with varied lactate concentrations were used; for inter-assay precision*,* a total of 28 measurements were performed over 14 days.

3.3.6 Statistical analysis

Microsoft Excel (Version 2010) was used to collect data for all microdialysis studies. Statistical analysis was performed using GraphPad Prism 8.4.3 (GraphPad Software, San Diego, USA) and was best considered separately for each of the three phases of the study protocol, as well as the method comparison study. *p* values are summarised as follows: $p > 0.05$ (ns, not significant), ≤ 0.05 (*), ≤ 0.01 (**), ≤ 0.001 (***).

3.3.6.1 Equilibration

To assess the effect of catheter-related trauma, metabolite concentrations were compared at 10, 30 and 60 min using repeated measures one-way analysis of variance (ANOVA). The start of dialysate collection was used as a surrogate for time of catheter insertion. Catheters were grouped by flow rate because it was unknown if different flow rates would affect the equilibration time; animals with missing values were excluded. Following the recommendations of Maxwell and Delaney (2004) for analysing repeated measures data, sphericity was not assumed therefore a Geisser-Greenhouse correction (Greenhouse and Geisser, 1959) was applied based on the value of epsilon. Where the ANOVA yielded a significant result, Tukey's multiple comparisons tests were carried out post hoc to assess which of the group means differed (i.e. comparing 10 vs 30 min, 10 vs 60 min, 30 vs 60 min). In addition, individual and mean data are presented for each flow rate across the first hour of dialysate collection.

3.3.6.2 Calibration by flow rate method

In vitro RR was calculated directly by dividing the mean dialysate concentration, C_{dial}, at each flow rate (n=4 for flow rates of 5.0, 3.0, 2.0 and 1.0 µL/min; n=3 for 0.5 and 0.3 µL/min) by the known concentration of the Calibrator A solution, C_{soln}.

$$RR\ (\%)\ = \frac{C_{dial}}{C_{soln}} \times 100$$

In vivo RR was calculated by the flow rate method (Stahle et al., 1991). Firstly, values obtained from the two catheters were averaged for each piglet as these represented biological replicates. Individual data (n=6) were pooled to generate mean dialysate concentrations for the five analytes of interest and the flow rate was plotted against

1/mean concentration. Simple linear regression was then performed, with the resulting regression equation used to calculate the true tissue concentration at zero flow (representing C_{soln}) and calculate RR, as shown above.

3.3.6.3 Arterio-interstitial analyses

Pearson correlation was performed to evaluate the relationship between arterial lactate and each analyte. It was not possible to pair microdialysis and arterial sampling as part of the study protocol because ABGs were undertaken flexibly in response to physiological monitoring for individual piglets (maximum sampling procedures limited the total volume, and therefore number, of blood samples that could be obtained during each study). Therefore, microdialysis samples were included only if an ABG was obtained within 5 min (±) of the midpoint of dialysate collection, providing 78 time-paired samples at 1.0 µL/min and 76 pairs at 2.0 µL/min for correlation analysis.

Time series graphs were then used to compare trends in interstitial lactate, L/P and L/G ratios with arterial lactate for each piglet. All elapsed times are given relative to the time of endotracheal intubation. Baseline (immediately prior to hypoxia or sodium lactate infusion) and peak concentrations were used to calculate fold-changes in arterial and interstitial lactate, respectively. Time series graphs were also used to estimate the arterio-interstitial time lag by comparing change points in arterial and interstitial lactate profiles.

To check the assumption that multi-analyte ratios are independent of relative recovery, samples collected simultaneously from the two catheters on each piglet were directly compared using scatterplots with Pearson correlation. The Wilcoxon matched-pairs signed rank test was then used to compare ratios between the flow rates (n=171 pairs for L/P; 174 for L/G), as differences were not normally distributed.

3.3.6.4 StatStrip Xpress® method comparison

Agreement between lactate values obtained with the StatStrip and ISCUS*flex* was initially assessed by means of a scatter plot with Deming regression, which assumes errors in observations for both *x* and *y* variables. Bland-Altman plots with 95% limits of agreement were then used to graphically compare methods (Bland and Altman, 1986).

The differences were tested for normality using the D'Agostino-Pearson omnibus test. Intra-assay and inter-assay coefficients of variation (CV) were calculated to the assess the precision of the StatStrip Xpress® and ISCUS*flex* lactate assays.

3.4 Results

3.4.1 Equilibration

Data from the equilibration period, representing the first 60 min of dialysate collection, was used to assess the effect of microdialysis catheter insertion on interstitial metabolite concentrations. There were significant differences in glutamate concentrations during the first hour of dialysate collection at 1.0 µL/min (p=0.014), with values decreasing over time. A similar but non-significant trend was seen at 2.0 µL/min (p=0.056), suggesting that catheter-related trauma caused a transient increase in interstitial glutamate levels. Post hoc comparisons using the Tukey test confirmed a significant difference between concentrations of glutamate at 10 and 60 min (p=0.047) at a perfusate flow rate of 1.0 µL/min only.

In contrast, repeated measures one-way ANOVA showed no effect of time on interstitial concentrations of lactate, pyruvate or glucose at either flow rate. Figure 3.4 and Table 3.4 summarise the results for the equilibration period.

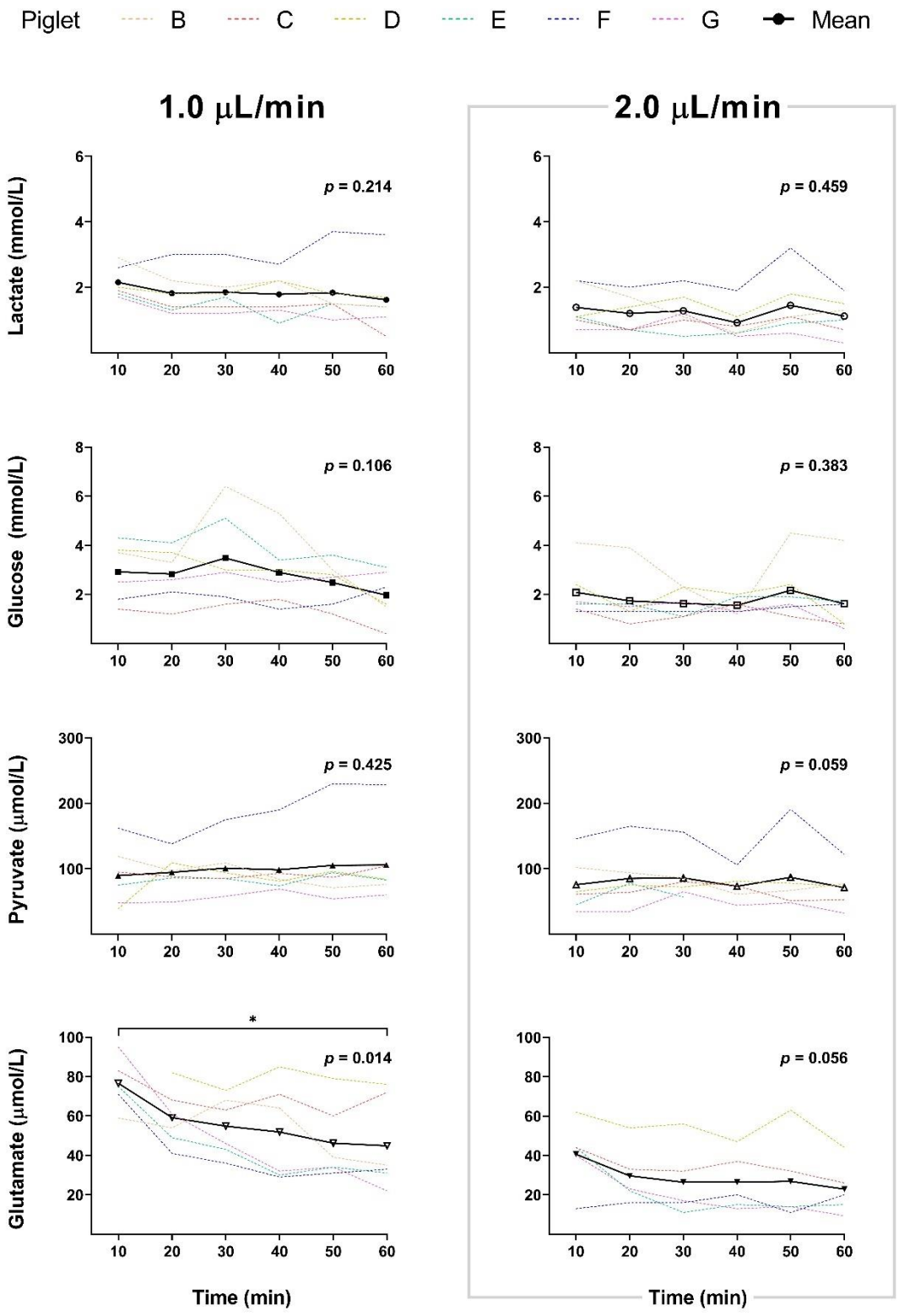

Figure 3.4 Effect of time since catheter insertion on metabolite concentrations. *Individual and mean analyte concentrations during equilibration (p values shown for repeated measures ANOVA). For glutamate, Tukey's multiple comparison tests were undertaken post-hoc with horizontal bar indicating significant difference (p=0.047) between 10 and 60 min.*

Table 3.4 Repeated measures one-way ANOVA results. *Analyte concentrations were compared at 10-, 30- and 60-min. DF, degrees of freedom; Epsilon, Geisser-Greenhouse's epsilon.*

Flow rate (µL/min)	Analyte	F (DFn, DFd)	*p*	*p* value summary	Epsilon
1.0	Lactate	F (1.072, 5.360) = 2.008	0.214	ns	0.536
	Glucose	F (1.368, 6.840) = 3.331	0.106	ns	0.684
	Pyruvate	F (1.575, 7.876) = 0.885	0.425	ns	0.788
	Glutamate	F (1.755, 7.019) = 8.652	0.014	*	0.877
2.0	Lactate	F (1.552, 7.759) = 0.784	0.459	ns	0.776
	Glucose	F (1.255, 6.273) = 0.977	0.383	ns	0.627
	Pyruvate	F (1.910, 7.639) = 4.249	0.059	ns	0.955
	Glutamate	F (1.367, 5.470) = 5.525	0.056	ns	0.684

3.4.2 Relative recovery and *in vivo* calibration

As discussed in Section 1.3.2, recovery *in vivo* is not the same as *in vitro* because analyte diffusion is affected by physical tissue properties and biological processes. Prior to the piglet studies, *in vitro* RR was calculated directly as shown in Figure 3.5A. RR exceeded 85% at perfusate flow rates of 0.5 and 0.3 µL/min for all analytes but decreased substantially at higher flow rates. Figure 3.5B demonstrates the principles of the flow rate method (Stahle et al., 1991) using *in vitro* data; in this example, the dialysate lactate concentration calculated by regression at zero flow (i.e. when the dialysate and surrounding medium are in equilibrium) closely approximated the true concentration of the solution (Calibrator A) used for *in vitro* studies.

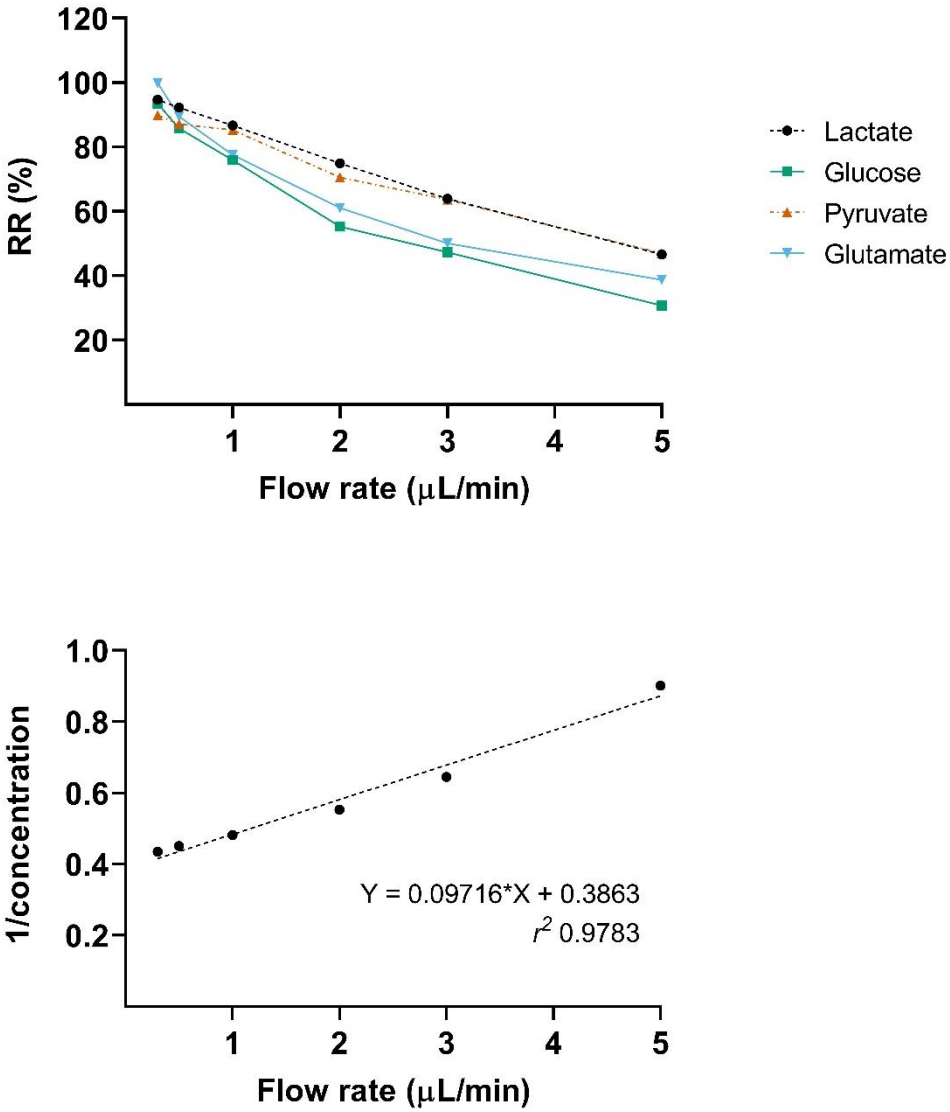

Figure 3.5 *In vitro* relative recovery. *(A) In vitro recovery of analytes by direct calculation. (B) Flow rate plotted against 1/mean dialysate lactate concentration. At zero flow, the lactate concentration calculated by regression was 2.59 mmol/L, closely approximating the mean concentration of the calibrator solution when tested in triplicate (2.44 mmol/L; manufacturer's stated concentration 2.55 mmol/L).*

For *in vivo* studies, RR was calculated by the flow rate method during the 'calibration' period. *In vivo* RR was considerably lower than that seen *in vitro* at all flow rates tested. Applying the flow rate method, linear r^2 values > 0.98 were obtained for all analytes (Figure 3.6). RR was very similar amongst the four analytes of interest, with maximum recoveries between 72.8 to 79.4% at a flow rate of 0.3 µL/min. At flow rates of 1.0 and 2.0 µL/min, as used throughout the rest of the *in vivo* study protocol, RR for lactate and the other analytes was around 50% and 30%, respectively (Table 3.5).

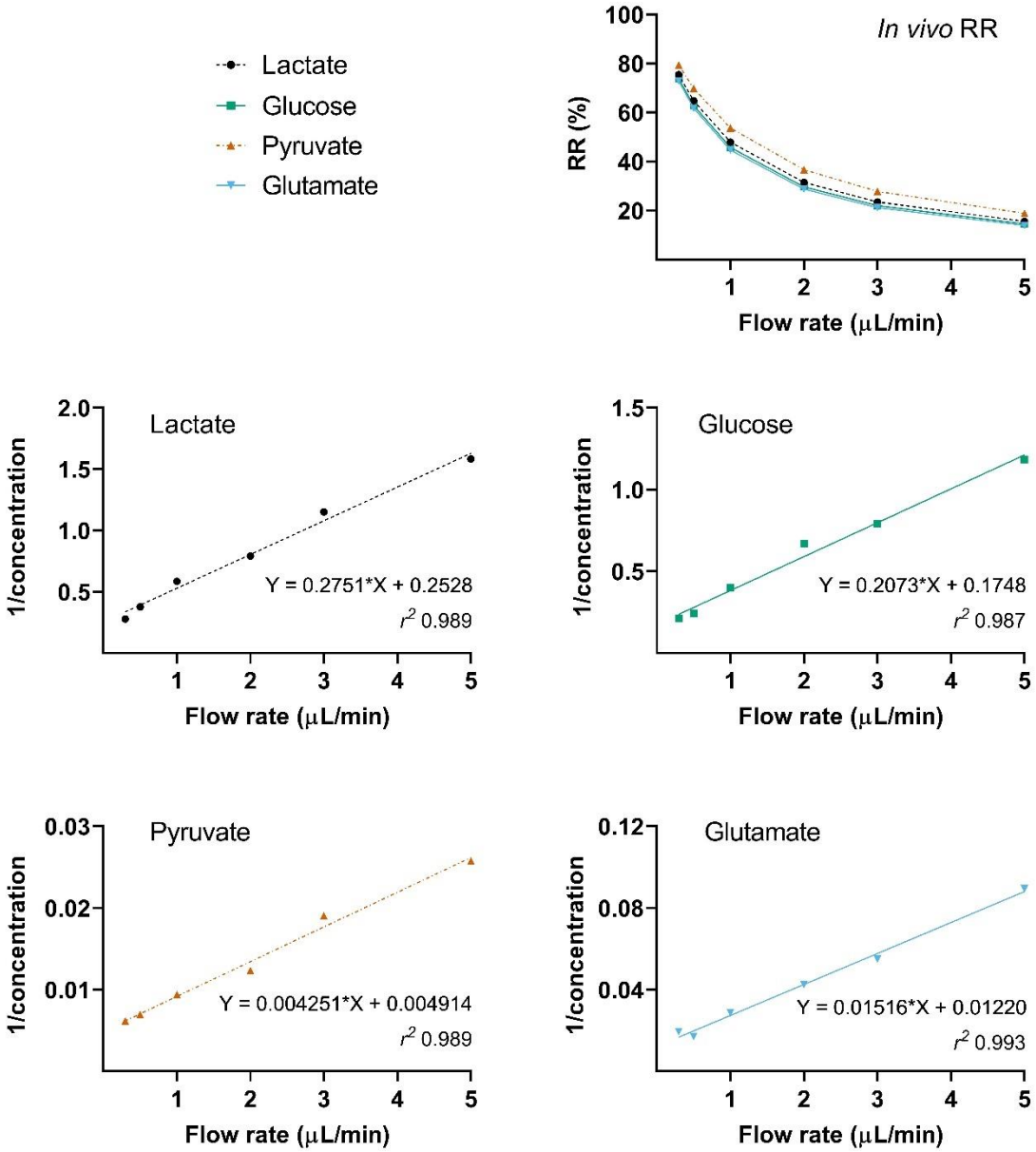

Figure 3.6 *In vivo* relative recovery. *(A) In vivo RR was similar for all analytes, but lower than in vitro RR. (B) Flow rate plotted against 1/mean dialysate concentration,*

showing linear r^2 values > 0.98 for all analytes, with the corresponding regression equations used to estimate in vivo RR.

Table 3.5 Summary of *in vivo* relative recovery (%). *Results are shown by flow rate.*

	Flow rate (µL/min)					
	0.3	0.5	1.0	2.0	3.0	5.0
Lactate	75.4	64.8	47.9	31.5	23.4	15.5
Glucose	73.8	62.8	45.7	29.7	21.9	14.4
Pyruvate	79.4	69.8	53.6	36.6	27.8	18.8
Glutamate	72.8	61.7	44.6	28.7	21.2	13.9

3.4.3 Relationship between arterial and interstitial lactate

To investigate the relationship between arterial and interstitial lactate, Pearson correlation was initially performed on time-paired arterial and dialysate measurements. There were strong positive correlations between arterial and interstitial lactate concentrations at both flow rates (Pearson's product-moment correlation coefficient, r=0.821 and 0.848 at 1.0 and 2.0 µL/min, respectively; both $p < 0.0001$), as shown in Figure 3.7. There were also significant positive correlations between arterial lactate and interstitial pyruvate (r=0.530 and 0.537 at 1.0 and 2.0 µL/min, respectively; $p < 0.0001$) and significant negative correlations with interstitial glutamate (r= -0.602 and -0.415, respectively; both $p < 0.0001$). Arterial lactate did not correlate with interstitial glucose alone at either flow rate. Multi-analyte ratios are considered separately below.

Individual time series data were used to further investigate the relationship between arterial and interstitial lactate in each piglet. These are presented by catheter flow rate in Figure 3.8, with interstitial values unadjusted for relative recovery. Expectedly, unadjusted microdialysis lactate values were lower than arterial values and the effect of different flow rates on lactate recovery was clearly evident.

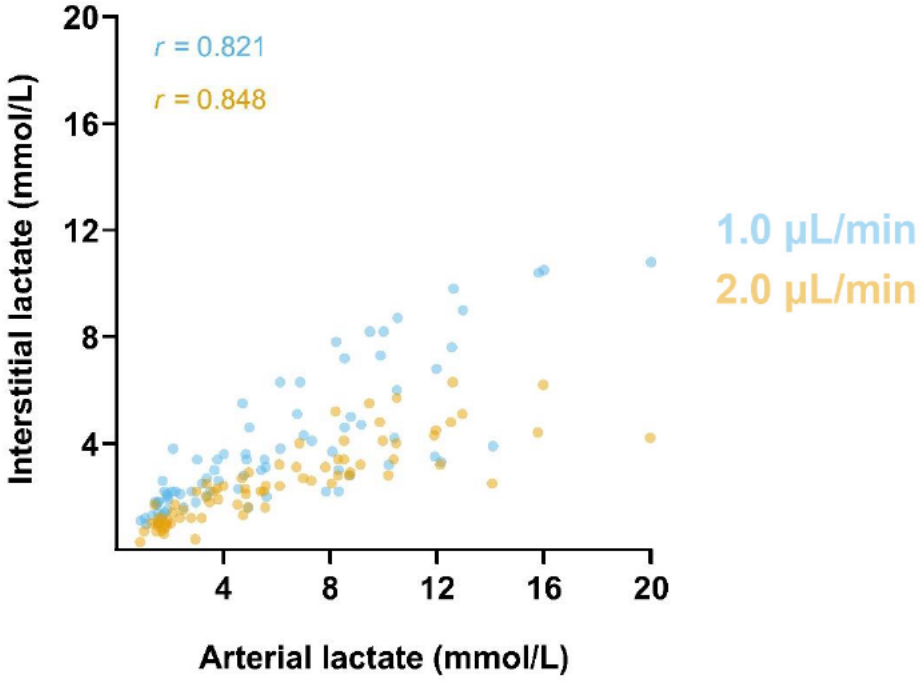

Figure 3.7 Correlation between arterial and interstitial lactate. *Pearson coefficient shown for respective flow rates (n=78 paired values at 1.0 µL/min, n=76 at 2.0 µL/min).*

During the equilibration and calibration periods, arterial lactate was generally stable below 4 mmol/L in all piglets except for H. In these steady state conditions, unadjusted dialysate lactate concentrations at MD flow rate of 1.0 µL/min were similar to arterial concentrations, and true interstitial concentrations exceeded those of blood at both flow rates (after adjusting for RR). As arterial lactate rose in response to hypoxia and/or sodium L-lactate infusion, interstitial lactate also increased. Interstitial lactate reflected the arterial lactate profile to varying degrees, with the poorest agreement in piglet B, in whom there were minimal changes in interstitial lactate despite a sharp rise in arterial levels. Trend agreement was also weaker in piglets C and D, in whom peak arterial lactate values did not exceed 6 mmol/L; however, the biphasic profile seen during hypoxia in piglet D was evident in both compartments. In piglets E through H, interstitial lactate mirrored both upward and downward trends in arterial lactate more clearly.

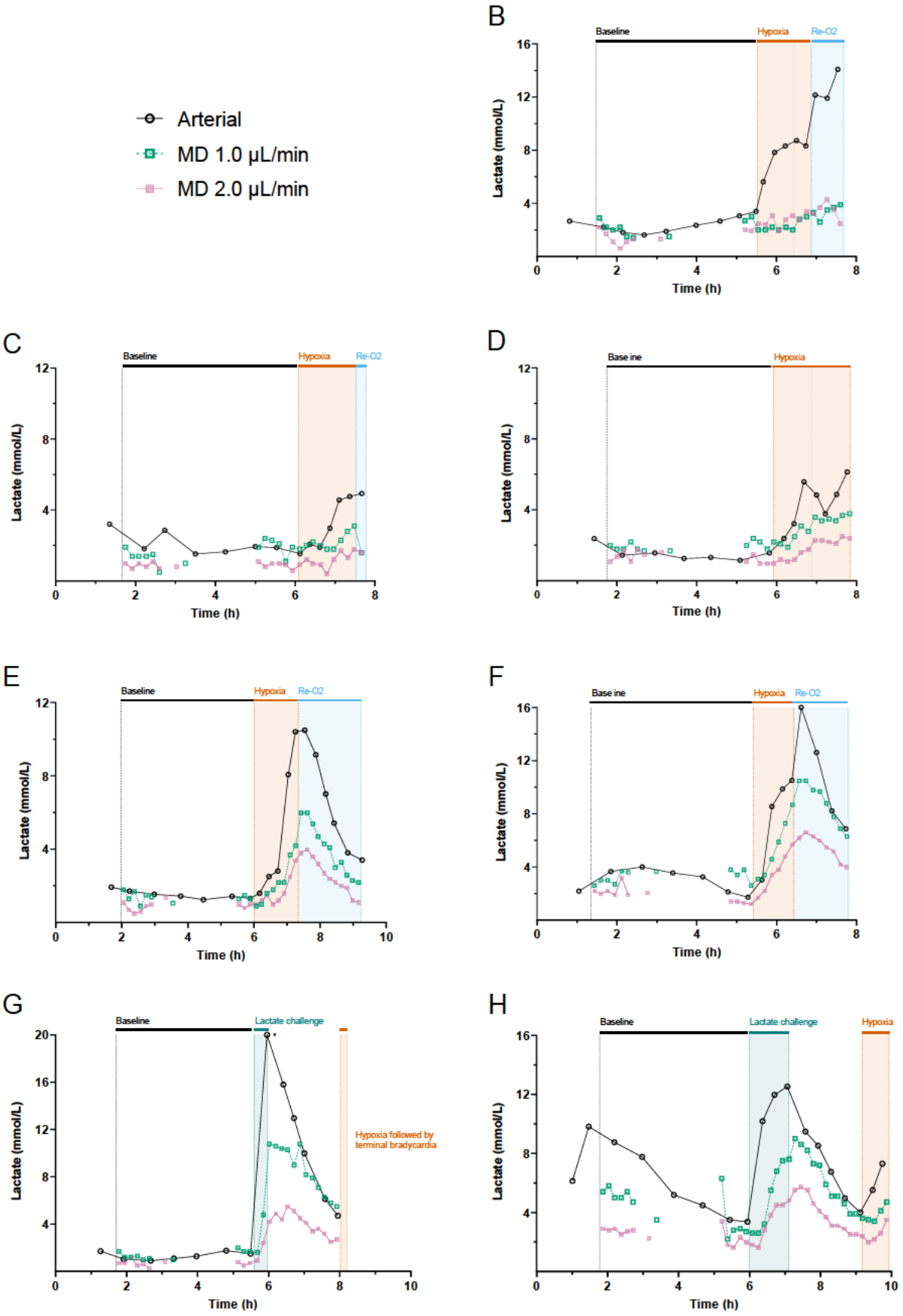

Figure 3.8 Individual time series for piglets B to H. *MD lactate values are unadjusted for RR. *Peak arterial lactate in piglet G exceeded the maximum measurement range of the epoc® blood analysis system (20 mmol/L).*

As with baseline values, peak interstitial lactate slightly exceeded peak arterial concentrations after adjusting for RR. However, relative changes in interstitial lactate (from baseline to peak) were considerably lower than those in the arterial compartment for all piglets. Table 3.6 shows individual and mean values for arterial and unadjusted interstitial lactate at baseline (immediately prior to hyperlactataemia) and peak, as well as the corresponding fold-changes in each compartment, calculated as follows:

$$\text{Fold-change } (\Delta) = \frac{[peak]}{[baseline]}$$

Fold-changes were of particular interest because they are independent of microdialysis recovery. Hence, a two-fold increase in arterial lactate should result in a two-fold increase in interstitial lactate (regardless of perfusate flow rate) assuming the compartments closely mirror each other across the range of expected concentrations.

Table 3.6 Arterial and interstitial lactate changes during hyperlactataemia. *Individual and mean concentrations in mmol/L of arterial (ART) and interstitial lactate (1.0 and 2.0 µL/min flow rates, unadjusted for RR), with corresponding fold-changes. Baseline values were taken immediately prior to hypoxia or lactate infusion. SD, standard deviation. † exceeded measurement range of epoc® device.*

Piglet		B	C	D	E	F	G	H	Mean (SD)
ART	*Baseline*	3.39	1.54	1.56	1.32	1.72	1.50	3.37	2.06 (0.91)
	Peak	14.1	4.93	5.58	10.5	16.0	20.0†	12.5	11.9 (5.45)
	Δ	4.15	3.20	3.58	7.95	9.30	13.3	3.72	**6.46 (3.86)**
1.0	*Baseline*	3.0	1.9	1.8	1.3	2.6	1.7	2.7	2.14 (0.62)
	Peak	3.9	3.1	3.6	6.0	10.5	10.8	9.0	6.70 (3.35)
	Δ	1.30	1.63	2.00	4.62	4.04	6.35	3.33	**3.32 (1.83)**
2.0	*Baseline*	1.9	0.6	1.0	1.0	1.2	0.7	2.0	1.20 (0.55)
	Peak	4.3	1.8	2.3	4.0	6.6	5.5	5.7	4.31 (1.78)
	Δ	2.26	3.00	2.30	4.00	5.50	7.86	2.85	**3.97 (2.06)**

3.4.3.1 Arterio-interstitial delay

In five piglets, there were clear change points in the time series indicated by an abrupt transition from an upward to downward trend (or vice versa) in lactate concentrations. For these change points, the median time difference between the interstitial and arterial compartments was 15 min (range 4 to 35, n=5). An example from piglet E is shown in Figure 3.9. Similar time lags were seen in piglets G and H following intravenous infusion of 2.0 M sodium L-lactate solution, as shown in Figure 3.8. In piglet G, interstitial lactate rose between 5 and 15 min after the infusion started, during collection of the second microdialysis sample. In piglet H, interstitial lactate rose between 15 and 25 min (third sample) after the infusion started.

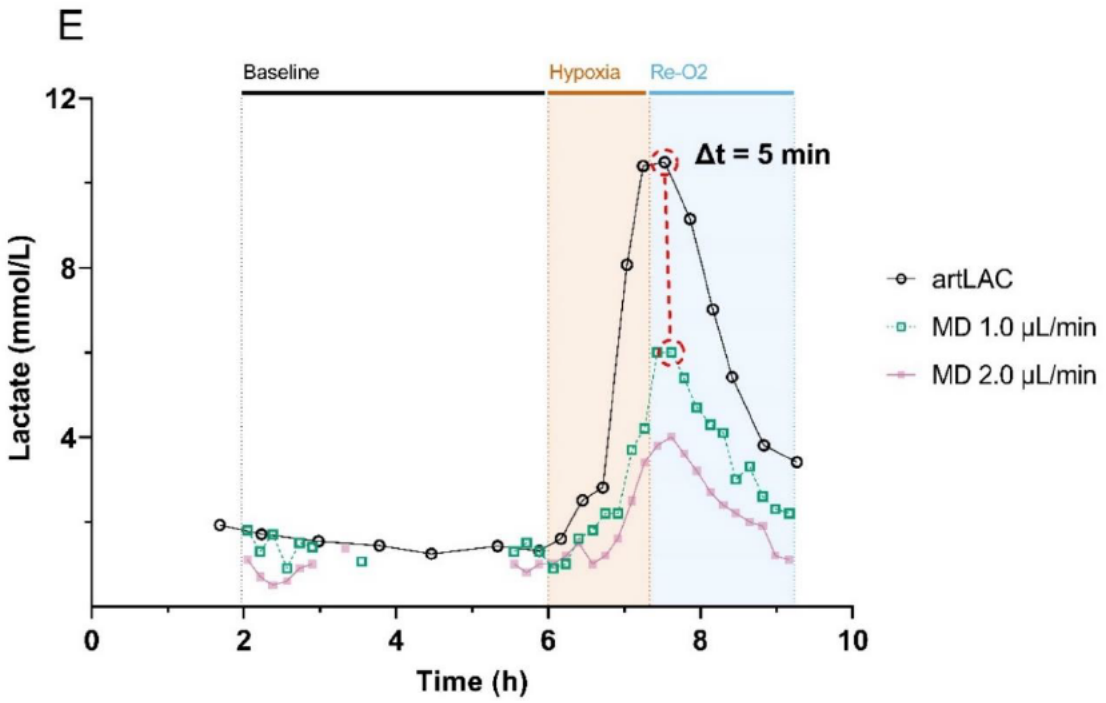

Figure 3.9 Representative example of arterio-interstitial delay. *Time delay (Δ t) between peak lactate concentrations in the arterial and interstitial compartments shown for piglet E.*

3.4.3.2 Multi-analyte monitoring

The relationship between arterial lactate and interstitial multi-analyte ratios was also investigated through Pearson correlation and time series graphs, as described above. There were moderate positive correlations between arterial lactate and interstitial L/P ratio at 1.0 µL/min (r=0.486) and 2.0 µL/ min (r=0.546). There were slightly stronger correlations between arterial lactate and interstitial L/G ratio at both flow rates (r=0.631 and 0.613, respectively). These results are shown in Figure 3.10, with representative time series for piglets E and F presented in Figure 3.11. Overall, the ratios reflected arterial lactate trends poorly in comparison with interstitial lactate alone. There was also marked variation in the absolute values of the ratios between piglets.

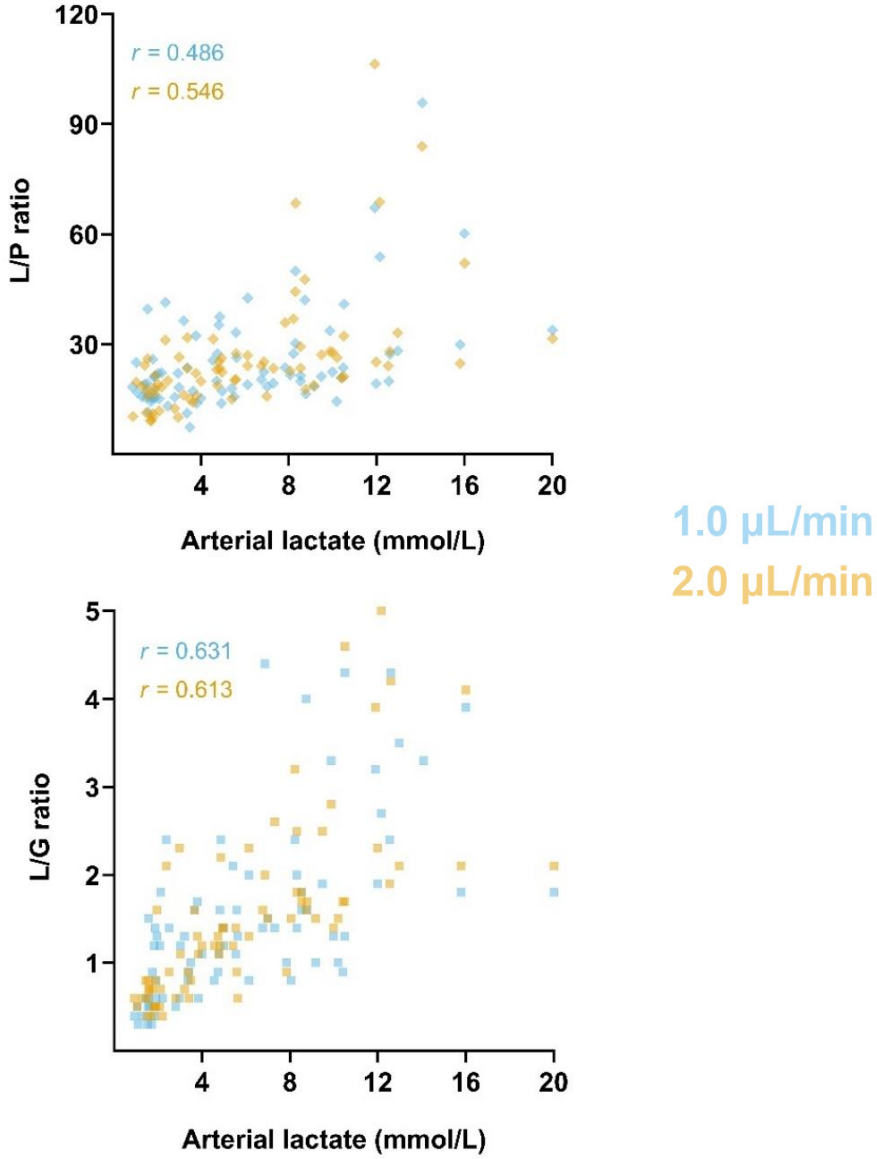

Figure 3.10 Correlation between arterial lactate and interstitial multi-analyte ratios. *Pearson coefficients shown for L/P and L/G ratios at respective flow rates.*

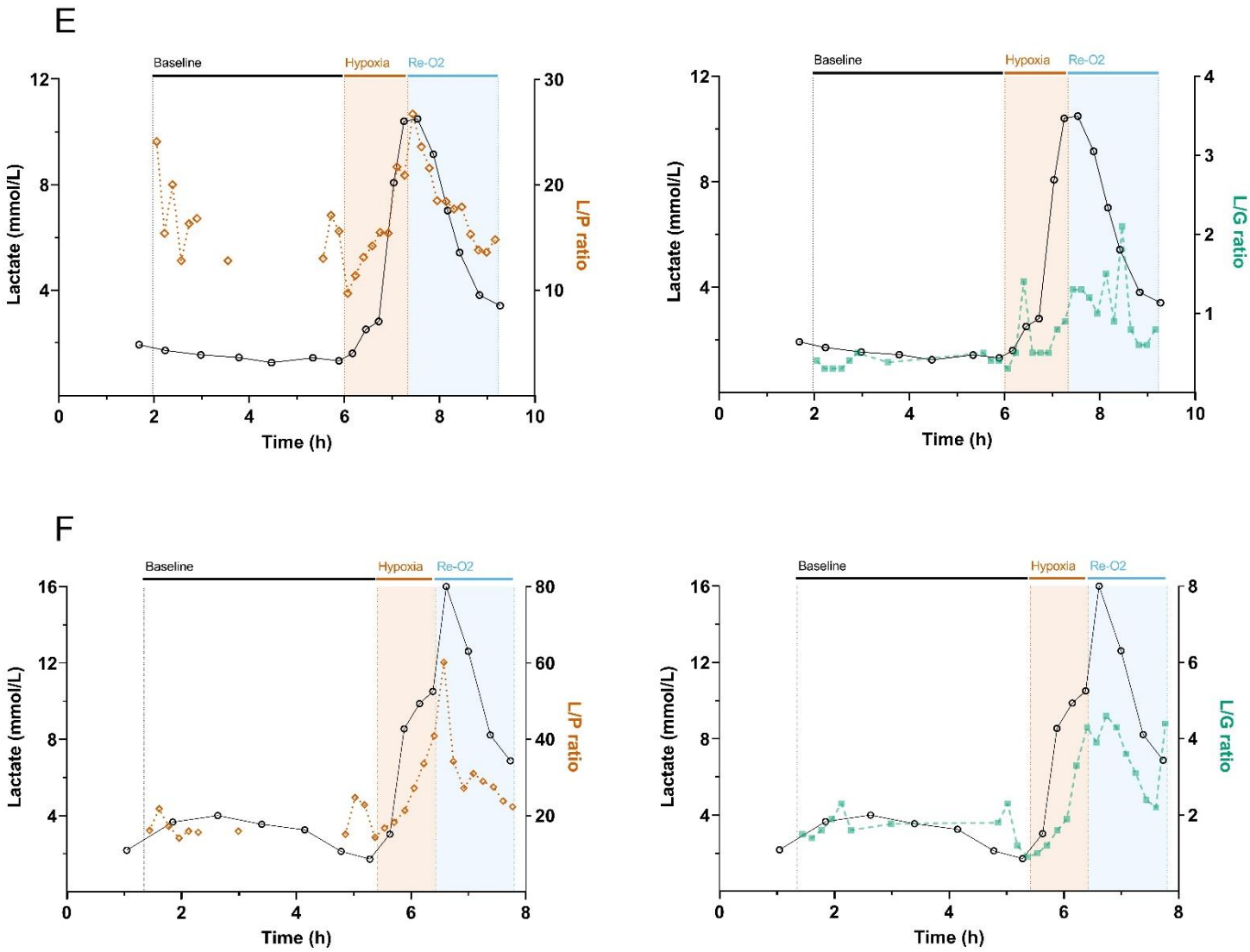

Figure 3.11 Representative time series of multi-analyte ratios. *Time series graphs of arterial lactate and interstitial L/P and L/G ratios for piglets E (upper panel) and F (lower). Note the wide variation between piglets in the values of both ratios, shown on right hand Y-axis.*

One of the potential advantages of using microdialysis to monitor multiple analytes is that ratios are independent of analyte recovery, in theory negating the requirement for 'calibration' *in vivo*. Because perfusate flow rate is the main determinant of recovery, as already demonstrated, paired data from the two catheters on each piglet were compared to confirm whether L/P and L/G ratios were independent of flow rate (n=171 pairs for L/P; 174 pairs for L/G). In Figure 3.12, scatterplots show both ratios were centred on the line of equity, albeit scatter increased at higher values. There was no significant difference between interstitial L/P ratios at 1.0 µL/min and 2.0 µL/min, nor between L/G ratios at these flow rates (Wilcoxon matched-pairs signed rank test: L/P = 0.7875, ns; L/G = 0.1933, ns).

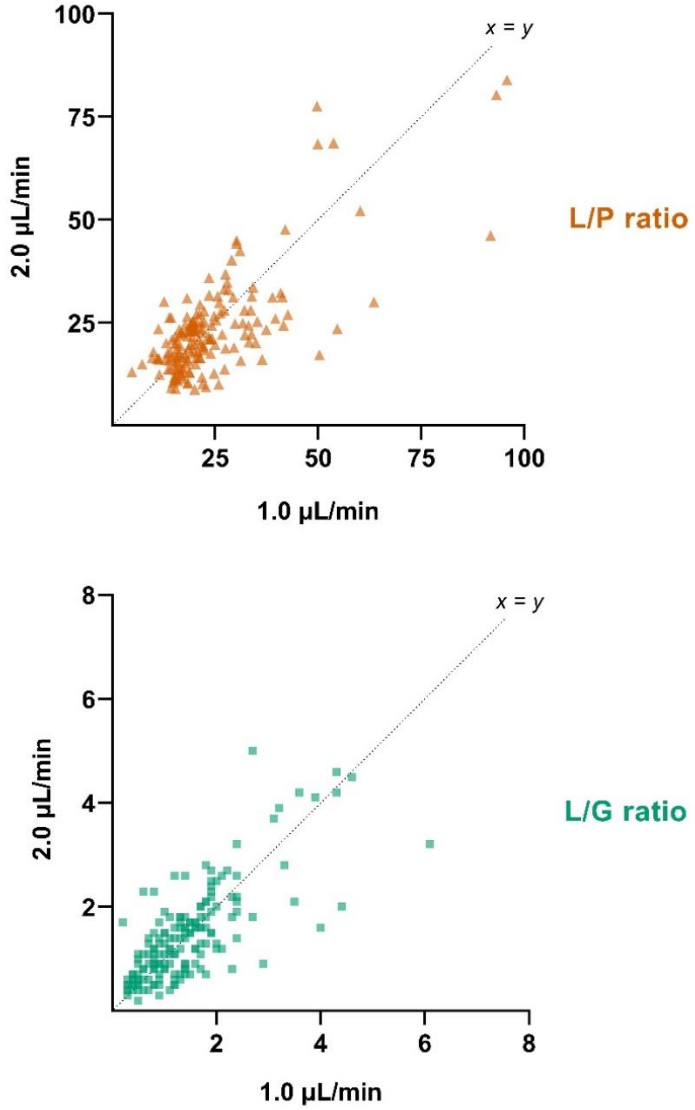

Figure 3.12 Paired comparison of multi-analyte ratios at different flow rates. *Scatterplot of L/P and L/G ratios at 1.0 and 2.0 µL/min, showing paired values centred on the line of equity.*

3.4.4 Evaluation of the StatStrip Xpress® Lactate Meter

3.4.4.1 Method comparison

There were eight failed analyses on the StatStrip due to 'ERROR' or 'LO' readings, thus providing a total of 126 paired measurements for comparison. An *xy* scatter plot (Figure 3.13A) confirmed that lactate measurements from the StatStrip and ISCUS*flex* were closely linearly related and provided an initial impression of agreement between the methods. The slope of the Deming regression line trended away from the line of equity ($x=y$, if methods agree perfectly) as lactate values increased, indicating a proportional negative bias. The 95% CI for the slope of the regression (0.665 to 0.734) did not include the line of equity, indicating the methods were not interchangeable.

This was better visualised by means of a Bland-Altman plot (Figure 3.13B), which showed a mean bias of -0.743 (95%CI -0.898 to -0.588) for lactate measured by the StatStrip Xpress®. The differences did not follow a Gaussian distribution but were negatively skewed as a result of the proportional bias. Non-normality was confirmed by the D'Agostino-Pearson omnibus test ($p < 0.0001$). Hence, the bias of the StatStrip Xpress® may be overestimated at lower lactate concentrations and underestimated at higher concentrations.

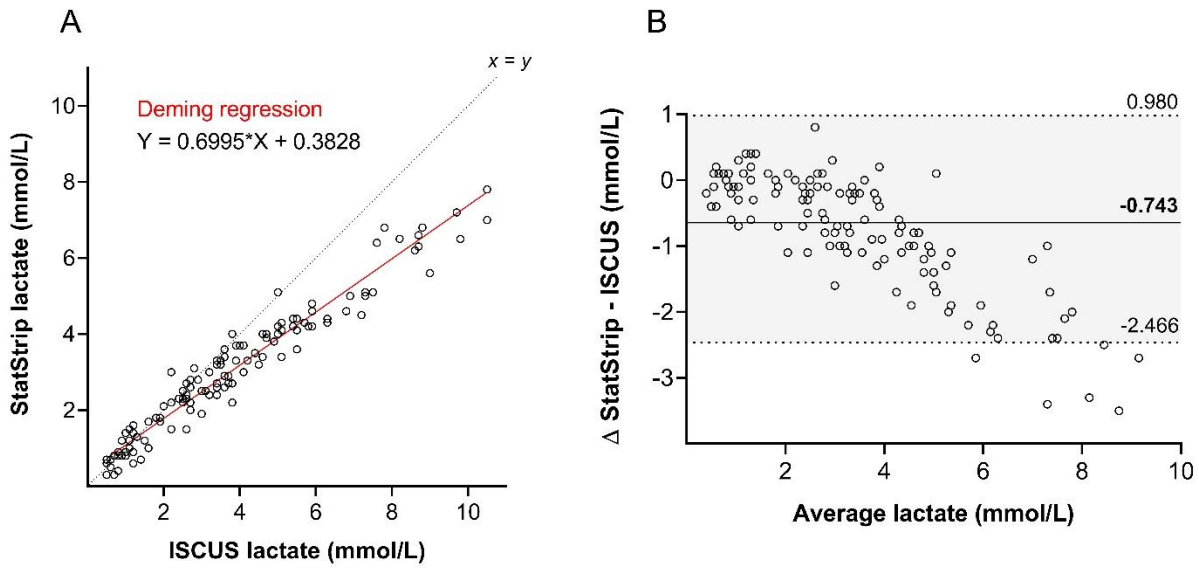

Figure 3.13 Method comparison of StatStrip Xpress® and ISCUS*flex* lactate. *(A) Scatterplot with Deming regression line (red) showing proportional negative bias of the StatStrip Xpress®. (B) Bland-Altman plot with 95% limits of agreement represented by the shaded area.*

When differences do not follow a normal distribution, Bland and Altman (1999) suggest either log transformation of the data or working directly with ratios. The latter approach was chosen because results are easier to interpret clinically; for example, ratios can be used as conversion factors to improve the agreement between two methods. In the resulting Bland-Altman plot of StatStrip:ISCUS ratio (Figure 3.14A), skewness was reduced but not removed entirely; most values fell below the mean when the average lactate value exceeded 4.0 mmol/L, which included approximately one third of dataset.

In practice, microdialysis-derived fetal interstitial lactate values above 4.0 mmol/L were considered unlikely as they would correspond to arterial lactate concentrations greater than 8.0 mmol/L (based on RR of approximately 50% at 1.0 µL/min flow rate). This is much higher than current clinical thresholds for intervention using fetal scalp lactate. Therefore, a secondary Bland-Altman analysis was performed excluding samples with an ISCUS*flex* lactate value > 4.0 mmol/L. The mean bias of the StatStrip Xpress® was smaller (-0.249, 95%CI -0.342 to -0.156) and the spread of differences around the mean approached a normal distribution (D'Agostino-Pearson test of normality p=0.03). Following the same steps above, normality occurred when the StatStrip:ISCUS ratio was plotted against average lactate, and this also provided a narrower confidence interval for the mean ratio of 0.914 (95%CI 0.867 to 0.961; Figure 3.14B). Hence, a conversion factor could be applied to allow the StatStrip Xpress® and ISCUS*flex* to be used interchangeably. However, the 95% limits of agreement remained wide so caution would be required in the clinical application of this approach.

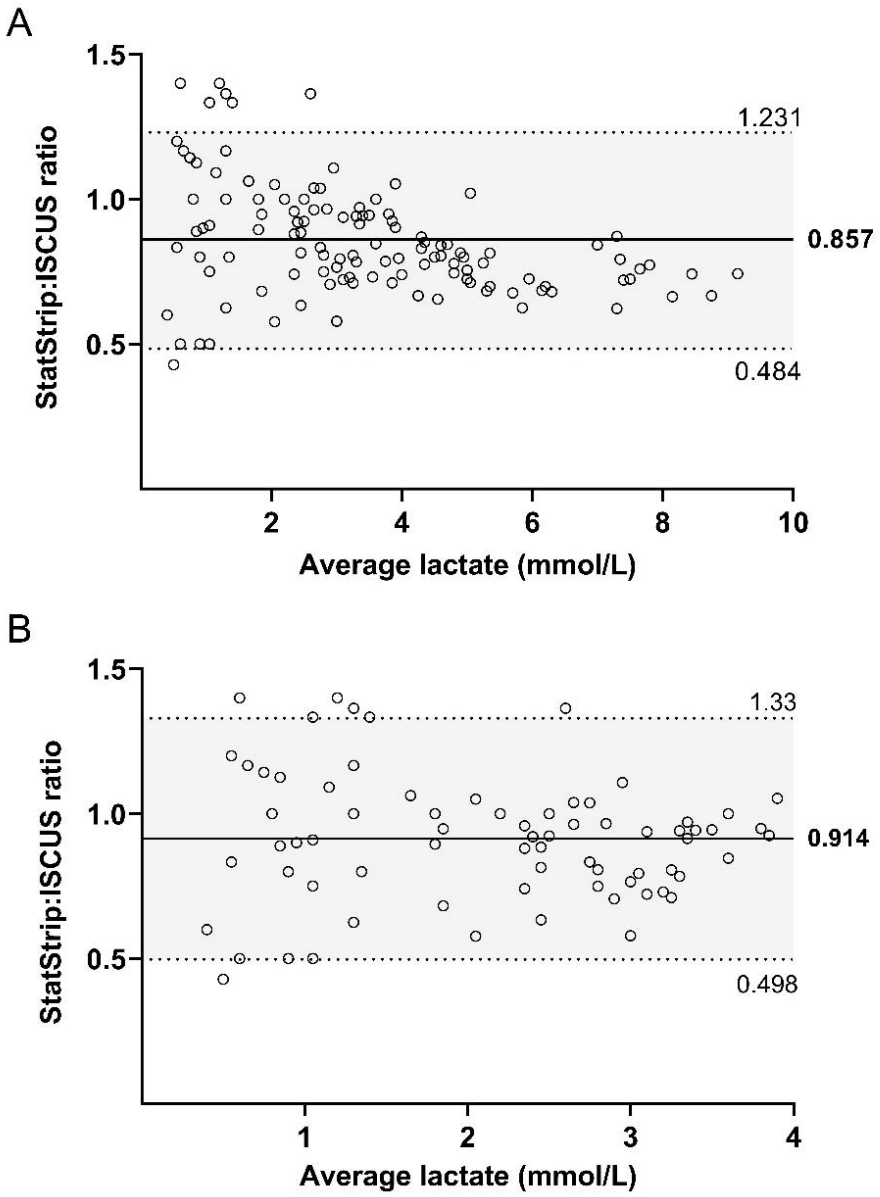

Figure 3.14 StatStrip Xpress® and ISCUS*flex* agreement by average lactate value. *(A) Bland-Altman plot of StatStrip:ISCUS lactate ratio against average lactate value for all paired measurements, with reduced skewness compared to Figure 3.13B. (B) Bland-Altman plot of StatStrip:ISCUS ratio for lactate values ≤ 4.0 mmol/L, showing normal distribution (D'Agostino-Pearson test, p=0.72). The 95% limits of agreement remained wide despite a narrower 95% CI for the mean ratio.*

3.4.4.2 Trend analysis using the StatStrip Xpress®

The above analyses focus on absolute values to compare the StatStrip Xpress® and ISCUS*flex*, however, one of the key advantages of continuous interstitial monitoring technology is that it provides information on metabolic trends, meaning clinicians are less reliant on single point values for decision-making. Trend data from the StatStrip was therefore also compared with that obtained by the reference ISCUS*flex* analyser. Figure 3.15 shows time series graphs for the four piglets for whom dialysate samples were also analysed with the StatStrip Xpress®. Although the proportional negative bias was evident at higher lactate values (F1 and H1), trends in interstitial lactate measured by the StatStrip were similar to those seen with the ISCUS*flex* and, more importantly, mirrored underlying changes in arterial lactate.

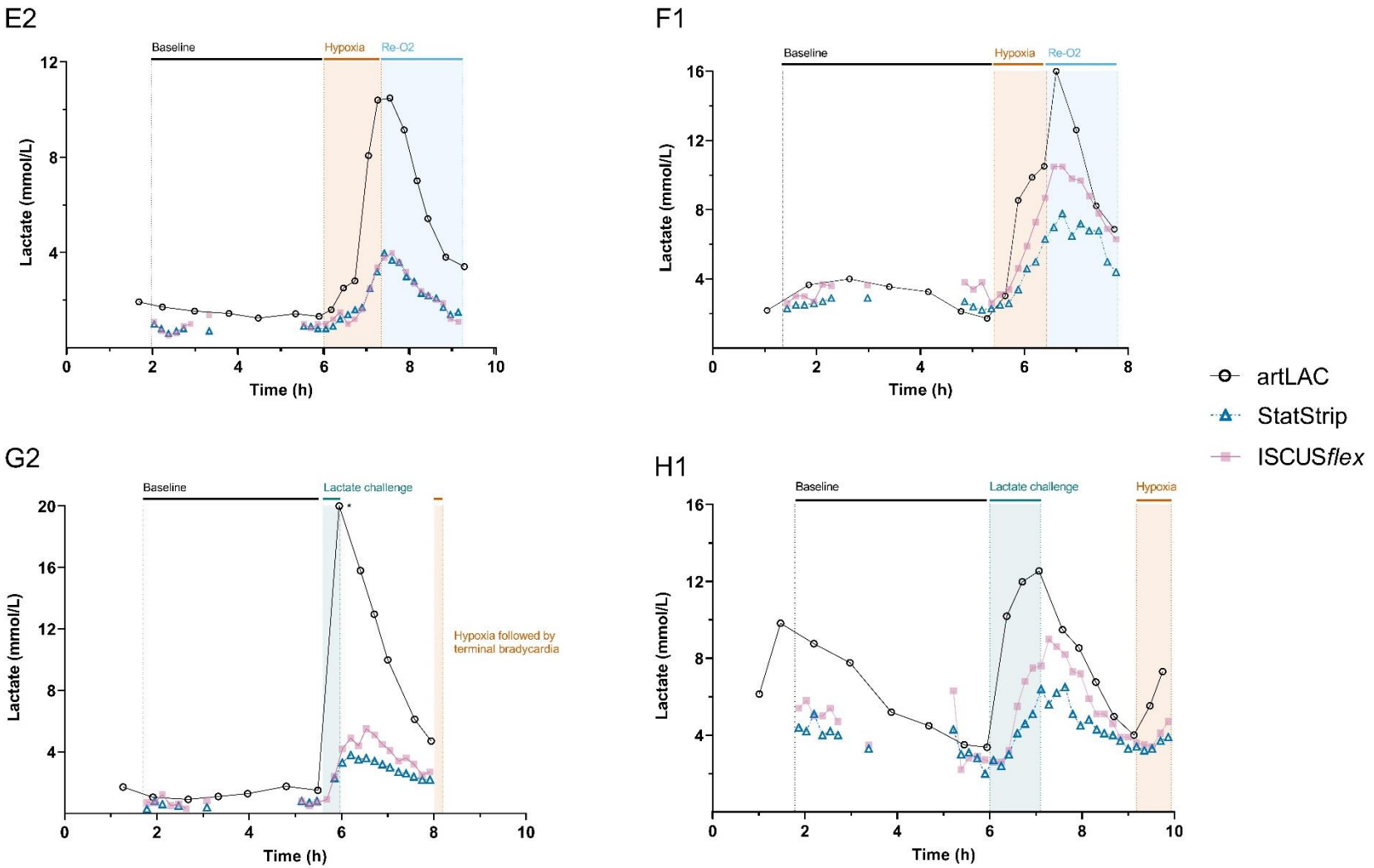

Figure 3.15 Trends in interstitial lactate measured by the StatStrip Xpress® and ISCUS*flex*. *Data presented by piglet and catheter flow rate (E2 = piglet E, flow rate 2.0 µL/min). The StatStrip's negative proportional bias is evident as lactate increases above 4 mmol/L.*

3.4.4.3 Assay precision

In addition to the method comparison study, the precision of the StatStrip Xpress® was evaluated and compared to the reference ISCUS*flex* analyser. Intra- and inter-assay CVs for all samples were below 10%. Precision of the StatStrip Xpress® appeared to decrease at higher lactate concentrations, whilst the ISCUS*flex* showed the opposite pattern, with CVs increasing at lactate concentrations below 2 mmol/L. Data are summarised in Table 3.7.

Table 3.7 Intra- and inter-assay precision of StatStrip Xpress® and ISCUS*flex*. *Samples designated according to lactate concentration and either by catheter (F2, G2) or as controls. The order of analysis was random for each run, but results are presented by increasing concentration. Inter-assay precision was evaluated over seven days using control samples only.*

StatStrip Xpress®	Sample	Mean (mmol/l)	SD	CV (%)
Intra-assay (n=6)	1 – F2 Low	1.63	0.052	**3.16**
	2 – Control Low	1.70	0.000	**0.00**
	3 – F2 Medium	3.40	0.179	**5.26**
	4 – F2 High	5.42	0.479	**8.85**
	5 – Control Elevated	7.47	0.698	**9.34**
Inter-assay (n=21)	Control Low	1.64	0.103	**6.26**
	Control Elevated	7.52	0.727	**9.67**

ISCUS*flex*	Sample	Mean (mmol/l)	SD	CV (%)
Intra-assay (n=6)	1 – G2 Low	0.567	0.137	**24.1**
	2 – Control Low	1.92	0.248	**13.0**
	3 – G2 Medium	2.95	0.217	**7.35**
	4 – G2 High	4.73	0.258	**5.46**
	5 – Control Elevated	10.78	0.325	**3.02**
Inter-assay (n=28)	Control Low	1.64	0.220	**13.4**
	Control Elevated	9.70	0.664	**6.84**

3.5 Discussion

The following section discusses the main findings of the study in relation to the existing microdialysis literature and considers the implications of this research for the development of a new fetal monitoring device. In concluding, the limitations of the current study are addressed and key areas for future research identified.

3.5.4 Relationship between interstitial and arterial lactate

In keeping with the findings of Tigchelaar et al. (2020), strong positive correlations between interstitial and arterial lactate were seen in the present study. Furthermore, interstitial lactate mirrored the major trends in arterial lactate seen in response to hypoxia and sodium lactate infusion. From the time series graphs, several important observations can be made regarding the relationship between these compartments.

Firstly, during the equilibration and calibration periods, resting interstitial lactate concentrations in the scalp appeared to exceed those of blood. Previous human studies of microdialysis in skin (Krogstad et al., 1996, Jansson et al., 1996, Petersen, 1999) and SAT (Jansson et al., 1990, Kopterides et al., 2012) have also shown this, leading the authors to propose these tissues as an important source of lactate. If the scalp remained a significant source of lactate production during hypoxia, then it is logical that interstitial concentrations would increase before, or in parallel with, arterial lactate. Accordingly, subcutaneous microdialysis might offer a more sensitive means of detecting tissue hypoperfusion than blood sampling. For example, Kopterides et al. (2012) found that SAT lactate increased before blood lactate in patients with shock. Lactate clearance in SAT has also been shown to precede falls in blood lactate in septic patients (Ilias et al., 2018). However, other human microdialysis studies (Petersen, 1999, Martinez et al., 2003, Ohashi et al., 2011) have reported conflicting results, with blood lactate leading changes in interstitial lactate. In the present study, arterial lactate preceded changes in interstitial lactate in all piglets, suggesting that the scalp is unlikely to be a significant source of lactate production during hypoxia. Hence, it may be more appropriate to view subcutaneous microdialysis as a window into the intravascular compartment, albeit one which is shifted-in-time or even distorted (Cobelli et al., 2016), rather than a means of directly assessing tissue hypoperfusion.

These results may be partly explained by the nature of the hyperlactataemia model used here. In the face of severe global hypoxia, the piglets' compensatory mechanisms were likely to be overwhelmed, resulting in a failure to preserve oxygenation to central organs such as the heart, brain and liver. As these highly metabolically active organs shift to anaerobic metabolism, they would be expected to become the primary source of lactate production and cause a rapid rise in blood lactate levels. The movement of lactate into the subcutaneous tissue depends, in turn, on a concentration gradient existing between the capillary endothelium and ISF, i.e. lactate will diffuse into the surrounding tissues once blood levels rise above interstitial concentrations to establish a positive arterio-interstitial gradient (Cengiz and Tamborlane, 2009). The lactate challenge largely replicates this dynamic, in that blood changes appear to drive lactate into the tissues. However, it is possible that the same compensatory mechanisms, in response to milder hypoxic-ischaemic insults, may lead to lactate production in skin before oxygenation of critical organs is compromised.

The presence, or not, of an arterio-interstitial lactate concentration gradient may help to explain the poorer agreement observed between the compartments in some piglets. For example, peak arterial lactate values in piglets C and D (4.9 and 5.6 mmol/L, respectively) were relatively low compared to the baseline interstitial concentrations (mean 2.9 and 3.5 mmol/L, respectively; values adjusted for RR). Therefore, a weaker gradient may have been present for the diffusion of lactate into ISF.

The smaller relative increase (or fold-change) in interstitial lactate during hypoxia and lactate infusion also lends support to the above observations. As baseline interstitial lactate concentrations are higher, the relative increase observed during hyperlactataemia was smaller than in the arterial compartment. Previous exercise studies in adults have reported similar results, with the relative increase in interstitial lactate concentration up to three times smaller than blood (de Boer et al., 1994, Ellmerer et al., 1998). Clinically, this is relevant because a significant increase in arterial lactate in the fetus may be less apparent when monitoring the interstitial compartment, particularly after accounting for microdialysis recovery. For example, a three-fold rise in arterial lactate from 2 to 6 mmol/L may only result in a two-fold rise in unadjusted interstitial lactate from 1 to 2 mmol/L (RR ~ 50%); hence, more precise analytical methods and narrower thresholds for intervention may be required.

Alternative explanations are likely to account for the data from piglet B, in whom the weakest agreement between interstitial and arterial lactate was observed. As discussed in Chapter 2, piglet B became progressively anaemic and hypovolaemic during the study due to heparin-induced coagulopathy. Peripheral vasoconstriction is an early response to hypovolaemia (Turner et al., 2020), therefore reduced blood flow to the scalp may have disrupted interstitial-blood dynamics and caused lower-than-expected tissue lactate concentrations. It is also plausible that the coagulopathy directly altered the local interstitial environment by causing an accumulation of blood in the extravascular space. There was visible evidence of haematoma formation at the catheter insertion sites in both piglets A and B, as shown in Chapter 2.

For the remaining piglets, there was a clearer relationship between interstitial and arterial lactate profiles. Overall, there also appeared to be more consistent agreement between the compartments than has been demonstrated in past microdialysis studies (Ellmerer et al., 2009, van den Heuvel et al., 2009, Kopterides et al., 2012), for which there are several possible physiological and methodological explanations. Most clinical microdialysis studies in humans have evaluated this relationship in peri-operative or critical care patients, with much lower peak arterial lactate values (below 5 mmol/L). For reasons already explained, interstitial lactate may poorly reflect trends in arterial lactate at these concentrations. It is also plausible that the hypodermis of the scalp provides a better site for microdialysis catheter insertion and sampling, in terms of its reproducibility and physiological validity. To date, SAT of the abdominal wall (Ellmerer et al., 2009, van den Heuvel et al., 2009, Ohashi et al., 2011) and upper thigh (Kopterides et al., 2012, Ilias et al., 2018) have been the most commonly utilised sites for sampling. However, Wolf et al. (2018) showed that subcutaneous lactate measurements obtained from the neck were more accurate than those from the abdomen, when compared with reference blood measurements, in an adult porcine model of hyperlactataemia. The scalp has a particularly rich arterial blood supply derived from branches of the common carotid artery, which also supplies the brain, so its perfusion may be preserved later than that of other peripheral tissues during compensatory adaptations to hypoxia. In view of the potential impact of blood flood on microdialysis recovery (Clough et al., 2002, de Lange, 2013), further research is required to formally investigate the effect of different patterns of hypoxia on scalp perfusion and arterio-interstitial lactate dynamics.

3.5.4.3 Interstitial monitoring in the intrapartum setting

The effects of labour, and its potential complications, on the fetal scalp must also be taken into account when evaluating its suitability as a microdialysis sampling site. Caput succedaneum, a diffuse soft tissue swelling of the presenting part of fetal scalp, is a common occurrence during labour as a result of mechanical forces acting on the fetal head. Some researchers have questioned the validity of capillary blood sampling from areas of caput succedaneum (Odendaal, 1974, Chandraharan and Wiberg, 2014). Caput succedaneum is particularly relevant because it typically forms on the area of the scalp that is most accessible, through the partially dilated cervix, for applying a monitoring device. Relatedly, 'blind' application of a microdialysis-based fetal monitoring device by digital vaginal examination during labour may be less reliable than directly visualised catheter insertion, as was undertaken here. Term human fetuses also have a larger scalp surface area than newborn piglets, which may result in greater variation between sampling sites (contralateral catheters were only separated by 3-4 cm on the piglets). This may present potential problems if, for example, the microdialysis device becomes dislodged and needs to be re-inserted; it is unclear if lactate values measured at a new site could be interpreted reliably, and without delay, to inform clinical decision-making.

In the current study, the agreement between scalp interstitial and arterial lactate within individual piglets appeared broadly similar for both catheters. However, there was some variation in the interstitial profiles obtained from the two catheters, even after accounting for differences in lactate recovery at their respective flow rates. Comparing data from the calibration period, when flow rates and sampling intervals were identical for both catheters, also showed evidence of variation within the piglets under steady-state conditions. Some degree of within-subject variation is expected due to biological variability and analytical imprecision (Røraas et al., 2016). For example, O'Brien and Murphy (2013) found a statistically significant difference in mean pH values between paired FBS samples obtained from a single scalp puncture. Importantly, within-subject variation in interstitial lactate concentrations in the current study generally appeared smaller than variation observed between subjects. There were two-fold differences in interstitial lactate levels at baseline in piglets C to G, while arterial lactate fell within a much narrower range from 1.32 to 1.72 mmol/L in the same piglets (piglet B was

excluded to a gradual rise in arterial lactate towards the end of the calibration period; piglet H was excluded due to elevated arterial lactate immediately following induction).

If these differences reflect true physiological variability in lactate metabolism between individuals, then using stationary thresholds for clinical intervention may not be appropriate: for example, a cut-off value of 4 mmol/L would represent a four-fold rise in interstitial lactate for some piglets, compared to a two-fold rise in others. This strengthens the theoretical argument for analysing lactate trends rather than making decisions based on absolute lactate values obtained in a discontinuous, 'snapshot' manner. Previous research to establish reference values for umbilical cord arterial pH and lactate also favours this approach. Wiberg et al. (2008) showed that median umbilical cord arterial plasma lactate concentrations increased from 3.9 mmol/L at 37 weeks' gestation to 4.9 mmol/L at 42 weeks' gestation. Hence, even amongst term singleton infants, normal variation in lactate concentrations is likely to exceed the stationary cut-offs used to interpret FSL results in practice (normal ≤ 4.1 mmol/L, abnormal ≥ 4.9 mmol/L; see also Chapter 1, Table 1.1). Kitlinski et al. (2003) demonstrated a similar linear relationship between gestational age and umbilical cord arterial pH. The reasons for the observed variation in interstitial lactate concentrations between (and within) subjects in the present study are unclear, but future studies should be appropriately powered to address these questions.

One of the key advantages of microdialysis, as demonstrated by the current study, is that it offers the potential to monitor trends in interstitial lactate. This may ultimately enable a shift in focus away from the traditional approach, which has been to compare absolute lactate or pH values against reference ranges and stationary cut-offs. Instead, each subject can act as its own control if an individualised baseline is first established under normal conditions. This is similar to STAN, which is only commenced in the presence of a normal CTG when 'there is certainty that the fetus is not already hypoxic' (Sacco et al., 2015). The presented data suggests that once normal interstitial lactate values have been established for each individual, a consistent upward trend from this baseline can be reliably interpreted to indicate an increase in arterial lactate, which may be a sign of evolving hypoxia.

3.5.4.4 Arterio-interstitial delay

Finally, this study sought to describe the temporal relationship between arterial and interstitial lactate. Clinically, a physiological delay of 15 min or less was considered likely to be acceptable for a microdialysis-based device capable of providing results in real or near-real time, as this is comparable to the time taken for an FBS to be performed and resulted (Tuffnell et al., 2006, Annappa et al., 2008). The median delay in the current study was 15 min, however, methodological considerations greatly restricted the temporal resolution of this data and prevented us from drawing any firm conclusions regarding clinical acceptability. Available data on lactate dynamics is limited to exercise studies in humans, with reported delays of between 7.8 to 10 min (de Boer et al., 1994, Ellmerer et al., 1998). However, the presented findings are generally consistent with the extensive literature on blood-interstitial glucose dynamics (Sternberg et al., 1996, Basu et al., 2013, Schiavon et al., 2015), outlined in Chapter 1. This was expected given the similarities between lactate and glucose and, more generally, the time-dependent nature of diffusion. There is a comparative lack of data on the time lag limited studies of interstitial lactate monitoring Concerning glucose, several authors have noted that the relationship between blood and interstitial concentrations, with respect to both magnitude and time, is affected by whether glucose levels are rising or falling (Aussedat et al., 2000, Kulcu et al., 2003, Wentholt et al., 2007). Aussedat et al. (2000) described this as the 'push-pull phenomenon', hypothesising that insulin-mediated uptake of glucose from the interstitial fluid into surrounding cells resulted in subcutaneous glucose concentrations decreasing earlier. Similar findings might be expected given the above discussion on blood-interstitial lactate gradients. That is, when lactate is rising, a longer delay will be observed because interstitial lactate only 'follows' once it has been surpassed by arterial lactate. When arterial lactate begins to fall, however, the gradient is reversed more quickly because peak concentrations in both compartments are similar at equilibrium, and therefore a shorter delay observed. There was no clear evidence of this in the current study: trend agreement and the delay between compartments appeared similar whether lactate was rising or falling, and regardless of the rate of such changes.

3.5.5 Implications for the development of a new fetal monitoring device

This study's findings also have important implications for the development of future fetal monitoring technology, specifically that which utilises microdialysis sampling.

Firstly, local tissue trauma following subcutaneous catheter insertion did not appear to influence interstitial concentrations of lactate, pyruvate, or glucose in our neonatal piglet model. Although these findings are consistent with previous studies in human skeletal muscle (Sørensen et al., 2018, Ashina et al., 2005), they cannot be generalised to other tissues or species. Andelius et al. (2019), for example, found that intracerebral lactate concentrations peaked 1-2 h after catheter insertion and only reached steady state at 4-5 h. This study is the first to report on dialysate concentrations of lactate and related metabolites within the first hour of subcutaneous catheter insertion. Crucially, these findings suggest that values obtained within 30 min of catheter insertion into the scalp are likely to be representative of true interstitial concentrations of these analytes. For a fetal monitoring device, an interval of 30 min from 'decision to test' to obtaining an initial result is therefore considered realistic: this would include 15 min for preparation of the monitoring system and transvaginal catheter insertion, followed by a washout period and first sample collection (10 min). As highlighted above, however, the device would ideally be applied before there are any concerns regarding fetal wellbeing so that baseline interstitial lactate levels can be established for that individual fetus. Once *in situ*, it would be capable of providing further results with minimal delay, either when prompted by clinicians or on a continuous, near real-time basis. Some delay will persist due to dead volume in the outflow tubing of the microdialysis system. This dialysate, reflecting biological events that have already occurred, equated to an approximate delay of 6 min at a flow rate of 1.0 µL/min in the current study. The total time taken for a change in arterial lactate to be detected by a microdialysis-based device must take this into account this sampling delay, in addition to any physiological lag between the two compartments, as discussed above.

In practical terms, the choice of perfusate flow rates used in the study was largely determined by the minimum volume required to manually process samples and evaluate multiple analytes using the ISCUS*flex*. Restricting future studies to lactate analysis alone would enable the collection of smaller dialysate volumes, which would, in turn, allow a reduction in the perfusate flow rate (to maximise lactate recovery) and/or sampling interval (to improve temporal resolution). Manually handling and

storing sample volumes below 10 µL is technically difficult and carries risks of sample evaporation and pre-analytical errors, therefore a flow rate of 1.5 to 2.0 µL/min with a sampling interval of 5 min may present a pragmatic balance for offline analysis of dialysate lactate in future *in vivo* studies. This would also reduce the sampling delay to approximately 3-4 min. A fully integrated device, in which microdialysis sampling is directly coupled to systems for sample separation and analyte detection, referred to as online microdialysis, would negate the need for sample handling and storage and make it possible to analyse sub-microlitre samples (Saylor and Lunte, 2015). The concluding chapter of this thesis discusses this further in relation to the proposed design of a fetal monitoring device.

In addition to reducing sample volume requirements, restricting the device to lactate analysis would simplify its development and technical complexity. This approach is further supported by the study's findings on multi-analyte monitoring. Although the L/P ratio has been suggested as a more specific marker of tissue hypoxia-ischaemia than lactate (Rimachi et al., 2012, Go et al., 2021), interstitial L/P ratios did not correlate with or reflect trends in arterial lactate as strongly as interstitial lactate alone. The reasons for this are unclear, although the wide variation in L/P ratios observed between piglets may reflect a compounding of analytical error (Holmes and Buhr, 2007). Unlike single analytes, ratios are subject to measurement error in both the numerator and denominator, with the latter having a proportionally greater effect on the ratio's final value. The precision of the ISCUS*flex* pyruvate assay was not formally evaluated; however, in support of this explanation, Tholance et al. (2011) estimated inter-assay CVs for L/P and L/G ratios to be nearly twice those of lactate, pyruvate and glucose.

Interstitial L/G ratios showed a slightly stronger correlation with arterial lactate, but poorer trend agreement, again, compared to interstitial lactate. Recently, Kastellorizios and Burgess (2015) found that subcutaneous ratios combining lactate and glucose were more sensitive than single-analyte monitoring for detecting metabolic changes and predicting exhaustion in a rat model of intense exercise. It is possible that the administration of intravenous glucose solutions in this study to avoid hypoglycaemia affected the ability of L/G ratios to reflect hypoxia-induced metabolic changes. This could be addressed by refining the glycaemic protocol in future studies. Notably however, there is very limited data relating to L/P ratios in umbilical cord blood at

delivery (Chou et al., 1998, Nordström et al., 1998), and none relating to L/G ratios. Hence, a much clearer relationship between these ratios and the reference standard of arterial lactate would need to be established to justify the additional complexities involved in monitoring multiple analytes simultaneously. In the current study, interest in multi-analyte monitoring stemmed largely from the fact that ratios are independent of relative recovery and so avoid the need for complicated calibration methods. However, this theoretical advantage becomes much less relevant if future clinical microdialysis studies move away from quantitative techniques towards trend analysis, as this thesis ultimately proposes.

Finally, although the majority of the data have been presented unadjusted for microdialysis recovery, the calculated recoveries for lactate, glucose, pyruvate, and glutamate in this study were consistent with those in recently published literature. Weir et al. (2018) used the flow rate method to estimate the following recoveries in human adipose tissue at a flow rate of 0.3 µL/min: 84% for lactate, 69-72% for glucose, 74-76% for pyruvate, and 80% for glutamate; figures which closely align with this study's findings. Directly comparing data from other studies is made difficult by the use of different microdialysis catheters, calibration methods and tissues. Similarly, the recovery realised *in vivo* with a fetal monitoring device would ultimately depend on the surface area of the incorporated microdialysis membrane, which is likely to be considerably smaller than that of the 63MD catheter used here. Tigchelaar et al. (2020) reported an *in vitro* lactate recovery of 24.7% at a flow rate of 1.5 µL/min for the microdialysis probe they incorporated into an FSE, approximately one third the recovery observed *in vitro* for the 63MD catheter. Nevertheless, these data serve as a useful starting point in terms of the subcutaneous dialysate lactate values expected and the necessary analytical performance (e.g. range, limit of detection, limit of quantification) of the proposed device.

3.5.6 Validation of the StatStrip Xpress® lactate meter

The ISCUS*flex* provided a reference multi-parameter microdialysis analyser which has been used extensively in pre-clinical and clinical studies. However, its cost and service requirements are high and present a potential barrier to researchers and clinicians, as was my own experience during this study. It was not possible to service an older model

of the ISCUS*flex* (CMA 600) owned by the University of Edinburgh and, after significant delays, a new analyser had to be loaned directly from the UK distributor (Linton Instrumentation, Norfolk, UK) to process the dialysate samples. Laboratory-based assays are an alternative reference method which were used for initial *in vitro* experiments (data not presented), but they were not considered appropriate for evaluating multiple analytes on several hundred small volume samples, due to the time constraints and the technical laboratory experience required. The StatStrip Xpress® Lactate Meter, although designed for testing whole blood, has been shown to be reliable for measuring lactate levels in amniotic fluid (Hall et al., 2014) and this study extends its potential application to the analysis of microdialysis samples.

The proportional negative bias observed with the StatStrip Xpress® is consistent with its performance on whole blood samples (Bonaventura et al., 2015, Iorizzo et al., 2019b, Graham et al., 2019). Bonaventura et al. (2015) postulated that the large negative biases seen at higher concentrations may reflect saturation of the lactate oxidase enzyme on the device test strips. However, there was no apparent clustering of StatStrip Xpress® measurements in this study at higher concentrations, which might be expected if this were the case. Indeed, the precision of the StatStrip decreased as lactate levels rose (as indicated by higher CVs), which is in contrast to previous findings in scalp and umbilical cord venous blood (Iorizzo et al., 2019b).

Nevertheless, the StatStrip's overall precision for analysing dialysate lactate appeared similar to its performance on whole blood: Bonaventura et al. (2015), for example, calculated an overall CV of 6%. Crucially, at lower lactate concentrations, the precision of the StatStrip Xpress® appeared better than the reference ISCUS*flex* lactate assay. This does not appear to be related to sub-optimal performance of the ISCUS*flex* in the current study: recommended procedures for handling samples were followed (Abrahamsson et al., 2008) and measured lactate values in control samples fell within the "Acceptable Control Range" on 27 of 28 occasions. In keeping with these findings, Tholance et al. (2011) reported a marked decrease in the inter- and intra-assay precision of the ISCUS*flex* below lactate concentrations of 1.2 mmol/L. Thus, the benefits of the ISCUS*flex* in minimising measurement bias may be counter-balanced by greater imprecision across the low-to-normal range of interstitial lactate

concentrations one would expect to see with subcutaneous microdialysis in most clinical settings.

For future studies, it appears that the systematic negative bias of the StatStrip Xpress® means that it should not be used interchangeably with the ISCUS*flex.* Even if its use were restricted to lower lactate concentrations, as previous authors have suggested (Graham et al., 2019), the 95% limits of agreement between the methods in the current study remained wide, despite a small mean difference. However, this should not completely discount the potential advantages of POC meters, in terms of their accessibility, costs, and turn-around times, compared to traditional benchtop analysers and laboratory-based assays. The trend data provided by the StatStrip Xpress® in the current study further highlights the potential value of this strategy for microdialysis research. For *in vivo* studies, one possible approach would be to use the StatStrip to analyse both arterial and dialysate samples, which would reduce any bias introduced by the use of different analytical methods between the compartments. This would also reduce blood and dialysate sample volumes, enabling more frequent sampling and improving the temporal resolution of the data.

3.5.7 Limitations and areas for future research

Several of this study's strengths and limitations relate to neonatal piglet model itself and have been outlined in Chapter 2. As with the physiological and ABG data presented in that chapter, direct comparisons of interstitial lactate concentrations between piglets were restricted by the lack of protocol standardisation in this exploratory study. However, the frequency of both arterial and interstitial sampling enabled detailed time series to be presented for individual piglets, which few previous studies of interstitial lactate monitoring have done (Tigchelaar et al., 2020, Ellmerer et al., 2009, van den Heuvel et al., 2009). Importantly, individual profiles suggested the possibility of weaker agreement between arterial and interstitial compartments in piglets with lower peak arterial lactate concentrations, a finding which was not apparent in the initial paired correlation analysis. This observation has not been reported in past studies of interstitial lactate monitoring, discussed at the start of this chapter and in Chapter 1, possibly because peak blood lactate concentrations have generally been much lower than those seen in the current study. However, differences in experimental protocols, species, and

microdialysis sampling sites, means it is not possible to confirm if blood lactate concentrations alone explain these discrepant findings.

Relatedly, because the hypoxia and sodium lactate infusion protocols described here targeted higher lactate concentrations, it would be important to replicate these studies in piglets with normal and mild-to-moderately elevated blood lactate (below 6 mmol/L), as is more likely to be encountered in clinical practice. Developing protocols to simulate gradually evolving and subacute intrapartum hypoxia would help address this question and improve the applicability of our data to the setting of fetal monitoring. Another key step for future studies would be to extend the duration of subcutaneous microdialysis monitoring to a minimum of 24 h, which reflects the maximum expected duration of device use, taking into account pregnancies in which the device may be applied prior to the onset of established labour. As most previous human studies of subcutaneous microdialysis have involved sampling over several consecutive days, it is not anticipated that this would present significant problems. Nevertheless, this would also help to exclude certain problems related to the microdialysis system, such as membrane biofouling and delayed effects of catheter-related trauma.

There are two other main limitations related to the application of microdialysis in the present study. Firstly, although microdialysis sampling was purposefully restricted to the scalp hypodermis to ensure the data were relevant to the intended application, it was not possible to directly compare data from contralateral catheter sites because of the use of different perfusate flow rates. This limited our ability to evaluate biological variation within subjects. McCormack and Holmes describe biological variation as the ‘noise attributable to normal physiological processes when repeated measurements are made over time in an individual’ (McCormack and Holmes, 2020). Understanding biological variation, as well as analytical and pre-analytical sources of variation, is therefore critical to accurately interpreting serial measurements of interstitial lactate in future studies. Taking skin biopsies from catheter sites for histological analysis and comparison may further improve understanding of the local tissue response to catheter insertion, and whether this contributes to any variation observed.

Secondly, the microdialysis and arterial sampling schedule used allowed only for an approximate determination of the arterio-interstitial time lag. Because the timing of arterial sampling was not fixed, but rather guided by each piglet’s physiological status,

the observed change points in arterial lactate may not have reflected the true peak and nadir values. This was unavoidable due to maximum blood sampling protocols, nevertheless, future refinement of the protocol would enable ABG sampling to be undertaken on a standardised schedule and in conjunction with dialysate sampling for *a priori* paired comparisons. Sampling at 10 min intervals further limited the temporal resolution of our microdialysis data. As discussed above, a higher sampling frequency could be readily achieved by increasing the perfusate flow rate and/or collecting smaller dialysate volumes. With respect to the intravenous sodium L-lactate infusion, for example, sampling at a perfusate flow rate of 2 μL/min and interval of 2 min would allow much earlier detection of changes in interstitial lactate. In the case of glucose, previous studies have used intravenous boluses of radio-labelled tracer glucose with even more frequent dialysate sampling to obtain high-resolution (1 min) tracer curves in the blood and interstitial compartments (Basu et al., 2013, Schiavon et al., 2015). Similar methodology could be employed with radio-tracer lactate, which has been used extensively to study lactate metabolism in other tissues (Herrero et al., 2007, Faubert et al., 2017, Hasenour et al., 2020).

Finally, as highlighted in the previous chapter, the absence of a sham-operated group (no hypoxia and/or lactate challenge) is a key limitation of this study. For example, the decision not to collect tissue specimens for histopathological analysis was made primarily because there was no control group to act as a comparator. Whilst this study did not seek to correlate interstitial lactate with histopathological evidence of hypoxic injury, evaluating the association between interstitial lactate and end-organ injury, independent of arterial lactate, would be an important step towards demonstrating the clinical applicability of microdialysis for fetal and/or neonatal monitoring.

3.6 Conclusions

This study explored the potential of using subcutaneous microdialysis to monitor the metabolic response to hypoxia and lactate infusion in newborn piglets. Overall, there was a strong positive correlation between interstitial and arterial lactate. Trends in interstitial lactate mirrored those seen in blood in most piglets, with an estimated delay of 10 to 20 min. In keeping with the findings of clinical microdialysis studies in humans,

however, there was individual variation in the closeness of agreement between the compartments. The current findings nevertheless demonstrate physiological proof of concept for interstitial monitoring as a novel approach to detecting perinatal hypoxia and address important questions about the feasibility of the microdialysis technique in the intrapartum setting. Further studies are required to accurately determine the time delay between compartments and investigate arterio-interstitial dynamics at lower blood lactate concentrations, as would be expected during fetal and/or neonatal monitoring.

This study also provides the first description of the use of POC meters for analysing dialysate samples. Whilst these findings indicate that the StatStrip Xpress® should not be used interchangeably with the reference ISCUS*flex* analyser, it provided similar information on interstitial lactate trends, particularly at lower lactate concentrations. Moreover, the accuracy and precision of the StatStrip Xpress® for analysing interstitial lactate was comparable to its reported performance in whole blood samples, for which it is already widely used in clinical practice. Therefore, it may offer an accessible, cost-effective means of undertaking offline microdialysis ‘at the bedside’ in future clinical and research studies.

Chapter 4 A qualitative study of UK obstetricians' views about intrapartum fetal monitoring technology

4.1 Introduction

Chapter 1 outlined the basic principles of intrapartum fetal monitoring from a physiological perspective and discussed the central role of fetal heart rate monitoring. Various adjunctive technologies to CTG were introduced and the evidence for each summarised, along with their practical and theoretical limitations. Based on available data, we have seen *what* adjunctive technologies are currently used to monitor intrapartum fetal wellbeing in the UK and other developed countries, as well as evidence for variations in practice across different maternity care settings. However, this paints an incomplete picture of the present landscape of fetal monitoring because it offers little insight into *how* and *why* different technologies are used.

There is growing recognition that intrapartum fetal monitoring is a complex process. Poor outcomes result not just from incorrect CTG classification, but from delays in escalating and/or acting on abnormal fetal heart rate patterns (NHS Resolution, 2019, RCOG, 2017). This resonates with the conclusion of the INFANT authors that "most adverse outcomes associated with preventable substandard care seemed to involve failure to take appropriate management decisions once the [CTG] abnormality had been recognised" (INFANT Collaborative Group, 2017). The interplay of fetal monitoring with other key elements of intrapartum care, such as risk recognition, human factors and team communication, further adds to this complexity. In the latest Each Baby Counts report (RCOG, 2020), there were, on average, six critical factors contributing to potentially avoidable harm for each baby. It is perhaps unsurprising then that national initiatives to improve CTG interpretation and training have not fully addressed the problems highlighted in the first confidential enquiry over 20 years ago (Confidential Enquiry into Stillbirths and Deaths in Infancy, 1993). Similarly, the introduction of a new fetal monitoring device may not translate into improved outcomes unless researchers understand how clinicians view that technology and use it in their

everyday clinical practice. A recent progress update from the Early Notification scheme, a national programme for reporting infants born with a potentially severe brain injury following term labour, recommended that future research must look beyond purely 'technical' solutions to explore the context in which intrapartum fetal monitoring occurs and the social mechanisms underpinning this process (NHS Resolution, 2019). Qualitative research allows one to understand "how things work in particular contexts" (Mason, 2002) and therefore lends itself well to this type of enquiry.

This chapter begins with a review of existing qualitative studies on healthcare professionals' views of intrapartum fetal monitoring, including recent experiences of introducing STAN into clinical practice. Past literature relevant to the implementation of new fetal monitoring technology within the NHS is addressed, and the rationale for undertaking a qualitative interview study of UK obstetricians is then presented.

The remainder of this chapter is structured into five sections. Section 4.2 outlines the aims and key research questions of the study, which were informed by the reviewed literature. Section 4.3 provides an in-depth discussion of the study's methodological approach, including sampling, participant recruitment and details the processes of data collection and thematic analysis, and ethical considerations of the research. The main findings of the study are presented in Section 4.4 through five inter-connected themes: 'the best we've got'; 'the developing obstetrician'; 'the socio-cultural context'; 'changing practice'; and 'future directions. Finally, Section 4.5 further explores these themes in relation to the original research questions and existing literature. This section considers the wider implications of my research for fetal monitoring practice and concludes by summarising the study's findings, its strengths and limitations, and identifying possible areas for future research. There are two appendices at the end of the thesis related to this chapter: the participant information sheet and the interview topic guide.

4.1.1 Understanding current fetal monitoring practice

Much of the existing qualitative literature on professional views of fetal monitoring relates to the increasing use of continuous CTG monitoring in place of IA. These studies have also predominantly or exclusively sampled midwives, whose roles span the care

of both low- and high-risk women during labour. Although these findings do not relate directly to the use of adjunctive technologies, they help to frame understanding of current fetal monitoring practice in the UK and the attitudes of midwives and doctors towards technology on labour ward.

4.1.1.1 Professional views of intrapartum fetal monitoring technology

Smith et al. (2012) performed a systematic review and meta-synthesis of eleven studies exploring professional views of intrapartum fetal monitoring, seven of which took place in the UK. All studies addressed the use of fetal heart rate monitoring rather than adjunctive technologies. Only two doctors were interviewed across the included studies, compared to 124 midwives. Reassurance and perceived protection against litigation through the use of CTG emerged as a prominent theme in the meta-analysis. Although the CTG provided 'proof' of fetal wellbeing that was not achievable through IA, professionals often felt that it was overused, which eroded their skills and contributed to the medicalisation of childbirth. Midwives' trust in the accuracy and reliability of CTG varied, a theme expanded upon by Altaf et al. (2006), who identified three different orientations towards the use of CTG: faith, caution and scepticism. These attitudes were influenced by individuals' clinical backgrounds; for example, those typically looking after low-risk women were more likely to view CTG as encouraging the 'deskilling' of midwives, whilst those routinely caring for high-risk women generally expressed confidence in the use of CTG (Altaf et al., 2006).

Midwives in past studies (Altaf et al., 2006, Hindley and Thomson, 2007) have also reported disagreements with doctors in relation to CTG interpretation and decision-making, occasionally giving rise to conflict within the team. Some midwives perceived doctors as relying too heavily on CTG and were therefore reluctant to accept their clinical judgement at face value, particularly if doctors were considered junior or inexperienced (Altaf et al., 2006). However, the only study to enrol both midwives and doctors (McKevitt et al., 2011) did not report any differences in the attitudes of the two professional groups towards CTG technology. Multi-disciplinary training and the use of shared codes of practice – for example, standardised systems for CTG classification and clear escalation policies – were highlighted by McKevitt et al. (2011) and previous

authors (Hindley and Thomson, 2007) as helping to enhance communication and collaboration between different professional groups and so avoid conflict.

Most recently, Mayes et al. (2018) interviewed eight doctors and ten midwives at a single Australian centre introducing STAN as part of a pilot RCT. Participants, particularly midwives, viewed STAN as being incongruent with their current philosophy of intrapartum care and this broadly aligned with perceived difficulties in using the technology. Both midwives and doctors expressed reservations about the evidence for STAN and its relevance to their population, along with a desire to see results from using the technology first-hand before accepting its value. Many midwives and doctors also reported problems around staff education and training to facilitate implementation and the need for ongoing support in this respect. Finally, a lack of endorsement by key team members and professional bodies was seen as a barrier to uptake.

The qualitative literature summarised above, as well as available quantitative data on the use of adjunctive technologies (discussed in Section 1.2.5), suggests considerable variation in practice in current obstetric practice in developed countries. During conception of the qualitative study presented in this chapter, I also supervised a University of Edinburgh BSc student (Isabel Thomas) project which surveyed 103 healthcare professionals, via online questionnaire, on their attitudes toward adjunctive fetal monitoring technologies. Almost three quarters of the participants sampled worked in one of three tertiary UK units (Royal Infirmary of Edinburgh; St George's Hospital, London; and the Royal Victoria Infirmary, Newcastle-upon-Tyne) yet there were striking differences in their attitudes to FBS and STAN. This highlighted the need for a contemporary, in-depth qualitative exploration of UK practice and also helped to inform the sampling strategy chosen for this study.

4.1.1.2 Dissemination and implementation of STAN

Several studies have been published on the implementation of new fetal monitoring technology which are also relevant to our understanding of current and future practice. Amer-Wåhlin et al. (2005) identified multiple barriers to behavioural change affecting the acceptance of STAN during a multi-centre Swedish RCT, which manifested as protocol violations during the study. These related to the environment, the personal characteristics (or practice style) of clinicians, and prevailing opinions within the team.

The authors observed a tendency for younger, less experienced midwives and doctors to accept change more readily. For others, retraining through audit and group case-based discussions increased personal experience and confidence in the technology and facilitated acceptance of the new guidelines. Opinion leaders, some of whom were initially hesitant, also came to act as 'catalysts' for implementation. After retraining, there was a significant reduction in operative deliveries for fetal distress and in the frequency of FBS in the CTG plus STAN group compared to the CTG only group. The authors argued that, for successful implementation and adoption, users must have time to gain familiarity with new technology, supported by active involvement from opinion leaders and strategies for regular feedback and follow-up (Amer-Wåhlin et al., 2005). This is consistent with published experiences of introducing STAN in the UK (Chandraharan et al., 2013) and Finland (Timonen and Holmberg, 2018), as discussed in Section 1.2.2, where a gradual 'learning curve' was paramount to the eventual success of the technology.

4.1.1.3 Adjunctive technologies within the NHS

The above literature suggests that at least some of the barriers faced during the implementation and adoption of new healthcare technology are shared, whilst others appear to be unique to a particular technology in a particular context. For example, fetal scalp lactate is a simple innovation which presents few of the perceived barriers to adoption that STAN does; it is quicker, has a lower failure rate, and has been shown to perform as well as FBS for pH measurement in two RCTs (Westgren et al., 1998, Wiberg-Itzel et al., 2008). Hence, it remains unclear why fetal scalp lactate has not been accepted into routine NHS practice, as it has in other countries, long after being endorsed by the RCOG (RCOG, 2015) and NICE (NICE, 2017). Examples of this research-to-practice gap are widely reported in other fields (Mallonee et al., 2006, Munro and Savel, 2016) but our understanding of the specific challenges faced in the clinical translation of intrapartum fetal monitoring technology in the UK remains limited.

Identifying barriers, and potential facilitators, to translating new technology is also important for researchers planning and evaluating clinical trials of adjunctive technologies. To date, most RCTs have focused on pre-specified clinical outcomes ('Does it work?') rather than the processes involved in implementing the intervention

('How and why it works or does not?'). Process evaluations are designed to explore the latter questions and can help researchers to distinguish between interventions that are "inherently faulty and those that are badly delivered" (Oakley et al., 2006). They are especially important in evaluating complex interventions across multiple sites, where the same intervention may be perceived and implemented in different ways. While there has been progress in recent years towards routinely collecting and reporting data on process outcomes, as in the INFANT trial of computerised CTG interpretation (INFANT Collaborative Group 2017), there remains limited evidence around how interventions related to intrapartum fetal monitoring are perceived and implemented, and ultimately effect changes in clinical practice.

4.1.2 Rationale for study

The above literature highlights the potential impact of individual (e.g. training and knowledge, clinical experience, personal beliefs) and contextual (e.g. unit size and composition, workload, leadership) factors on intrapartum fetal monitoring practices. However, significant gaps remain in our understanding of current UK practice and the barriers that exist to implementing new interventions and organisational change on the labour ward. Specifically, there is limited understanding of how doctors perceive, use and interact with fetal monitoring technology. Doctors are ultimately responsible for initiating adjunctive tests of fetal wellbeing, such as FBS, and making decisions based on the information that those tests provide. It is therefore crucial that researchers seek to understand fetal monitoring technology from their perspective, as primary end users working within complex and highly pressurised environments.

In their clinical and extended roles – as trainers, opinion leaders and managers – within maternity healthcare systems, doctors are also key stakeholders in the development of new fetal monitoring technology. Their 'buy-in' is fundamental for the successful translation of future technologies into routine practice, as demonstrated by previous studies on implementing STAN in different healthcare settings (Amer-Wåhlin et al., 2005, Doria et al., 2007, Mayes et al., 2018). Exploring the views of UK obstetricians on the development of a new fetal monitoring device, which this thesis aims to provide proof-of-concept for, is therefore a logical and necessary step.

4.2 Aims and objectives

The overarching aim of this qualitative study was to explore and understand how and why obstetricians in the UK use adjunctive technologies to monitor fetal wellbeing during labour. The key objectives were to describe and help explain variation in intrapartum fetal monitoring practices and attitudes at the level of individual clinicians and maternity units; and to inform future research into the development and implementation of new intrapartum fetal monitoring technology, as discussed in the remainder of this thesis.

4.2.1 Research questions

The specific research questions which the study sought to address were:

- What are obstetricians' experiences of using adjunctive technologies for intrapartum fetal monitoring in the NHS?
- What factors shape their attitudes towards fetal monitoring; and how does this influence their use of available technologies in practice?
- Do obstetricians think there is a need for new technology to monitor fetal wellbeing during labour? What would the key features of such technology be?
- What do obstetricians consider the main barriers and facilitators to implementing new fetal monitoring technology in clinical practice to be?
- What research questions about fetal monitoring do obstetricians think should be prioritised?

4.3 Methods

4.3.1 Ethical approval and sponsorship

Ethical approval for Qual-IFY (**Qual**itative study of UK obstetricians' views of **I**ntrapartum **F**etal monitoring technolog**Y**) was granted by the University of Edinburgh School of Philosophy, Psychology and Language Sciences Ethics Review Committee on 1st August 2019. The study was co-sponsored by NHS Lothian and the University of

Edinburgh through the Academic and Clinical Central Office for Research and Development (ACCORD).

4.3.2 Study design

This was a qualitative study collecting data through individual semi-structured interviews with UK obstetricians. The study was conducted, wherever possible, in accordance with the COnsolidated criteria for REporting Qualitative research (COREQ; Tong et al., 2007). All specialty trainees (ST; further denoted by their current year training in seven-year programme e.g. ST3), specialty and associate specialist (SAS) doctors, and consultants in Obstetrics and Gynaecology with a current NHS role in providing high-risk intrapartum care were considered eligible for inclusion. There were no exclusion criteria.

Interviews were considered the most appropriate methodology to allow an in-depth exploration of obstetricians' experiences of using fetal monitoring technology and how their views, along with external factors appeared to shape practice (Britten et al., 1995, Pope and Mays, 1995). In contrast to focus groups, individual interviews also provided a safe environment for participants to express personal opinions, outwith existing professional hierarchies and the influence of group dynamics (Hofmeyer and Scott, 2007, Sim and Waterfield, 2019). Telephone interviews were chosen over face-to-face interviews as they provided the most efficient means of collecting data from a geographically dispersed sample of healthcare professionals. Past qualitative research supports the use of telephones as an alternative to face-to-face interviews as the content and quality of data collected are similar (Miller, 1995, Sturges and Hanrahan, 2004, Drabble et al., 2016, Block and Erskine, 2012). Telephone interviews were also considered to be more acceptable to doctors, as participants, because they were likely to take less time and afford more scheduling flexibility. Finally, conducting interviews remotely eliminated the need for local Research and Development approvals from individual healthcare trusts and removed any onus from participants or units to host a visiting interviewer, whilst minimising resource and travel costs for the research team.

4.3.3 Sampling and recruitment

Purposive sampling was used to help attain a diverse sample of units from several UK regions and units with experience of using different adjunctive technologies for intrapartum fetal monitoring. The study specifically aimed to recruit from units that had participated in the INFANT trial of computerised CTG interpretation and/or used STAN. Recruitment commenced on 14 August 2019 with initial promotion of the study through the members' forum of the British Intrapartum Care Society, a multi-disciplinary network promoting excellence in intrapartum care, of which I am a member. When potential participants self-referred, they were asked to forward study information onto other colleagues within their unit who may be interested in participating – a process known as 'snowballing' (Mason, 2002). In this manner, the study aimed to recruit three to five participants of varied career grade from each unit, to ensure a breadth of experience and opinions. Trainees, for example, are more likely to have worked in multiple units in recent years due to the rotational nature of Obstetrics and Gynaecology specialty training. As a result, they may offer different perspectives on the culture within their current unit, compared to consultants and SAS doctors in long-term posts.

The target sample size when recruitment commenced was 20 participants, which was considered sufficient to capture a diversity of perspectives and experiences and to explore the potential impact of both individual and contextual factors. Based on previous interview studies of healthcare professionals (Lawton et al., 2016, Lawton et al., 2020), this recruitment target was also considered appropriate to achieve 'sampling adequacy' as evidenced by theoretical saturation and replication, which refers to the point at which no new data or themes emerge during analysis (Bowen, 2008). From an early stage, however, it became apparent that snowballing alone would be insufficient to achieve the target sample size and distribution. Further convenience sampling was therefore undertaken through collaboration with the UK Audit and Research Collaborative in Obstetrics and Gynaecology (UK-ARCOG), a national trainee-led network, and directly via academic and clinical collaborators of the research team.

Detailed study information, including the PIS and data protection information sheet, was distributed to 39 potential participants from 18 units. A total of 16 obstetricians from nine units consented to participate and all were interviewed. In line with the guidance from the study's co-sponsors, recruitment was suspended prematurely on

23 March 2020 in response to the evolving COVID-19 pandemic. After analysing the available transcripts, the research team agreed that the collected data was of sufficient depth and quality to answer the research questions and the study was ended permanently on 1 July 2020.

4.3.4 Consent

Once self-referral or consent to contact had been established, potential participants were sent the participant information sheet (PIS; Appendix 5.5.1) and data protection information sheet. Participants were given at least 48 hours to review this information and ask any questions prior to enrolling. Oral consent was confirmed and audio-recorded using a predefined script at the start of each interview (Appendix 5.5.1). The process of obtaining oral consent by telephone has been used for qualitative interview studies of health professionals in the past (Sanders, 2019) and was chosen to minimise the administrative burden for participants and researchers in conducting interviews remotely across multiple sites.

4.3.5 Data collection

A semi-structured interview topic guide with open questions was developed based on the literature, my own knowledge of the subject and with input from my clinical and non-clinical supervisors. The questions were intended to generate interview content suitable to answer the research questions and study aims. The topic guide was piloted on two trainees not affiliated to the study (interviews not included in the final sample) and underwent several changes in structure and content at this stage. Further minor revisions were made after reviewing the first few transcripts, following which the topic guide (Appendix 5.5.2) did not change materially. This served as a flexible structure for the interviews, however participants were encouraged to discuss related experiences which were important to them in order to collect rich and in-depth accounts. The open-ended nature of the interview also gave participants the opportunity to raise issues which were potentially unforeseen at the study's outset, in keeping with the exploratory nature of this research.

Interviews were conducted by telephone at a time convenient to participants and digitally audio-recorded using an encrypted recording device. The interviews were completed between 19 September 2019 and 31 March 2020 and all were conducted in one sitting, with a mean duration of 45 minutes (range 23-74 min).

Files were transferred securely to a third-party company for verbatim transcription and all transcripts were double-checked for consistency and, where possible, to clarify inaudible passages. Field notes were taken during the interviews and at this stage of 're-listening'; raw audio files were revisited on several occasions for this purpose and to facilitate familiarisation with the data. Finalised transcripts were anonymised and any demographic information which may have indirectly identified participants was removed prior to data analysis.

4.3.6 Data analysis

Interviews were analysed using thematic analysis, a method for identifying, analysing and reporting "patterns of shared meaning (themes) across the dataset" (Braun and Clarke, 2006) that capture something important in relation to the research questions. In contrast to other qualitative methods, thematic analysis does not strictly align itself with a particular epistemology or philosophical stance and this theoretical flexibility makes it accessible to researchers with limited experience of qualitative research. My analysis broadly followed the steps outlined by Braun and Clarke (2006):

1. Familiarisation with the data
2. Searching for themes and coding
3. Reviewing, defining and naming themes
4. Writing the report

Although presented linearly, data collection and analysis were undertaken concurrently as an iterative, reflexive process. Immersion in the data began by re-listening to interviews when checking the accuracy of transcripts; anonymised interview transcripts were then read repeatedly with notes and preliminary coding ideas generated on paper at this stage. QSR NVivo 12 Pro qualitative data analysis software (QSR International Pty Ltd 2019, Chadstone, Vic., Australia) facilitated subsequent coding and data management.

Coding and theme development were largely inductive in nature and driven by the content of the data, whilst acknowledging the subjectivity of the researcher as integral to the process of data analysis. However, a more deductive approach was utilised to specifically address *a priori* themes and capture data that was relevant to answering the research questions, consistent with King and Brooks' (2017) description of template analysis. In this manner, emerging and *a priori* themes were clustered into meaningful groups following preliminary data analysis, with broader themes encompassing one or more levels of more narrowly focused subthemes.

Both research supervisors (JL, FCD) reviewed the first four transcripts in full to inform this preliminary analysis, and to provide detailed feedback on the topic guide and my interviewing technique. The use of multiple coders is essential to maximise rigour in qualitative analysis (Tong et al., 2007, Nowell et al., 2017), and the coding template and evolving themes were discussed regularly during subsequent team meetings to ensure consensus and enhance the dependability and confirmability of the study findings. Two selected transcripts were reviewed by JL at a later stage in the development of the coding template. The coding template was finalised following completion of data collection and coded datasets were reviewed to develop more nuanced interpretations of the data and identify illustrative quotations.

4.3.6.1 Use of supervision

Supervision by JL, a highly experienced non-clinical qualitative researcher, and FCD, a clinical academic obstetrician, were integral to the conduct of this study from conception, through data collection and analysis, to synthesis of the findings. This was particularly important given my personal professional background and potential biases, as well as my lack of qualitative research experience. For example, JL noted several instances in early transcripts where participants had not fully articulated their responses because there was an assumption of shared, tacit knowledge (van Braak et al., 2018) with myself, which may not have occurred with a non-clinical researcher. Strategies to avoid this and increase the depth of data were therefore discussed and successfully applied in subsequent interviews. As outlined above, both supervisors reviewed multiple transcripts in full and contributed significantly to development of the final coding template and thematic analysis in keeping with COREQ's recommended

standards for qualitative reporting (Tong et al., 2007). This collaborative effort allowed me to negotiate the learning curve of qualitative interviewing and analysis, as well as strengthening the credibility of the study's findings.

4.3.6.2 Reflexivity statement

I, the principal investigator and interviewer, undertook the Qual-IFY study as part of my Doctor of Medicine (MD) thesis. I am a 36-year-old white British male and completed this research whilst employed as Academic Clinical Fellow by NHS Lothian, during which I was also a member of the team providing intrapartum care at the Royal Infirmary of Edinburgh. I did not have prior experience of conducting qualitative research but received formal training during this study through the Wellcome Trust Clinical Research Facility ('An Introduction to Qualitative Research Methods' and 'Using Mixed Methods'). At the time of conducting this study, I had over seven years of experience as a specialty trainee in Obstetrics and Gynaecology, working in Scotland and the North East of England, and have a clinical interest in high-risk intrapartum care. As such, I recognise that my own experiences and beliefs around fetal monitoring, as well as my motivations for undertaking the study, may have influenced the findings presented herein at several stages: from how the research questions were formulated; to how interviews were conducted and responses elicited from participants; to how data were analysed, interpreted and presented. At the same time, I accepted Braun and Clarke's (2019) concept of reflexive thematic analysis, in which themes are "creative and interpretive stories" produced at the intersection of the data and the researcher's knowledge, analytical skills and theoretical assumptions. In this sense, it was not considered appropriate to abandon my preconceptions or formally bracket interviews, as some have suggested (Tufford and Newman, 2010), but instead to engage in thoughtful reflection with the data and analytical process.

Participants were aware that I was a clinical researcher, a position which afforded me enhanced opportunities for recruitment through professional networks and may have influenced their willingness to engage in the study. This common ground was also vital in building rapport with participants and enabled me to collect rich accounts despite the brief and remote nature of the study visits. Conversely, having a deep level of shared understanding and/or past experiences with some participants proved

challenging as an interviewer because of the potential for ideas and concepts to be communicated implicitly without direct verbalisation, as previously discussed.

I had previously worked with two of the participants and personally knew a third, however all participants were provided with the same study information outlining my interest in this area. The wider application of this research in informing the development of new fetal monitoring technology was not declared to participants, as it may have influenced their responses.

4.4 Results

4.4.1 Characteristics of participants

Table 4.1 shows the characteristics of the 16 interview participants, who were recruited from nine units across seven UK regions (excluding the East Midlands, East of England, South West, Wales and Northern Ireland). Difficulties with sampling and recruitment, which are discussed in the conclusion of this chapter, resulted in fewer participants (one to three per unit) from a higher number of units than expected. Most worked at medium (2500–5000 births per annum) or large-sized maternity units (over 5000 deliveries per annum), based on delivery data from the National Maternity and Perinatal Audit (https://maternityaudit.org.uk/). In terms of career grade, the sample included four ST1-4 trainees, five ST5-7 trainees, six consultants and one SAS doctor, who was grouped with the consultants during data analysis based on their clinical experience.

Table 4.1 Characteristics of study participants.

Participant	Career grade	Region	Unit code	Unit size
R1	ST5-7	Scotland	A	Medium
R2	ST1-4	West Midlands	B	Large
R3	Consultant/SAS	West Midlands	B	Large
R4	Consultant/SAS	South East	C	Medium
R5	ST1-4	Yorkshire & Humber	D	Large
R6	ST5-7	North West	E	Medium
R7	Consultant/SAS	London	F	Medium
R8	Consultant/SAS	North West	G	Large
R9	Consultant/SAS	Yorkshire & Humber	D	Large
R10	ST5-7	North West	G	Large
R11	ST1-4	North West	E	Medium
R12	ST1-4	Scotland	A	Medium
R13	ST5-7	Scotland	A	Medium
R14	Consultant/SAS	Scotland	H	Medium
R15	ST5-7	North East	I	Small
R16	Consultant/SAS	North East	I	Small

4.4.2 Themes

Analysis of the data revealed five broad themes and 11 corresponding subthemes, which are presented in detail below and summarised in Table 2.2. Illustrative quotations are identified by participant number.

Table 4.2 Themes and subthemes. *Five themes and 11 subthemes identified during thematic analysis, with illustrative quotes.*

Theme	Subthemes	Illustrative quotes
'The best we've got' - perceptions of FBS	'Buying time' and reassurance • Managing CTG uncertainty	You would only use FBS if you are not sure; if you are sure of the CTG and the decision, you would just make the decision (R2, ST1-4)
	• Prioritisation tool	We use it more for timing purposes or to buy a bit of time...(R5, ST1-4)
	• Medico-legal protection	I suppose it's to cover your own back really…(R11, ST1-4)
	Limitations of FBS	I'm going to do this really invasive test which you're going to feel is really demeaning…that's going to give us an answer that we may or may not trust. (R15, ST5-7)
The developing obstetrician	Clinical competence and autonomy	There is a difference between consultants and registrars in how confident they are with CTGs and how quick they do an FBS...(R9, consultant/SAS)
	Understanding the whole picture	I'm definitely much more heavily concentrating on the CTG interpretation, the risk factors and everything else about the clinical scenario, not just the FBS…(R1, ST5-7)
The socio-cultural context	Unit culture	I know how they feel in this trust…this is how they want me to practice, this is how they would do it if they were here...(R11, ST1-4)
	The role of guidelines	It very much felt that the culture was much more you follow NICE to the letter (R8, consultant/SAS)

Table 4.2 Themes and subthemes *(continued)*

The socio-cultural context *(cont.)*	The learning environment	If the outcome is fine nobody will care. But if the outcome is not fine then suddenly there is like 100 eyes looking at those notes (R2, ST1-4)
Changing practice	Barriers • Lack of resources	The state of the NHS and funding at the moment means that there are things that are much less costly and that are known needs that we have…(R8, consultant/SAS)
	• Low readiness to change	I think it's good to be sceptical, but it's not a bad thing to try things out…(R9, consultant/SAS)
	Facilitators • Regional approach	If there is support from the network…and we have that strength in numbers that this is what's happening across our patch, then hopefully people will buy into it (R16, consultant/SAS)
	• Opinion leaders	All the midwives came back after this [CTG masterclass]…and they were so anti-FBS…(R11, ST1-4)
Future directions	Filling the gap • Declining role of FBS	I suspect that we won't be doing FBSs in many units...(R7, consultant/SAS)
	• Scepticism regarding computerisation	I feel that obstetrics is more of experience. Artificial intelligence will not play much in obstetrics…(R14, consultant/SAS)
	Prioritising training	The more money that goes into CTG training and education, I think, the better...(R16, consultant/SAS)

4.4.2.1 'The best we've got' – perceptions of FBS and adjunctive technologies

FBS was the only adjunctive technology available to participants in their current units and was reported as being used regularly in all except one unit. Almost all participants described attaching some value to FBS as a tool for intrapartum decision-making, whilst also acknowledging its limitations or expressing specific reservations about its use. Several described feeling that there were no alternative means to assess fetal wellbeing more reliably during labour. The theme's title – 'the best we've got' (R10, ST5-7) – reflected this conflicted, but necessary, use of FBS. The main concerns reported by participants centred around the accuracy of FBS, its ability to predict clinical outcomes, and the invasive nature of the procedure itself. Several participants commented that FBS often correlated poorly with umbilical cord gases or the condition of the baby at birth and questioned the physiological validity of capillary scalp blood to reflect oxygenation of the fetus' central organs. Many described specific cases of false positive FBS results that had led them to undertake potentially unnecessary operative deliveries.

> There's certainly been cases where I've done an FBS, it's been borderline or it's been abnormal, and we've gone to theatre, and [the baby] has come out screaming…so I guess, when it's normal, that's fine; when it's not normal, that's…you know, perhaps in my experience, that's not necessarily an indication that it's a compromised [baby]. (R6, ST5-7)

Some participants explained that their concerns about the validity and clinical utility of FBS were heightened by how invasive they felt the procedure was for both the mother and baby.

> As an obstetrician I always remember thinking [FBS] is just about the worst thing that happens to a woman. You're in the throes of labour, you've got someone doing this to you, your partner's thinking, my God, what are they up to? The whole set-up is just so horrible and if it made a huge difference I think then we would look into it but it never really made that much of a difference…. (R7, consultant/SAS)

Although few reported finding the procedure technically difficult to perform, concerns about contamination (e.g. with amniotic fluid or lubricant) or failed analysis of samples were common amongst participants and seen as another factor that potentially limited the reliability of FBS.

> If you know you've taken a good sample, it's not, you know, a contaminated sample, then you're more reassured. But actually, if you thought, you know, that was a really difficult FBS, I'm not quite sure then, maybe I won't rely entirely on my…normal result. (R12, ST1-4)

4.4.2.1.1. 'Buying time'

Despite these limitations, the vast majority of participants, across all regions and training grades, reported using FBS in their routine practice. FBS was most frequently viewed as a means of 'buying time'; that is, participants described performing FBS either to delay or avoid more definitive interventions, such as caesarean section, when faced with CTG concerns. Most participants who reported using FBS in this manner described performing it when they were confident of getting a normal result, which, in turn, delayed the need for immediate additional actions in accordance with the NICE guidelines on the interpretation of FBS results (discussed in Section 1.2.1). The same participants said that they would be more reluctant to perform FBS if they had genuine concerns about fetal wellbeing.

> I feel like I use it in a way that if I feel like I need to buy time, so if I think clinically I do feel like this baby is coping really well with labour but because there are CTG changes, do an FBS – I'm confident that it will be normal and then that allows the patient to continue labouring and hopefully progress and have a natural delivery. If I'm actually very concerned about the CTG I won't bother with an FBS. If I think this baby is not coping and needs to deliver, I would deliver that baby. (R11, ST1-4)

Similarly, several trainees and consultants reported using FBS as a prioritisation tool to 'buy time' when there was more than one case on the labour ward requiring potential obstetric intervention. Less experienced trainees were most likely to describe

performing FBS for this reason, particularly out-of-hours or when direct senior support and theatre resources were limited.

> So, if it [CTG] isn't perfect and the consultant is tied up in theatre they'll often just do an FBS just in case…it might make it better. Or at least will ask the consultant and if they're tied up then they might say do an FBS so that I know whether I need to open a second theatre or not. So, we use it more for timing purposes or to buy a bit of time I think. (R5, ST1-4)

4.4.2.1.2. Clinical reassurance

Despite the aforementioned concerns about its physiological validity, most participants also viewed FBS as a source of reassurance when there were uncertainties around CTG classification or persistent intermediary concerns. Some participants explained that a normal FBS result offered more objective evidence of fetal wellbeing, even if there was a low suspicion of fetal compromise based on the CTG. This use of FBS could be driven by an internal need for validation, as suggested in the first quote below; or by a desire to reassure other members of the intrapartum care team, particularly where there were divergent opinions about CTG interpretation. Several participants described having performed FBS to appease the concerns of midwives, rather than because they deemed it clinically necessary.

> You kind of think, oh I've been looking at this CTG for ages, and ages, and ages, and probably, you know, we should think, or at least think about doing something else. And I think, if I do an FBS, it will be normal, but I guess it's kind of giving you that little bit of backup. (R6, ST5-7)

> I don't want to be that person who didn't do an FBS and then two hours later they will say 20 people were telling you to do an FBS…so you just say, okay, that's fine. Five minutes of my life to show the FBS normal, fine. (R2, ST1-4)

4.4.2.1.3. Medico-legal protection

As well as providing reassurance to support clinical decision-making, some participants reported feeling medico-legally protected by performing FBS. Although an abnormal FBS result was not seen as a prerequisite for deciding to deliver a baby, several participants described using a normal result to formally justify their decision not to act. Trainees were more likely than consultants to use FBS in this manner, citing the importance of adhering to guidelines and being able to defend their practice in the event of an adverse outcome. Although trainees raised this as an issue of medico-legal liability, the language used when recounting personal experiences suggested that they were also driven by a fear of criticism from colleagues, discussed below in *'the learning environment'* subtheme.

> I suppose it's to cover your own back really, because you can't ignore…you can't do nothing, you know, with a pathological CTG. That doesn't make sense for anyone – for me, for the patient, for the trust, that doesn't make sense. So I think you've got proof I suppose in a way. You know, that's in national guidance, it's in our local guidance that says, yeah, you've done the right thing, now you have this [normal result] … the advice would be to continue. (R11, ST1-4)

4.4.2.2 The developing obstetrician

It was clear from participants' accounts of using FBS that their approach to intrapartum fetal monitoring had evolved throughout their training. Indeed, cumulative experience and career stage emerged as the most important individual factor influencing participants' reported use of FBS and their attitudes towards adjunctive technologies and CTG; hence this theme was conceptualised as the developing obstetrician.

Most participants described using FBS less as they progressed through training to become a consultant, which mainly reflected a growing confidence and independence in CTG interpretation and intrapartum decision-making. For example, senior trainees and consultants often highlighted specific clinical scenarios where they had either found FBS helpful or felt it should be avoided, suggesting a restrictive approach to its use. This contrasted with less experienced trainees, who described using FBS more openly for additional reassurance of fetal wellbeing and medico-legal purposes, as discussed above.

> Even if you're still following NICE Guidelines, I think that you naturally do less fetal blood samplings the more experience that you get, it's just how you might interpret decelerations, how you might classify them...but definitely as you go on in your career, you do intervene less I would say…. [FBS] is an adjunct, so the skill is in knowing when you need to do it, rather than just using it as a backup, that, oh, I'm not sure about the CTG, let's do an FBS. (R16, consultant/SAS)

In keeping with these findings, some senior trainees and consultants described having developed their own approach to CTG interpretation through years of experience. Greater self-efficacy in fetal monitoring and higher degrees of clinical autonomy appeared to mitigate the influence of external factors, such as changing guidelines, on these individuals' practice.

> I honestly must say that I, most of the time, still do what I always did. The CTG guidelines have changed over the years so many times, because we still don't know what the best way is; but in those years, I found my way a little bit…what I think is the best way so far. (R9, consultant/SAS)

Clinical experience also manifested in a greater awareness of the 'full clinical picture' (R15, ST5-7) and the ability to integrate multiple sources of information when making intrapartum decisions. For example, senior trainees and consultants were more likely to discuss careful patient selection and the need to undertake a holistic assessment – taking into account pre-existing maternal and fetal risk factors, intrapartum events, and progress in labour – when interpreting CTGs and considering the use of FBS.

> I think the main distinguishing factor tends to be the patient's risk factors. And by that, I mean, if it's a very high risk pregnancy in terms of, it's a small baby, doppler changes, that sort of stuff, my threshold for actually using it, I would be probably more inclined to use caesarean section, rather than FBS…I think you have to select your patients carefully. (R3, consultant/SAS)

4.4.2.3 The socio-cultural context of intrapartum fetal monitoring

Almost all participants reported variation in fetal monitoring practices amongst the different units where they had worked, even units within the same region or NHS trust. Such variation ranged from the practical aspects of intrapartum care, such as the use of CTG stickers and central monitoring systems in some labour wards, to deeper-seated differences in how units approached CTG interpretation and use of adjunctive technologies. Three interlinked subthemes emerged in relation to the socio-cultural context of fetal monitoring: the maternity unit culture, the role of guidelines, and the learning environment.

4.4.2.3.1 'This is how they want me to practise' – maternity unit culture

Most participants identified distinct cultures within each unit around the processes of intrapartum fetal monitoring, differences which were especially apparent when trainees rotated between units – '[unit] is quite pro-FBS compared to…' (R11, ST1-4) – or when personal beliefs came into conflict with new social and organisational norms. In such situations, individuals typically appeared to adopt the dominant culture of the unit, whether consciously or subconsciously.

> I found a very different approach to the process of fetal monitoring interpretation in the clinical setting when I came to [region] compared to how I've been taught about it in my earlier training…and to be fair I didn't actually question this as a trainee when I transferred deaneries. I just kind of thought, okay, well, that's what I used to do…and this is, I've got to learn how this new approach of how [region] does it. So I did because it was a bit of probably culture and integration into culture…. You take on the culture of a new team, and that's what happened to me and I can see that retrospectively. (R8, consultant/SAS)

Conversely, experienced participants seemed more willing to defend views which deviated from those of the wider unit. This was consistent with the findings discussed above, and suggested a balance between internal and external influences that evolved as participants progressed through their career.

> I think I definitely have a different position and standing point in [FBS] than most of my colleagues…because coming from a different country, you will do a lot of things different, but I can almost always explain why I'm doing things, and then people understand it, and then people are okay with it as well. (R9, consultant/SAS)

4.4.2.3.2 The role of clinical guidelines in practice

The identity of each unit also related to how fetal monitoring guidelines were applied in everyday practice, despite all units using the same NICE classification system for intrapartum CTGs. The majority of participants reported a rigid 'tick box' approach to implementing NICE guidance in the units within which they currently worked, which was often reinforced by the use of stickers to document intrapartum CTG reviews. Most participants said that they felt strict adherence to the NICE classification system generally increased the use of FBS and other interventions in labour, and described concerns about the possible repercussions of not following guidelines.

> I would find the NICE guidelines, probably the same as everyone else, that it's just a check list and you end up intervening more than you feel that you should and people are scared of not following the NICE Guidelines. (R16, consultant/SAS)

Participants from some units described adopting a more flexible view of the guidelines as a framework around which to base intrapartum assessments. Several of these participants said they had been, or were, directly involved in work to re-structure and improve fetal monitoring training in their units (or at a regional level). Such initiatives appeared to have strongly influenced attitudes and practice at both an individual and unit level. This training was generally described as placing less emphasis on the NICE classification criteria and more on the fetal physiology underlying the observed CTG changes, in line with the principles of 'physiological CTG interpretation' (see Section 1.1.4.1). Most participants said that they had observed a reduction in the use of FBS as a result of these changes.

> What we've found, which again it just happened over time, was we started looking at CTG differently, we trained altogether, we started learning from each other and cases…because again when you have electronic intrapartum care you can just use those CTGs over and over again in different formats to be able to train and teach each other…and actually we just didn't need to use FBS. (R7, consultant/SAS)

4.4.2.3.3 The learning environment

Local fetal monitoring training and the learning environment on each labour ward also appeared to influence how participants used CTG guidelines and FBS. All units employed e-learning packages for fetal monitoring, such as the Perinatal Training Programme provided by K2 Medical Systems, but participants generally described placing a higher value on face-to-face training. The availability and nature of such training varied, from weekly CTG clubs and mandatory study days in some units to nothing in other units. Participants explained that CTG teaching in many units had been incorporated into risk management reviews of adverse outcomes. While some viewed these as positive learning experiences, several trainees described a fear of criticism or blame which had subsequently affected their clinical practice. The potential power of such negative feedback was underlined by the language used in different trainees' accounts (e.g. "bollocking", "persecutory").

> If the outcome is fine nobody will care. But if the outcome is not fine then suddenly there is like 100 eyes looking at those notes. You didn't write this, you didn't do this. There was a CTG, look at that; that was at half an hour pathological deviation, you should have acted then…. You know, it's like yeah, I don't have two hours to look at the CTG, I have two minutes. And it looked fine so…as I said, outcome driven and no matter what you do it is not correct if the outcome is poor. (R2, ST1-4)

Participants also reported varied experiences with respect to informal fetal monitoring training; for example, by discussing cases and their management with colleagues in real time on the 'shop floor'. Although most described an open culture on their current labour ward where all team members were encouraged to voice their opinions, professional hierarchies were still seen to play an important role. For example, less

experienced trainees said that they were unlikely to question decisions made by consultants, which had a detrimental effect on their own learning.

> I did sometimes feel at [unit] it was a persecutory environment of fear on occasion, especially as a junior trainee. So you would be less keen to discuss your thoughts about a CTG for fear of persecution or ridicule, whatever adjective you wish to pull off, which didn't always add to learning. (R15, ST5-7)

4.4.2.4 Changing practice

The remaining themes, which related to participants' experiences of changing practice and their ideas on the future of intrapartum fetal monitoring, were identified in a more deductive approach during data analysis. Hence, they remained closely linked to predefined research questions around the development and implementation of new fetal monitoring technology and future research priorities in this field.

None of the participants had worked in a unit where new fetal monitoring technology, such as fetal scalp lactate, had recently been introduced. However, two units had made significant changes to the content and delivery of local fetal monitoring training and a further unit was involved in planning similar changes across their region in the coming years. Several participants also drew on experiences in other areas of maternity care to discuss perceived barriers and facilitators to changing practice. One of the main barriers identified was a lack of financial and human resources. Several participants highlighted the challenges already faced in providing CTG training of sufficient quality and frequency, specifically in terms of staff availability and leadership, and felt that this would present even more of a challenge with the introduction of new technology.

> They were starting to try and introduce the physiology-based CTG interpretation. However, the consultant who started to introduce that left and there is nobody else who really has the experience to lead on that. (R10, ST5-7)

For those participants who had been involved in implementing changes in fetal monitoring practice, such as introducing STAN or physiological CTG interpretation, the training required to support implementation was considered equally as important as

the intervention itself. Relatedly, these participants suggested that a potential advantage of introducing new technology or guidelines was that it presented an opportunity for wider organisational change.

> If we make the change to physiological interpretation, the biggest thing will be making a change to how we train our staff, that's, kind of, one of our drivers for it as well…just changing a guideline or just introducing a new technology on its own, is not going to do anything unless enough money is put into the people that are going to be looking after it. (R16, consultant/SAS)

4.4.2.4.1 Readiness to change

Participants also identified low readiness to change and poor 'buy-in' amongst stakeholders, especially clinicians, as potential barriers to changing practice. Some participants drew a connection between low readiness to change and cultural tribalism, in which members display a very strong loyalty to the shared values or norms of their group above all others – 'we do it here this way because we've always done it this way' (R9, consultant/SAS). Where changes had been successfully implemented and adopted in the long term, persistence combined with positive feedback (e.g. through audit cycles or first-hand experience of benefit) had been critical to securing buy-in over time.

> So after maybe two to three years, everyone started buying into it. But when I first wanted to introduce it, a cohort of consultants never accepted it…. Some of the healthcare workers accepted it. Some of the junior doctors accepted it. So the buy-in was not from everyone. (R14, consultant/SAS; discussing the introduction of a maternity sepsis care bundle)

> I think that it's human nature to be sceptical…but it's not a bad thing to try things out, and that's why I think [STAN] worked so well. Because we were part of the trial, we found out that it really worked well. And it's kind of sometimes that you need to see things before you believe in it. (R9, consultant/SAS)

4.4.2.4.2 Facilitating change

Two other important facilitators emerged in relation to changing practice. The first was the use of regional clinical networks to encourage stakeholder engagement (both clinicians and management) and implement change simultaneously across multiple units or trusts. Three consultants cited examples of this type of regional approach, which had offered the benefits of standardising practice for trainees rotating between units and enabling the use of shared resources (training materials, business plans).

> I think, you know, in a little [district general hospital]…that's quite a thing to kind of stand alone and say, we are not going to follow NICE guidelines…. I think it would need to be a regional thing in [region], to have kind of a bit of backup and, you know, a bit of kind of support between the units, and stuff. And I struggle to see, like my unit just doing that. (R6, ST5-7)

Opinion leaders within the specialty also acted as potential facilitators. The majority of participants had either personally attended or referred during their interview to advanced CTG masterclasses led by Edwin Chandraharan, a consultant from St. George's Hospital in London, which are provided by Baby Lifeline Training Ltd (https://www.babylifelinetraining.org.uk). Participants described these masterclasses as having had a marked effect on their own and colleagues' attitudes towards intrapartum fetal monitoring, particularly the use of FBS.

> They'd pretty much all been on the advanced CTG course, all of the midwives and most of the doctors, so they were a lot happier…interpreting CTGs and putting it into the context of the labour. And also they were very much more wary about doing FBSs.... (R5, ST1-4)

> The guy who does the Baby Lifeline, Edwin [Chandraharan]…who came and gave his very powerful lecture, his…'FBS is a knife crime'. I did see a significant decrease in the feelings of the midwifery staff towards [FBS] at that point, which had quite a profound impact on culture I would say within [region].... (R15, ST5-7)

4.4.2.5 Future direction of intrapartum fetal monitoring

Looking beyond their direct experiences of changing practice, participants voiced mixed views about how intrapartum fetal monitoring would evolve in the future. All acknowledged the limitations of CTG and existing adjunctive technologies, with several indicating a trend away from FBS in current practice. However, few said that they could envisage a new approach to fill this gap and many voiced scepticism about computerisation and the value of artificial intelligence. Continued work to optimise training in CTG interpretation and management was therefore seen by participants as the key priority for future researchers and funders.

4.4.2.5.1 Filling the gap

Almost all participants reported being open to new fetal monitoring technology, but they offered few ideas about what this might entail. Most said that they struggled to imagine something capable of replacing or reducing the present reliance on continuous CTG monitoring. The perceived lack of alternative methods to those currently in use also resonated with the conflicted acceptance of FBS that was discussed in the earlier theme: 'the best we've got'.

> A lot of people say, we need to step away completely from CTGs and we need to find something else, yeah, maybe, but how are you going to do that? What else can you look at…but we can try and optimise it, of course, as much as we can. And I still think that the CTG has a major role in that, I can't see anything else so far, what is going to take over. (R9, consultant/SAS)

Most participants expressed specific reservations about the potential role of artificial intelligence in the future, some citing the results and/or reflecting on their own experiences of the INFANT trial, which demonstrated no benefit to computerised CTG interpretation (INFANT Collaborative Group 2017). In a much broader sense, several participants perceived new technology as posing the risk of detracting from holistic, patient-centred intrapartum care.

> I don't think I would rely on a computer because I think CTG interpretation isn't pattern recognition because that's not going to take into account the risk factors, it's not going to take into account the progress and the clinical picture at that time. So I wouldn't feel confident in relying on a computer to give me an answer as to how well that mum and that baby are. (R10, ST5-7)

Participants raised concerns that clinicians would give undue weight to the information provided by adjunctive monitoring technologies, compared to other elements of the individualised risk assessment and clinical picture. This was also reflected in participants' reported experiences of using STAN.

> It was unsafe to continue using a piece of equipment [STAN] which the registrars, who ultimately are there more than the consultants and everyone else, felt uncomfortable using...so we stopped using it because people were putting it on pre-terminal CTGs, or women who were infected, they had meconium or failure to progress, they just needed delivery…not because of the CTG, if that makes sense. (R7, consultant/SAS)

Relatedly, about half of participants saw the role of FBS declining in the near future, attributing this either to a gradual cultural shift or an increasing awareness of the limited evidence base for FBS. Several participants had already noted a reduction in FBS use within their unit, but some felt that a more significant step change ('leap') in policy at either regional or national levels would be required for practice to change.

> I think [FBS] will die out a bit just because none of the consultants like it so they're teaching none of the trainees to like it, so we won't do it when we become consultants. (R5, ST1-4)

> There is still value attributed to FBS in [region] which I think therefore will mean I don't think it will disappear or if it does it will take a much longer time and much more evidence for that to disappear, or something like a national driver…. (R8, consultant/SAS)

4.4.2.5.2 Prioritising and improving training

Although all participants acknowledged the limitations of existing methods, developing new monitoring technologies was not identified as a key priority. Rather, staff training was the most frequently identified priority to improve the standard of intrapartum fetal monitoring. As most units relied heavily on e-learning to provide regular training in CTG interpretation, participants recognised a specific need to increase the availability of face-to-face training, with an emphasis on underlying fetal physiology. Several also stressed the importance of multidisciplinary team (MDT) learning and described how training in fetal monitoring should be expanded to incorporate additional elements, such as human factors and decision-making, in much the same way that obstetric skills drills and simulation training has done.

> I always think that one of the most important things that needs to happen again is the training about intrapartum care has to be MDT and I think it has to be whether you are a consultant or a junior doctor, one of the…. (R7, consultant/SAS)

> I would actually want more training in terms of management…to say what to do in this situation because we all, yeah, as I said, we all can read the guidelines, but it's the dynamic decision-making that experience will give you and training is a good way to get that experience without putting in the time. (R2, ST1-4)

Several participants simultaneously called for greater standardisation with respect to fetal monitoring training (e.g. national qualification and competency assessment tools) and clinical practice (e.g. infrastructure, documentation and guidelines) – 'it would be good if we are all singing from the same hymn sheet' (R11, ST1-4).

4.5 Discussion

This section summarises the key findings of this qualitative study in relation to the original research questions and existing literature. The study's main strengths and limitations are addressed, as well as its implications for current clinical practice. In

concluding, areas for future research are considered and the above findings are related to the remainder of the research presented in this thesis.

4.5.1 Summary of findings

The findings of this study suggest that FBS still plays an important role in intrapartum fetal monitoring within the UK. Participants who said they performed FBS regularly acknowledged its limitations, and often described feeling conflicted in its use; nevertheless, they attributed value to FBS as a clinical tool and/or safety mechanism. Personal attitudes to FBS and intrapartum fetal monitoring evolved with experience, reflecting the development of clinical competence and autonomy. The interplay between obstetrician's individual autonomy, the social and cultural norms of each maternity unit, and various external controls, influenced how FBS was used in practice.

Moving forward, participants identified improvements to staff training for intrapartum fetal monitoring as the key research and policy priority. Although they recognised a need for better methods to monitor fetal wellbeing, there was scepticism about the role of 'complex' technology and the realities of implementing change within the intrapartum care setting. Regional clinical networks and opinion leaders were seen as positive facilitators of practice change.

4.5.1.1 The current role of FBS

Chapter 1 highlighted the lack of contemporary UK data on the use of FBS and other adjunctive technologies. The data presented in this chapter suggests that FBS remains widely used in UK maternity units, however, attitudes towards its use may be shifting. Many participants in this study reported their own practice or that of colleagues starting to move away from FBS and there is indirect evidence supporting this observation. For example, Rose et al. (2018) noted a significant decrease in the proportion of women in whom fetal acidosis was confirmed by FBS prior to undergoing caesarean for suspected fetal distress in their time-trends analysis of term births in a Scottish unit. Furthermore, in a recent multi-centre retrospective study of FBS (Al Wattar et al., 2019), several sites withdrew from data collection due to the low number of FBS tests performed. This contrasts with data from Denmark, where the use of FBS has increased

in recent decades (Jørgensen and Weber, 2014). The reasons for this shift in UK practice are not yet clear, although several participants linked this to a transition towards physiological CTG interpretation within their unit or region. The direct and indirect impact of physiological CTG masterclasses, provided by Baby Lifeline Training, was also evident in this study.

Amongst participants who continued to perform FBS regularly in their practice, most described feeling conflicted in their use of this test, in keeping with previous studies of professionals' views of fetal monitoring technology (Altaf et al., 2006, Blix and Öhlund, 2007, Hindley and Thomson, 2007). However, reservations about performing FBS were often superseded by participants' perceived need to act. The tendency for healthcare professionals' decision-making to default to action over inaction, even when the benefits of intervention are not clear, has been highlighted by past healthcare researchers (Shim et al., 2008, Sinha, 2017, Seymour et al., 2020) and social commentators (Taleb, 2013). In a study of midwives' attitudes towards CTG monitoring, Hindley and Thomson (2007) described this paradox as "being seen to be doing something" despite "not necessarily believing what was seen". Such behaviour was particularly evident in trainees' accounts of using FBS, however, there is evidence of such behaviour across all grades of clinicians. A survey of 1192 UK obstetricians (Ennis et al., 1991), the majority of them consultants, found that many methods of fetal surveillance continued to be used despite being deemed inaccurate, either for "medico-legal reasons" or as an "aid to clinical judgement". Similar themes emerged in a systematic review and thematic analysis of professionals' views of intrapartum CTG monitoring (Smith et al., 2012).

Reassurance and perceived protection against litigation were also two of the main reasons offered by study participants for continued use of FBS. Just as midwives have described CTG tracings as offering hard copy 'proof' of fetal wellbeing that is not available with intermittent auscultation (Altaf et al., 2006), obstetricians herein described FBS as providing more objective evidence of fetal wellbeing than CTG, and hence a more credible defence of their clinical judgement. Concerningly, the language used and descriptions provided often implied a lack of psychological safety amongst trainees, particularly around fetal monitoring education. In many of the units, informal CTG training had been incorporated into risk management meetings where the focus

was on reviewing adverse outcomes. Hollnagel et al. (2015) describe this type of safety management system as Safety-I, which "presumes that things go wrong because of identifiable failures or malfunctions of specific components". The result of such an approach, the authors argue, is that safety becomes defined by the occurrence of, typically rare, adverse outcomes rather than high frequency, acceptable outcomes which happen every day "when things go right" (Hollnagel et al., 2015). In the current study, this appeared to contribute to a defensive style of practice amongst less experienced obstetricians, some of whom reported performing FBS even when they felt it was not clinically indicated. With increasing clinical experience, however, these themes of contradiction, reassurance and medico-legal protection became less prominent. Thus, most consultants in the study offered narrower perspectives on the value of FBS and its role within their personal approach to fetal monitoring.

4.5.1.2 Individual autonomy within the socio-cultural context of labour ward

The relatively fixed attitudes and behaviours of experienced obstetricians reflected not only their increasing clinical competence, but also the emergence of individual autonomy. Autonomy is a multidimensional concept: within the framework of competency-based medical education and training, it has traditionally been linked with improved clinical decision-making skills, increased responsibility for patient care, professional accountability, and a readiness for independent practice (Armstrong, 2002, Skår, 2010, Salvatore et al., 2018, Sawatsky et al., 2022). In the current study, key features of autonomy included self-efficacy in CTG interpretation, the ability to integrate complex information when undertaking intrapartum assessments and making decisions, and reduced adherence to external constraints, such as clinical practice guidelines (Martin et al., 2017, Andri, 2022). Senior obstetricians were also more likely to demonstrate a clear professional identity or practice style related to fetal monitoring. Goyert et al. (1989), who analysed variation in caesarean section rates in a single US hospital, referred to this as the 'physician factor'. Contemporary research has also highlighted the impact of obstetricians' personal beliefs and characteristics on intrapartum decision-making (Panda et al., 2018).

By contrast, trainee participants described adapting, whether consciously or not, to the social and organisational norms of the units in which they worked. These norms defined

the unique culture of each unit, relating both to technical aspects of fetal monitoring (e.g. how guidelines were applied, value attributed to FBS) and to accepted standards of social behaviour on labour ward (e.g. interdependency of staff, clinical escalation, authority structures). For trainees, integration into this culture was accepted as a means of feeling connectedness to others and even took precedence over their own knowledge and belief system at times. Previous research has highlighted the positive impact that "feeling part of a team" has on junior doctors' wellbeing and morale (Singh et al., 2019, Salles et al., 2019). This may be particularly important for doctors in specialty training programmes, such as those in the current study, who rotate regularly between units and must gain the trust of new peer groups repeatedly. Henceforth, trainees may be more likely to prioritise social connectedness above other innate psychological needs related to their personal and professional growth, such as competence, autonomy and contribution (Ryan and Deci, 2000, Ten Cate et al., 2022, RCM, 2021). In addition to a desire for social connectedness, deference to professional hierarchies and power differentials within the multi-disciplinary labour ward team is also likely to play an important role in trainees adopting the behaviour and practices of their current colleagues.

Taken together, these findings suggest: firstly, that obstetricians' attitudes to FBS evolve throughout their training and career as they develop competence (and, in some cases, subsequent expertise) in intrapartum fetal monitoring and decision-making; and, secondly, that their use of FBS in clinical practice reflects a dynamic balance between individual autonomy and a wide range of contextual factors, including the immediate socio-cultural environment of the labour ward.

4.5.1.3 The challenge of changing

The findings of Qual-IFY are also relevant to the research presented in the remainder of this thesis, and to others looking to develop new fetal monitoring technology. Many of the organisational barriers to implementing change identified by participants in this study have been reported in earlier studies of intrapartum fetal monitoring (Parer, 2003, Amer-Wåhlin et al., 2005, Mayes et al., 2018), or in the wider literature on implementation science (Battista, 1989, Greenhalgh, 2005, Langhan et al., 2015). Providing adequate training and support to staff during implementation was seen as

the biggest challenge within the limited resources of the NHS. Although few participants had direct experience of STAN, the complexity of this technology and high burden of knowledge acquisition required for its use seemed to typify these concerns. Somewhat contradictorily, it is possible that the simplicity of fetal scalp lactate may also have hindered its implementation: because almost no education or organisational infrastructure was required to support its implementation, there may have little impetus for UK obstetricians to change their existing behaviours by adopting fetal scalp lactate in place of FBS for pH measurement.

Looking forward, all participants acknowledged the limitations of CTG and FBS and recognised the unmet clinical need for better methods to detect babies at risk of intrapartum hypoxia. However, their enthusiasm towards future technology was tempered by an underlying scepticism of 'computerisation' and the potential for over-reliance on any one aspect of the holistic intrapartum assessment. These themes emerged again when participants reflected on past experiences of using STAN and computerised CTG interpretation. Similarly, Mayes et al. (2018) reported feelings of distrust towards technology amongst midwives and obstetricians following the introduction of STAN in an Australian hospital.

The declining role of FBS and failure of newer adjunctive technologies to be adopted should be bourne carefully in mind by researchers seeking to develop new fetal monitoring technology. Engaging both midwives and obstetricians as stakeholders in the development of such technology from an early stage will be crucial to fostering trust and facilitating successful implementation. Giving clinicians the opportunity to trial new technology can further encourage buy-in (Langhan et al., 2015), as one participant described in relation to their own experience of STAN.

4.5.2 Strengths and limitations

To my knowledge, this is the first qualitative interview study of UK obstetricians on the use of adjunctive technologies for intrapartum fetal monitoring. My sample included obstetricians with a wide range of clinical experience from maternity units in several UK regions, including a number of participants who had previously used STAN and/or computerised CTG interpretation. This encouraged a representative cross-section of

obstetricians from different sized units and allowed the study to explore variations in practice at a local and regional level. Conducting interviews by telephone with oral audio-recorded consent enabled the collection of rich data from a geographically dispersed sample of healthcare professionals. The practical advantages of this approach, in terms of scheduling and reduced administrative burden for participants, facilitated both initial engagement with the study and subsequent data collection. The latter fact was reflected in the duration of the interviews (mean 45 min) when compared to Mayes et al. (2018), who experienced difficulties interviewing midwives and doctors 'on shift' in a busy clinical environment and reported an average interview duration of 13 min.

At the same time, there were several disadvantages to the sampling strategy used and recruitment was further hindered by the COVID-19 pandemic. Recruiting through professional and clinical academic networks with the use of snowballing may have generated a more homogeneous sample of obstetricians who were likely to have an interest in intrapartum care. For example, several of the consultants had specialist experience and/or additional roles related to intrapartum fetal monitoring (e.g. labour ward lead, member of national guideline development committee). Only two of the participants worked in small-sized maternity units (less than 2500 births per annum), which also may have resulted in fewer generalist obstetrician-gynaecologists being captured in the sample. In addition, only one participant from the London region was recruited and that unit appeared to be an outlier in the final dataset for several reasons: FBS was not performed in the unit regularly; it had previously used STAN; and it had introduced physiological CTG interpretation alongside NICE guidelines. Such regional variation was expected at the study outset, based on my own knowledge of UK practice and recent literature (Chandraharan et al., 2013, Al Wattar et al., 2019). Hence, additional efforts were made to recruit directly from London and smaller units, but this was ultimately prevented by the COVID-19 pandemic. As a result, these findings may not be generalisable to this region specifically, nor to the full spectrum of maternity units in the UK. Whilst the importance of generalisability in qualitative research is debated (Carminati, 2018), Lincoln and Guba (1985) argued that by providing a comprehensive description of the participants and the research process, readers are able to assess whether findings are transferable to their own setting. By following the consolidated criteria for reporting qualitative studies (Tong et al., 2007), this study has

strived to provide such descriptions and henceforth offer a valuable perspective on current fetal monitoring practice within the NHS.

With respect to recruitment, my professional background made it relatively easy to establish initial contact with potential participants through various channels. However, expanding that interest to other doctors in each unit proved challenging and placed the onus on the 'index' participant to actively facilitate recruitment (e.g. distributing study information and sending follow-up emails). So, although I originally anticipated recruiting three to five participants from most units, the final sample included fewer participants from a higher number of units. This may have offered advantages in terms of the breadth of data collected (more regions sampled) but potentially limited the depth at which the study was able to explore other areas, especially in relation to the culture of individual units.

When interpreting the findings of Qual-IFY, it must be remembered that intrapartum fetal monitoring is a multi-disciplinary process therefore including only doctors risks the possibility of presenting one side of a complex, multi-faceted story. During the study's conception, the option of combining individual interviews and multi-disciplinary focus groups was considered. Whilst this may have offered greater insight into team dynamics and the social context of intrapartum fetal monitoring, focus groups presented several practical and methodological challenges, including scheduling concerns and the need for experienced focus group facilitators, combined with a higher volume, intensity, and complexity of data collection (hence greater risk). Furthermore, professional hierarchies may have influenced group dynamics and affected the kinds of insights and information participants felt comfortable disclosing in individual interviews. Although not feasible within the time-scales of my own research, collecting alternative sources of qualitative data alongside the interviews would nevertheless have enhanced the analysis and provided opportunities to triangulate findings.

4.5.3 Implications for current fetal monitoring practice

Notwithstanding these limitations, this study has important implications for maternity services providing fetal monitoring in high-risk pregnancies. Firstly, it is evident that FBS is often performed by trainees because of a lack of direct senior support, not just

during the complex task of CTG interpretation, but when they are faced with making difficult clinical decisions and prioritising on a busy labour ward. Therefore, maternity units should begin by taking additional steps to support less experienced obstetricians and midwives, who find themselves faced with managing increasingly complex pregnancies and meeting ever-higher expectations of care. It must be acknowledged that consultant presence on UK labour wards has already increased in recent decades, in line with the standards set out in *Safer Childbirth* (RCOG, 2007); however, more formalised processes for involving senior clinicians in fetal monitoring and intrapartum decision-making may be required to adequately support the learning needs of the team. For example, Lowe and Beckmann (2016) showed that direct review of abnormal CTGs by a consultant obstetrician prior to performing fetal scalp lactate was associated with half the number of tests actually being performed, as well as reductions in caesarean section for fetal distress, instrumental birth, and the proportion of babies born with a cord pH < 7.10. In small and medium-sized maternity units, where consultants may be less physically present on the 'shop floor', this can be facilitated by remote access to real-time digital CTGs and maternity records through systems like K2 Guardian™, already widely used in the UK. However, support need not be provided only by consultant obstetricians. Senior midwives with demonstrated expertise in fetal monitoring are equally capable of supporting fellow midwives and obstetricians. Indeed, Liberati et al. (2021) identified multidisciplinary learning and flexible hierarchies, in which skills and experience are valued above seniority or professional roles, as key features of safety in maternity units.

The need for better support has already been recognised at a national level. The Saving Babies' Lives Care Bundle Version 2 (NHS England, 2019) recommended that every consultant-led unit appoint a Fetal Monitoring Lead responsible for improving the standard of intrapartum risk assessment and fetal monitoring, yet few participants in this study could identify such a person or role within their unit. The interim report of the Ockenden review (Department of Health and Social Care, 2020), which is investigating failings in maternity care at the Shrewsbury and Telford Hospital NHS Trust, has extended these recommendations by calling for the appointment of a Lead Midwife and Lead Obstetrician to champion best practice on fetal monitoring in every unit. The data presented here underscores the need for additional frontline support and leadership

on intrapartum fetal monitoring and should offer further encouragement to maternity units to urgently meet these recommendations.

This study also suggests that that the provision of face-to-face fetal monitoring training in many UK maternity units is lacking, whether in quantity and/or quality. On this point, it is important to note that all interviews were conducted prior to the COVID-19 pandemic, therefore this finding does not reflect disruption to training resulting from social distancing. Rather, the shift towards remote learning in the wake of the pandemic would be expected to have further restricted opportunities for face-to-face training and increased the observed reliance on online fetal monitoring resources.

In some units, the nature of face-to-face fetal monitoring training may even be having have a detrimental effect on the learning environment; for example, CTG training undertaken as part of adverse event reviews appeared to entrain a fear of criticism and blame amongst some trainee participants. This 'find and fix' approach to safety is common within NHS maternity care but promotes the view that adverse outcomes occur when something goes wrong (Hollnagel et al., 2015) and may negatively impact upon psychological safety around fetal monitoring, as well as contributing to defensive clinical practice. An alternative approach is for units to adopt a Safety-II approach, as was recently suggested in a report into delays in intrapartum interventions (Healthcare Safety Investigation Branch, 2020). In the current context, this means establishing systems for regularly reviewing educationally appropriate cases with both positive and negative outcomes, where the focus is on understanding variability in the everyday performance of the MDT (Hollnagel et al., 2015). This is crucial to creating a safe environment on the labour ward which promotes informal learning opportunities and prompt, appropriate escalation of concerns.

The argument for Safety-II extends also to multidisciplinary simulation training. Traditionally, 'skills and drills' training has focused on the management of obstetric emergencies, such as shoulder dystocia, eclampsia and major post-partum haemorrhage. Programmes like PROMPT (PRactical Obstetric Multi-Professional Training), widely adopted in the UK and other developed countries (Shoushtarian et al., 2014, Weiner et al., 2016), have been pivotal in increasing knowledge around human factors and situational awareness amongst maternity care providers. However, these factors are equally important in the day-to-day functioning of labour ward teams;

for example, how obstetricians and midwives manage uncertainty around CTG interpretation, prioritise clinical activity, and resolve disagreements on treatment decisions. Face-to-face training should, therefore, evolve beyond provisioning of information on fetal physiology and CTG interpretation, to incorporate simulated MDT scenarios with a focus on the social context of fetal monitoring.

4.5.4 Future research directions

This study also helps bring to light areas for future researchers and policy makers to consider. As already noted, an important and natural extension of this research will be to explore the use of adjunctive fetal monitoring technologies from the viewpoint of the midwife providing one-to-one intrapartum care. Although midwives are unlikely to perform FBS, the value they ascribe to a normal result is likely to influence how CTG concerns are escalated within the team and acted upon. For example, several participants described instances in which they had performed FBS in order to appease a concerned midwife, rather than because they deemed it clinically necessary. Furthermore, Mayes et al. (2018) noted that the majority of midwives in their study had worked in the same unit for over a decade and therefore strongly aligned themselves with the organisational norms and culture of that unit. Beyond any variance in professional background, these factors could result in very different perspectives to that of the obstetricians interviewed here, a majority of whom had worked in multiple units and/or regions in recent years.

Recruiting midwives of varied experience, including labour ward coordinators, is also necessary to examine the social interactions and team dynamics that underscore multidisciplinary intrapartum care. The use of other qualitative methodologies, such as ethnography and longitudinal case studies, would further enhance the collection of rich data related to the social context of fetal monitoring. Lame et al. (2019) have recently published a protocol for an ethnographic study of intrapartum fetal monitoring in three UK maternity units and further research in this area should be prioritised urgently, in line with the recommendations of the Early Notification Scheme progress report highlighted at the outset of this chapter. More immediately, organisations can engage in a range of small-scale exercises to reflect upon, measure and improve culture within their maternity units (Al Nadabi et al., 2019, Simpson et al., 2019, RCM, 2021).

Finally, it is critical that process evaluations are embedded into future trials of fetal monitoring technologies to better understand how these interventions are implemented in different contexts. For example, site-level data from the INFANT trial (INFANT Collaborative Group, 2017) suggests that clinicians may have responded to CTG concerns differently in each unit: the time from the last red level of concern (indicating a potentially pathological CTG) to delivery varied from around thirty minutes in some units to nearly two hours in others. If such variation in fetal monitoring practice exists between sites, then the same intervention may be implemented and received in different ways, particularly when that intervention is complex (Petticrew, 2011), as in the case of adjunctive technologies. This makes it difficult to draw accurate conclusions about the effectiveness of the study intervention. In the case of STAN, previous research has shown that the successful implementation of new technology is not a tick box but a gradual, evolving process which must be tailored to the changing needs of each context (Amer-Wåhlin et al., 2005, Doria et al., 2007, Timonen and Holmberg, 2018). Whilst it may not be realistic to replicate this learning curve within a multi-site randomised controlled trial, integrating process evaluations into future trials of intrapartum interventions will help researchers understand how context influences outcomes, and is an important step towards facilitating sustainable improvements in maternity care across the NHS.

4.6 Conclusions

In summary, this chapter has explored the current role of FBS from the perspective of UK obstetricians and identified a range of individual and contextual factors that appear to influence fetal monitoring practice. FBS remains widely performed within the NHS, yet there were marked differences in attitudes towards fetal monitoring and evidence of variation in practice both at the level of individual participants and maternity units. The real-life use of adjunctive fetal monitoring technologies appears to reflect the interplay between the attending obstetricians' clinical competence and autonomy, the socio-cultural context in which they practice, and the influence of wider external factors, such as clinical practice guidelines. These findings support the view proposed

by Lame et al. (2019), in which intrapartum fetal monitoring is best understood as a "complex socio-technical process" requiring the coordinated actions of a multi-disciplinary team of individuals, who must integrate and respond to multiple sources of clinical information over time and in highly pressurised environments.

To date, our understanding of this process has been limited because prior studies have focused largely on the clinical effectiveness of CTG and adjunctive technologies, rather than the processes underlying their use. Therefore, further research is urgently needed to fully characterise the social, cultural, and organisational features of maternity units that influence intrapartum fetal monitoring, alongside continued efforts to improve staff training in fetal monitoring, as highlighted by participants in this study.

Chapter 5 Discussion

5.1 Summary of findings and implications

Every day in the UK, babies and mothers come to harm because of the limitations of existing methods of intrapartum fetal monitoring, mainly CTG and fetal blood sampling. A better means of detecting babies at risk of evolving hypoxia may be to continuously monitor lactate and related metabolites within the interstitial fluid, in a similar manner to continuous glucose monitoring devices. Prior to undertaking this research, however, it was not known if interstitial lactate reflected changes in blood lactate that occur during hypoxia. This thesis explored the potential of subcutaneous microdialysis of the scalp as a novel means of monitoring fetal wellbeing during labour, by testing the hypotheses that interstitial lactate reflects trends in arterial lactate in response to hypoxia in newborn piglets.

This is the first study to investigate the relationship between interstitial and arterial lactate in a perinatal model, and the findings presented in **Chapter 3** are broadly consistent with existing animal and human studies of interstitial lactate monitoring. Firstly, there was a strong positive correlation between interstitial and arterial lactate (r=0.821 to 0.848), similar to that reported by Tigchelaar et al. (2020). Secondly, interstitial lactate trends reflected major trends in arterial lactate following both hypoxia and intravenous sodium lactate infusion. As with past studies of clinical microdialysis (Ellmerer et al., 2009, van den Heuvel et al., 2009), there was variation amongst individual piglets in the closeness of agreement between the compartments. This may be related to differences in peak lactate concentrations in the current study: for example, interstitial lactate profiles mirrored those of blood much more clearly in piglets whose arterial lactate exceeded 8 mmol/L. Based on current understanding of arterio-interstitial lactate dynamics, I have presented a plausible explanation for this novel observation, which may have important implications for the clinical application of interstitial lactate monitoring, as discussed below.

Finally, the delay in lactate changes between blood and ISF was approximately 15 min in the piglets, which is in keeping with available data for both lactate and glucose. For a microdialysis-based fetal monitoring device, the time lag of the microdialysis system itself (around 5 min based on suggested flow rates and tubing dimensions) must also be considered. However, these preliminary data indicate that a device monitoring interstitial lactate continuously should be capable of detecting changes in fetal arterial lactate within 20 min. As the main purpose of the proposed device is to detect gradually evolving or subacute hypoxia, in which metabolic acidosis occurs slowly – pH falls by 0.01 every 2-3 min in subacute hypoxia (Chandraharan, 2017) – this is likely to be clinically acceptable. As already highlighted, this is similar to the time currently taken to obtain an FBS result in practice.

This study also provides the first description of the use of the StatStrip Xpress® POC meter for lactate measurement of dialysate samples. The StatStrip Xpress® has previously been shown to reliably measure lactate in amniotic fluid, however a notable limitation of the study of Hall et al. (2014) was that the minimum lactate concentration tested was 7.7 mmol/L. This thesis has confirmed the same device's performance across a more physiological range of lactate concentrations, which one would expect to recover at typical flow rates used for clinical microdialysis. Although results from the StatStrip Xpress® were shown not to be interchangeable with the ISCUS*flex,* it may offer a more accessible means of offline microdialysis in future research and clinical studies. An additional advantage of using the StatStrip Xpress® to analyse dialysate and blood samples simultaneously is that it would minimise analytical sources of variation between the compartments.

Having demonstrated physiological proof of concept for the potential value of interstitial lactate monitoring around the time of birth, **Chapter 4** sought to explore the current and future role of adjunctive technologies within intrapartum care in the UK. Even a device capable of detecting intrapartum hypoxia with perfect sensitivity and specificity relies on effective implementation by clinicians, as end users, to reduce harm avoidable harm to babies and mothers. Hence, this type of enquiry was considered vital from early in the conception of my research, not least because there is a paucity of recent data in relation to this important area of clinical practice. The findings of the qualitative study presented here suggest that FBS plays an important role in current fetal

monitoring practice within the UK, although evidence from this and other studies (Rose et al., 2018, Al-Watter et al., 2019, East et al., 2021) indicates its use may be declining. Participants who said they performed FBS regularly acknowledged its limitations, and often described feeling conflicted in its use; nevertheless, they attributed value to FBS as a clinical tool and safety mechanism when making difficult intrapartum care decisions. Personal attitudes to FBS and related aspects of intrapartum monitoring evolved with experience, reflecting the development of clinical competence and autonomy. This interplay between obstetrician's individual autonomy, the social and cultural norms of each maternity unit, and various external factors, ultimately influenced how FBS was used in everyday practice.

Moving forward, doctors in this study identified improvements to staff training on intrapartum fetal monitoring as the key research and policy priority. This was consistent with their own experiences of fetal monitoring training, which many reported as being insufficient in quality and/or quantity. As previously highlighted, the direct impact of the COVID-19 pandemic on junior doctor education and training (Seifman et al., 2022) and the wider pressures facing the maternity workforce in the pandemic's wake (RCOG, 2022) are only likely to have exacerbated this issue in the time since these interviews were conducted.

It was hoped that the qualitative data collected would also complement the overarching aims of this research by exploring obstetricians' ideas about new fetal monitoring technology: what that technology might look like, and any specific design or functionality criteria which were considered important. In this regard, most participants recognised a need for better methods to monitor fetal wellbeing, however, there was scepticism about the role of 'complex' technology and the realities of implementing changes in practice within the intrapartum setting. Participants also offered very few ideas about potential new approaches to monitoring. This may be due to the abstract nature of the topic, and it is noteworthy that no information was disclosed to participants about the microdialysis research being undertaken, nor the concept of monitoring interstitial lactate, because of concerns that this may have influenced their responses. It is possible that alternative qualitative methodologies, such as focus groups, might have encouraged more open dialogue around new approaches to fetal monitoring and provided greater insight into questions related to device design and specification.

5.1.1 Microdialysis-based intrapartum fetal monitoring

In the final year of this research, Tigchelaar et al. (2020) published on the use of a custom microdialysis probe incorporated into a spiral fetal scalp electrode to monitor interstitial lactate in three adult rats exposed to hypoxia. There are several advantages to this design: FSEs are widely used in current practice and can be applied by midwives and doctors at digital vaginal examination from early labour onwards (cervical dilatation of $\geq$ 1-2 cm); they are considered acceptable by clinicians and women in terms of their level of invasiveness; and they provide the opportunity for simultaneous monitoring of the fetal heart rate. This latter point is important because any microdialysis-based device would have to be used in conjunction with fetal heart rate monitoring to allow detection of acute intrapartum events, such as cord prolapse, which require intervention within minutes i.e. before changes in interstitial lactate may be detected.

The concept of integrating microdialysis sampling into a FSE had been forefront in my mind during conception and planning of the piglet studies. In collaboration with engineers at Heriot-Watt University, I undertook parallel work to incorporate a microdialysis membrane into a curved hollow hypodermic needle (data not shown) based on the FSE design shown in Figure 1.3. Unfortunately, time constraints of my research, including restrictions to laboratory access following the national lockdown in March 2020, meant it was not possible to complete this work to a prototype stage which could be evaluated either *in vitro* or *ex vivo*. The findings presented in this thesis, although obtained from commercially available microdialysis catheters, nevertheless address important questions about the application of this technique to fetal monitoring.

One concern was that clinical microdialysis studies to date have included prolonged equilibration periods before sampling in order to avoid any effect of catheter insertion on interstitial metabolite concentrations (Hammarlund-Udenaes, 2017). However, consistent with previous findings in skeletal muscle (Sørensen et al., 2018, Ashina et al., 2005), this study demonstrated that interstitial lactate concentrations are not affected by catheter-related trauma, which means that dialysate lactate levels could be reliably interpreted as soon as they become available to clinicians. Regardless, preparation of the microdialysis system and catheter insertion in early labour, prior to the onset of any intrapartum hypoxic stress or associated CTG concerns, would be a prerequisite for the accurate interpretation of interstitial lactate point results and trends.

As with STAN, this approach enables clinicians to establish what is normal for each fetus and more easily recognise subsequent deviation from normal (noting, in the current study, that there were two to three-fold differences in interstitial lactate concentrations amongst piglets before hypoxia was induced). The argument for a more individualised approach has also been made in relation to FBS interpretation, supported by population-level data on variation in umbilical cord blood lactate (Wiberg et al., 2008) and pH (Kitlinski et al., 2003). As previously discussed, this may also negate the need for 'calibration' methods to adjust for lactate recovery *in vivo*. Whether any pre-defined threshold for intervention, based either on absolute interstitial lactate values or observed trends, would be clinically appropriate and practical in the context of microdialysis-based monitoring remains to be determined.

As with any diagnostic test, finding the optimal balance between sensitivity and specificity is a crucial step when defining thresholds for interstitial lactate at which clinical intervention should occur. When used in conjunction with continuous CTC, interstitial lactate thresholds could be set primarily with a view to improving specificity, i.e. reducing false positive diagnoses of fetal distress based on CTG alone, which commonly contribute to unnecessary obstetric intervention. However, unlike FBS, which is performed only if a CTG abnormality has already been recognised, interstitial lactate monitoring may actually improve the detection (sensitivity) of hypoxia and/or other pathophysiological mechanisms which contribute to adverse perinatal outcomes (e.g. intrauterine infection, meconium aspiration) by serving as an independent marker of fetal wellbeing. This is a key potential advantage over current adjunctive fetal monitoring technologies.

Based on the data presented in **Chapter 3** and existing FSE designs, I have also suggested certain parameters for a microdialysis-based fetal monitoring device, such as perfusate flow rate (minimum 1.5 µL/min) and outlet tubing length (maximum 15 cm, equating to approximately 5 µL dead volume). However, these parameters would depend on the final device design, especially the surface area of the microdialysis membrane and resulting lactate recovery. Assuming recovery is closer to that seen with the custom probe described by Tigchelaar et al. (2020; *in vitro* RR approximately 25% at 1.5 µL/min), *in vivo* lactate recovery may be as low as 10% in practice. In theory, trend data should be preserved regardless of RR, however, lower recoveries would

invariably condense results across a narrower range of concentrations. For example, a rise in interstitial lactate from 2 to 8 mmol/L results in dialysate levels increasing from 1 to 4 mmol/L at 50% RR (similar to current study), but only from 0.2 to 0.8 mmol/L at 10% RR. Detecting such changes would demand lactate sensors with a very high degree of analytical precision. As discussed above, this may be compounded by the fact that relative changes in interstitial lactate were lower than those seen in blood, a finding which is consistent with past studies (de Boer et al., 1994, Ellmerer et al., 1998).

Optimising probe recovery and analytical precision are just two examples of the practical challenges faced in developing a microdialysis-based fetal monitoring device. There are several considerations further downstream in the device development pathway which this thesis has not explored, such as user interface, cost-efficient manufacturing, and regulatory approvals. Nevertheless, the data presented in this thesis may be applied much more readily to neonatal setting, where subcutaneous microdialysis of glucose has already been demonstrated (Holzinger et al., 2006). Microdialysis may be of particular benefit in this population because it avoids the need for repeated blood sampling in critically ill neonates, often over a period of days to weeks, and so avoids the risk of iatrogenic blood loss (Counsilman et al., 2019). The existing availability of clinically-approved microdialysis catheters and analytical platforms presents an attractive avenue for future researchers to investigate the potential of interstitial lactate monitoring.

5.2 Strengths and limitations

The strengths and limitations of this research have been discussed in detail within their respective chapters and are summarised below.

The key strength of the work presented in **Chapters 2 and 3** is the use of newborn piglets. Piglet models of perinatal HI are widely established and their anatomical and physiological similarities to the term human fetus enhances the applicability of the findings presented here to the proposed application of fetal monitoring. Moreover, by providing a detailed description of the development and ongoing refinement of the hyperlactataemia protocol, this thesis aims to establish a reproducible animal model which is ideally suited to large-scale studies of fetal and neonatal monitoring

technology in the future. In this regard, the accessibility and lower cost of studying newborn piglets, compared to ovine models of *in utero* hypoxia, is considered an important advantage.

Despite the promising data presented from these studies, the small sample size limited the ability to draw robust conclusions about the relationship between arterial and interstitial lactate. This was exacerbated by the premature loss of one piglet, in whom microdialysis was not performed, whilst data from a further piglet (B) should also be interpreted with caution. The small sample also prevented standardisation of the study protocol, as multiple refinements were required to achieve target hyperlactataemia and to address unforeseen experimental problem, such as coagulopathy, haemodilution, and glycaemic control. Whilst this restricted comparisons between the piglets in subsequent data analyses, these refinements were critical to meeting the study's primary objective to investigate the relationship between arterial and interstitial lactate. A related limitation of this work is the absence of a control group, which may have provided valuable information on interstitial lactate and the microdialysis technique itself over a longer period of steady-state conditions. This is also a critical step forwards to control for biological and experimental variables in future studies.

The limitations of the qualitative study presented in **Chapter 4** relate primarily to recruitment and sampling. Sixteen obstetricians were interviewed, which was lower than the target sample size of 20 participants, and participants from small maternity units and the London region were under-represented in the final sample. In addition, it is possible that the use of snowballing and recruitment through professional and academic networks may have resulted in the study interviewing obstetricians with higher levels of interest in intrapartum care and fetal monitoring. These limitations must be balanced against the strengths of this study, particularly the use of telephone interviews and verbal consent to gather rich data from a geographically dispersed sample of healthcare professionals. This was achieved within strict resource and time constraints, notwithstanding the impact of the COVID-19 pandemic, discussed separately below. As a result, this study has successfully provided a much-needed contemporary perspective on intrapartum fetal monitoring practices within the UK.

5.3 Impact of the COVID-19 pandemic

The work presented in this thesis was impacted significantly by the COVID-19 pandemic. In the UK, the pandemic led to a national lockdown from late March 2020 and all non-essential research activity at the University of Edinburgh was halted at this time. This meant suspending recruitment for the qualitative study, which impacted upon the final sample size and make-up, as discussed above. At the time of lockdown, funding had also been secured and plans approved for four more piglet studies, which would have allowed further refinements to the protocols for both hypoxia and sodium lactate infusion. Specifically, the sequence was to be reversed such that piglets underwent the hypoxic insult first then a secondary lactate challenge with revised sampling schedule. This aimed to improve the precision of estimates on the arterio-interstitial delay, whilst minimising the risk of compromising hypoxia-related data.

In addition to preventing further data collection, restrictions on laboratory access during the lockdown delayed processing of the microdialysis samples. Analysis of samples from the first three study days (September to December 2019) had already been delayed because it was not possible to repair a microdialysis analyser owned by the University of Edinburgh. After securing the loan of an ISCUS*flex* directly from Linton Instrumentation, its delivery was then delayed by several months during lockdown. As a result, all microdialysis samples were batch analysed in the final month of my research programme (July 2020), after the laboratory re-opened and the ISCUS*flex* was delivered. With the benefit of hindsight, this data may have prompted earlier amendments to the experimental protocols for *in vivo* studies, particularly in relation to the frequency and timing of sampling, as well as the use of sodium lactate infusion.

5.4 Future directions

This thesis has generated several questions for future research regarding interstitial lactate monitoring and its potential application for intrapartum fetal assessment.

Firstly, a larger sample size is required to evaluate inter-individual variation in the closeness of agreement between arterial and interstitial lactate. These studies should involve randomised allocation of piglets to control and experimental groups and, ideally, the inclusion of different patterns of hypoxia within experimental groups to

better replicate the mechanisms of hypoxia seen during labour. Relatedly, the focus should be on arterial lactate concentrations within the established reference range for umbilical cord arterial lactate (2.0 to 9.5 mmol/L, equivalent to the 3rd and 97th centiles at 40 weeks' gestational age; Wiberg et al., 2008), rather than achieving extreme peak values as was the objective in this exploratory study. As the findings presented here have raised the possibility of weaker agreement between the compartments at lower arterial lactate concentrations, it would be crucial to examine this relationship in piglets with blood lactate levels between 3 to 7 mmol/L, which is also the range in which thresholds for interpreting FBS and FSL fall.

Additional areas to address in future piglet studies include:

- Extending the duration of subcutaneous microdialysis to a minimum of 24 h;
- Fixed arterial and microdialysis sampling to facilitate paired comparisons;
- Use of radio-labelled lactate to accurately quantify the arterio-interstitial delay;
- Comparison of dialysate data from multiple sampling sites in the same pig; and
- Tissue specimen collection to evaluate the correlation between interstitial data and histopathological evidence of HI injury.

Finally, the data presented here and in the recent study by Tigchelaar et al. (2020), warrant continued collaboration with engineers at Heriot-Watt University to develop a custom microdialysis catheter based on an FSE design. This workstream would be supported by ongoing refinement of the piglet model presented in **Chapter 2**.

The findings of the qualitative study presented here also highlight important areas for future research. In addition to recruiting more participants from small-sized maternity units and the London region, a recognised limitation of this study, it is vital to seek the perspective of midwives on intrapartum fetal monitoring in high-risk pregnancies, including the use of FBS and other adjunctive technologies. Concentrating recruitment in only two or three units with a higher number of participants from each, including midwives and obstetricians of varied experience, would give researchers a better opportunity to explore the impact of team dynamics on practice and encourage fuller characterisation of the 'culture' of individual units. An attractive option would be to sample purposively from units which have been successful in implementing changes

in fetal monitoring practice, such as the introduction of physiological CTG interpretation. This approach, known as deviant or extreme sampling, has been applied in a similar manner to identify important features of safety within a single high-performing UK maternity unit through a longitudinal ethnographic study involving observations, interviews and focus groups (Liberati et al., 2019).

Finally, this research has underscored some of the challenges faced in changing clinical practice, including the implementation of new approaches to fetal monitoring. If the vision of reducing avoidable harm around the time of birth is to be realised, future researchers must therefore ensure that their efforts to develop new monitoring technology or treatments are matched by a thorough understanding of the context in which such interventions take place, and that evidence relating to clinical outcomes is supported by robust process evaluations.

5.5 Appendix

5.5.1 Qual-IFY participant information sheet and oral consent script

A Qualitative study of UK obstetricians' views of Intrapartum Fetal monitoring technologY

We are inviting you to take part in an interview study. Before deciding whether you would like to take part, please read the following information carefully. Talk to others about the study if you wish and contact us if there is anything that is not clear, or if you would like more information.

What is the purpose of the research?

Improving how we monitor fetal wellbeing during labour is key to reducing avoidable harm for both mothers and babies around the time of birth. Although cardiotocography is the mainstay of monitoring in high-risk pregnancies, additional tests (adjunctive technologies) are commonly used in the UK to help identify babies at risk due to a lack of oxygen. However, debate about their value has led to variation in practice and uncertainty regarding directions for future research. Few studies have explored obstetricians' views of using adjunctive technologies for fetal monitoring. This is important if new technology is to be adopted and successfully implemented within the NHS.

The purpose of Qual-IFY is to improve our understanding of how and why obstetricians use additional tests, such as fetal scalp blood sampling and ST waveform analysis, to monitor the wellbeing of babies during labour.

Why have I been invited to take part?

You have been asked to take part as you are a qualified doctor who has a current NHS role in providing care to high-risk women in labour, or because one of your colleagues has suggested you would be a good person to talk to.

Do I have to take part?

It is up to you to decide whether you would like to take part. If you do take part, you will be given this information sheet to keep and be asked to provide oral consent at the time of your interview. You will still be free to withdraw at any time without giving a reason.

What will happen if I take part?

You will be contacted to arrange a convenient date and time for the interview, which will be a one-to-one conversation with a member of the research team by telephone. It is anticipated that your interview will last about 30 minutes, although this will depend on how much you have to say. You have the right not to answer any questions you don't want to, and you are free to stop the interview at any time without giving any reason at all.

The interview will be audio-recorded using an encrypted digital recorder and sent securely to a professional external company to be typed up (transcript).

What are the possible benefits of taking part?

There are no direct benefits to you taking part in this study, although the interview will present an opportunity to reflect on an important aspect of clinical practice. Unfortunately, we are unable to pay you for your time.

What are the possible disadvantages of taking part?

The main disadvantage is the time required for the interview itself. Every effort will be made to schedule this at your convenience and keep related correspondence to a minimum.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time without giving reason. If you choose to withdraw after your interview, any recordings, transcripts and data resulting from your involvement will be destroyed and will not contribute to the findings of the study.

What happens when the study is finished?

The information we have collected as paper copies will be stored under lock and key, while the electronic data can only be accessed with a secure password. Only the researchers, sponsors, regulatory authorities and auditors will have access to the data.

The data we collect will be used only for the purposes of this research. Recordings, transcripts and all study documents (paper and electronic), including identifiable information about you, will be kept for 3 years after the study has finished.

Will my taking part be kept confidential?

All the information we collect during the course of the research will be kept confidential and there are strict laws which safeguard your privacy at every stage. The professional transcription company will be held to the same levels of confidentiality as the researchers. Nothing that could identify you will be kept in the typed-up transcript and you will not be identifiable from any publications or presentations of the findings.

For details on what data will be held about you and who will hold and store this information please refer to the Data Protection Information Sheet.

What will happen to the results of the study?

The results of the study may be published in a scientific journal or presented at conferences, in addition to forming part of an MD (Doctor of Medicine) thesis. You will be asked if you wish to receive information on the overall findings in the form of a short report.

Who is organising and funding the research?

The study forms part of a doctoral research project. The study is funded by Tommy's and jointly sponsored by the University of Edinburgh and NHS Lothian (ACCORD).

Who has reviewed the study?

The study is sponsored by ACCORD (Academic and Clinical Central Office for Research and Development) and a favourable ethical opinion has been obtained from the University of Edinburgh PPLS Ethics Review Committee.

Researcher Contact Details

If you have any further questions about the study or if you wish to take part, please contact:

Dr Andrew Brown

Email:	Telephone: 07307 635710 or 0131 2422692

Independent Contact Details

If you would like to discuss this study with someone independent to seek general advice about taking part, please contact:

Dr Sarah Murray
Email:

If you wish to make a complaint about the study please contact:

Patient Experience Team
2 – 4 Waterloo Place, Edinburgh, EH1 3EG
Email: feedback@nhslothian.scot.nhs.uk
Telephone: 0131 536 3370

Thank you for taking the time to read this information sheet.

ORAL CONSENT SCRIPT

Participant ID:

Hello again, I'm Andrew Brown from the University of Edinburgh and I wanted to talk to you about the study 'Qual-IFY' which I gave you information about before. To recap, the broad aims of the study are to explore UK obstetricians' views of using adjunctive technologies to monitor fetal wellbeing during labour.

Are you still interested in taking part? *[Await confirmation]*.

I need to confirm some of the details of the study to make sure you understand what's involved. As the study is being carried out by telephone, I would like to audio-record this part of our conservation as a record of your consent to take part – is that OK? *[Await confirmation]*.

Please listen carefully and respond, with a simple Yes or No, to the following:

You have read and understood the participant information sheet (12 Jun 2019, v1.0) and the Data Protection Information Sheet (22 Jul 2019, v1.1) for the above study; and you have had the opportunity to ask questions and have had these answered satisfactorily.

You understand that your participation is voluntary and that you are free to withdraw at any time, without giving reason and without your legal rights or employment being affected.

You understand that relevant sections of data collected during the study may be looked at by individuals from the Sponsors (University of Edinburgh and NHS Lothian) or other regulatory authorities where it is relevant to your taking part in this research; and you give permission for these individuals to have access to your data.

You understand that data collected about you during the study may be converted to anonymised data; and you are happy to be quoted anonymously in any publicised materials.

You agree to your interview being audio-recorded and transcribed securely by a professional external company.

Would you like to receive a short report summarising the main findings of the study?

Finally, do you agree to take part in the study?

Name of person receiving consent	Date	Signature

1x original – into Site File as oral consent record form

5.5.2 Interview topic guide

QualIFY

TOPIC GUIDE

Note – The contents of the topic guide may be revised in light of findings that emerge during data collection and analysis, as is standard procedure in a qualitative study of this design.

Introduction i.e. statement of purpose, interview length approx 30 minutes

Background

- Demographic details – position, education (highest qualification), expertise, years in post
- Can you tell me about the unit you are working in? [birth rate, case mix, labour ward team]

Current fetal monitoring practice

- How do you monitor fetal wellbeing during high-risk labours? [CTG guidelines, central monitor]
- What, if any, adjunctive technologies do you use in addition to continuous CTG?
- Have you undertaken any recent training in intrapartum fetal monitoring?
- How would you describe the safety culture on your labour ward?

Experiences of adjunctive technologies

- Can you tell me about your experience of using FBS / STAN / computer-aided CTG analysis (depending on method currently or most recently used)?
- What do you like most about it? Dislike?
- What effect do you think it has on maternal outcomes in your unit? Neonatal outcomes?
- Have you used any other adjunctive technologies? Can you tell me about this?

Attitudes and influencing factors

- How have your views towards using adjunctive technologies changed over time? Why?
- How do other clinicians in your unit feel about…? What about midwives? [physician factor]
- Do your colleagues' views influence your practice? [team dynamics, opinion leaders]
- Are you aware of fetal monitoring practices in other UK regions and internationally?

New technology

- Do you think there is a need for new technology to monitor fetal wellbeing during labour? [cf. improving use of currently available methods]
- How might this technology work? Key features?
- How should new technology be evaluated before it is adopted into routine practice?
- Can you tell me about your experiences of (any) intrapartum trials?
- Would you consider using new technology based on local experience, i.e. if it were not recommended in national guidelines? Why / why not?

Implementing change

- Have you worked in a unit where new fetal monitoring technology was introduced? Can you tell me about this? [If not, has your unit considered introducing new technology e.g. POC lactate]

Qual-IFY
Interview topic guide
18 Sep 2019 v1.1

- What were the main barriers? How were these addressed?
- Did this change your practice and / or unit policy? If so, what were the facilitators to successful implementation and maintenance?

Future directions

- How do you think we can improve intrapartum fetal monitoring? [human factors, CTG training, 'AI', devices etc.]
- What steps would you like to see taken – at local, regional, national levels?
- Do you think it is a research priority in women's health? Why / why not?
- What key questions should future research address?

Wind up

- Reflecting back on this interview, how do you think the way we monitor fetal wellbeing during labour might change over the next 20 years?
- Do you have anything else you would like to add?

References

Abrahamsson, P., Johansson, G., Aberg, A. M., Haney, M., & Winsö, O. (2008). Optimised sample handling in association with use of the CMA 600 analyser. *J Pharm Biomed Anal, 48*(3), 940-945. doi:10.1016/j.jpba.2008.08.010

Adamsons, K., Beard, R. W., & Myers, R. E. (1970). Comparison of the composition of arterial, venous, and capillary blood of the fetal monkey during labor. *Am J Obstet Gynecol, 107*(3), 435-440. doi.org/10.1016/0002-9378(70)90572-7

Al Nadabi, W., McIntosh, B., McClelland, T., & Mohammed, M. (2019). Patient safety culture in maternity units: a review. *Int J Health Care Qual Assur, 32*(4), 662-676. doi:10.1108/ijhcqa-01-2018-0005

Al Wattar, B. H., Lakhiani, A., Sacco, A., Siddharth, A., Bain, A., Calvia, A., ... Castling, Z. (2019). Evaluating the value of intrapartum fetal scalp blood sampling to predict adverse neonatal outcomes: A UK multicentre observational study. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 240*, 62-67. doi.org/10.1016/j.ejogrb.2019.06.012

Alfirevic, Z., Gyte, G. M. L., Cuthbert, A., & Devane, D. (2017). Continuous cardiotocography (CTG) as a form of electronic fetal monitoring (EFM) for fetal assessment during labour. *Cochrane Database of Systematic Reviews*(2). doi:10.1002/14651858.CD006066.pub3

Allanson, E. R., Waqar, T., White, C. R. H., Tunçalp, Ö., & Dickinson, J. E. (2017). Umbilical lactate as a measure of acidosis and predictor of neonatal risk: a systematic review. *BJOG, 124*(4), 584-594. doi:10.1111/1471-0528.14306

Altaf, S., Oppenheimer, C., Shaw, R., Waugh, J., & Dixon-Woods, M. (2006). Practices and views on fetal heart monitoring: a structured observation and interview study. *BJOG, 113*(4), 409-418. doi:10.1111/j.1471-0528.2006.00884.x

American College of Obstetricians and Gynecologists. (2009). ACOG Practice Bulletin No. 106: Intrapartum Fetal Heart Rate Monitoring: Nomenclature, Interpretation, and General Management Principles. *Obstet Gynecol, 114*(1), 192-202. doi:10.1097/AOG.0b013e3181aef106

American College of Obstetricians and Gynecologists. (2014). Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. Report of the American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. *Obstet Gynecol, 123*(4), 896-901. doi:10.1097/01.AOG.0000445580.65983.d2

American College of Obstetricians and Gynecologists. (2015). Committee Opinion No. 644: The Apgar Score. *Obstet Gynecol, 126*(4), e52-e55. doi:10.1097/aog.0000000000001108

Amer-Wåhlin, I., Hellsten, C., Norén, H., Hagberg, H., Herbst, A., Kjellmer, I., ... Maršál, K. (2001). Cardiotocography only versus cardiotocography plus ST analysis of fetal electrocardiogram for intrapartum fetal monitoring: a Swedish randomised controlled trial. *The Lancet, 358*(9281), 534-538. doi:10.1016/S0140-6736(01)05703-8

Amer-Wåhlin, I., Kallen, K., Herbst, A., Rydhstroem, H., Sundstrom, A. K., & Marsal, K. (2005). Implementation of new medical techniques: experience from the Swedish randomized controlled trial on fetal ECG during labor. *J Matern Fetal Neonatal Med, 18*(2), 93-100. doi:10.1080/14767050500233191

Amer-Wåhlin, I., Ugwumadu, A., Yli, B. M., Kwee, A., Timonen, S., Cole, V., ... Vayssiere, C. (2019). Fetal electrocardiography ST-segment analysis for intrapartum monitoring: a critical appraisal of conflicting evidence and a way forward. *Am J Obstet Gynecol, 221*(6), 577-601.e511. doi.org/10.1016/j.ajog.2019.04.003

Andelius, T. C. K., Pedersen, M. V., Bøgh, N., Omann, C., Hjortdal, V. E., Pedersen, M., ... Henriksen, T. B. (2019). Consequence of insertion trauma – effect on early measurements when using intracerebral devices. *Sci Rep, 9*(1), 10652. doi:10.1038/s41598-019-47052-4

Andri, M. (2022). Clinical guidelines and clinical autonomy: exploring the missing link. *Journal of Health Organization and Management, 36*(3), 351-367. doi:10.1108/JHOM-11-2020-0438

Andriessen, P., Zwanenburg, A., van Laar, J. O. E. H., Vullings, R., Hermans, B. J. M., Niemarkt, H. J., ... Delhaas, T. (2018). ST waveform analysis for monitoring hypoxic distress in fetal sheep after prolonged umbilical cord occlusion. *PLOS ONE, 13*(4), e0195978. doi:10.1371/journal.pone.0195978

Annappa, R., Campbell, D. J., & Simpson, N. A. B. (2008). Fetal blood sampling in labour and the decision to delivery interval. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 141*(1), 10-12. doi.org/10.1016/j.ejogrb.2008.07.002

Armstrong, D. (2002). Clinical autonomy, individual and collective: the problem of changing doctors' behaviour. *Soc Sci Med, 55*(10), 1771-1777. doi:10.1016/s0277-9536(01)00309-4

Armstrong, L., & Stenson, B. J. (2007). Use of umbilical cord blood gas analysis in the assessment of the newborn. *Arch Dis Child Fetal Neonatal Ed, 92*(6), F430-434. doi:10.1136/adc.2006.099846

Aroni, F., Xanthos, T., Varsami, M., Argyri, I., Alexaki, A., Stroumpoulis, K., ... Iacovidou, N. (2012). An experimental model of neonatal normocapnic hypoxia and resuscitation in Landrace/Large White piglets. *J Matern Fetal Neonatal Med, 25*(9), 1750-1754. doi:10.3109/14767058.2012.663823

Ash, S. R., Rainier, J. B., Zopp, W. E., Truitt, R. B., Janle, E. M., Kissinger, P. T., & Poulos, J. T. (1993). A subcutaneous capillary filtrate collector for measurement of blood chemistries. *ASAIO Journal, 39*(3), M699-705.

Ashina, M., Jorgensen, M., Stallknecht, B., Mork, H., Bendtsen, L., Pedersen, J. F., ... Jensen, R. (2005). No release of interstitial glutamate in experimental human model of muscle pain. *European Journal of Pain, 9*(3), 337-337. doi:10.1016/j.ejpain.2004.09.004

Aukland, K., & Reed, R. K. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiological Reviews, 73*(1), 1-78. doi:10.1152/physrev.1993.73.1.1

Aussedat, B., Dupire-Angel, M., Gifford, R., Klein, J. C., Wilson, G. S., & Reach, G. (2000). Interstitial glucose concentration and glycemia: implications for continuous subcutaneous glucose monitoring. *American Journal of Physiology-Endocrinology and Metabolism, 278*(4), E716-E728. doi:10.1152/ajpendo.2000.278.4.E716

Ayres-de-Campos, D., Arulkumaran, S., & FIGO Intrapartum Fetal Monitoring Expert Consensus Panel (2015a). FIGO consensus guidelines on intrapartum fetal monitoring: Physiology of fetal oxygenation and the main goals of intrapartum fetal monitoring. *International Journal of Gynecology & Obstetrics, 131*(1), 5-8. doi:10.1016/j.ijgo.2015.06.018

Ayres-de-Campos, D., Spong, C. Y., Chandraharan, E., & FIGO Intrapartum Fetal Monitoring Expert Consensus Panel (2015b). FIGO consensus guidelines on intrapartum fetal monitoring: Cardiotocography. *International Journal of Gynecology & Obstetrics, 131*(1), 13-24. doi:10.1016/j.ijgo.2015.06.020

Ayres-de-Campos, D., Arteiro, D., Costa-Santos, C., & Bernardes, J. (2011). Knowledge of adverse neonatal outcome alters clinicians' interpretation of the intrapartum cardiotocograph. *BJOG, 118*(8), 978-984. doi:10.1111/j.1471-0528.2011.03003.x

Bailey, E., Frolova, A., Lopez, J., Raghuraman, N., Macones, G., & Cahill, A. (2019). Mild neonatal acidemia is associated with neonatal morbidity at term. *Am J Obstet Gynecol, 220*(1, Supplement), S145. doi.org/10.1016/j.ajog.2018.11.221

Bakker, J., Coffernils, M., Leon, M., Gris, P., & Vincent, J. L. (1991). Blood lactate levels are superior to oxygen-derived variables in predicting outcome in human septic shock. *Chest, 99*(4), 956-962. doi:10.1378/chest.99.4.956

Barthelmes, D., Jakob, S. M., Laitinen, S., Rahikainen, S., Ahonen, H., & Takala, J. (2010). Effect of site of lactate infusion on regional lactate exchange in pigs. *Br J Anaesth, 105*(5), 627-634. doi:10.1093/bja/aeq214

Baschat, A. A. (2006). The fetal circulation and essential organs – a new twist to an old tale. *Ultrasound in Obstetrics & Gynecology, 27*(4), 349-354. doi.org/10.1002/uog.2762

Basu, A., Dube, S., Slama, M., Errazuriz, I., Amezcua, J. C., Kudva, Y. C., ... Basu, R. (2013). Time Lag of Glucose From Intravascular to Interstitial Compartment in Humans. *Diabetes, 62*(12), 4083. doi:10.2337/db13-1132

Battista, R. N. (1989). Innovation and Diffusion of Health-Related Technologies: A Conceptual Framework. *International Journal of Technology Assessment in Health Care, 5*(2), 227-248. doi:10.1017/S0266462300006450

Beard, R. W., Morris, E. D., & Clayton, S. G. (1967). pH of foetal capillary blood as an indicator of the condition of the foetus. *BJOG, 74*(6), 812-822. doi:10.1111/j.1471-0528.1967.tb15562.x

Belfort, M. A., Saade, G. R., Thom, E., Blackwell, S. C., Reddy, U. M., Thorp, J. M., ... Van Dorsten, J. P. (2015). A Randomized Trial of Intrapartum Fetal ECG ST-Segment Analysis. *N Engl J Med, 373*(7), 632-641. doi:10.1056/NEJMoa1500600

Benfeldt, E., Hansen, S. H., Vølund, A., Menné, T., & Shah, V. P. (2007). Bioequivalence of Topical Formulations in Humans: Evaluation by Dermal Microdialysis Sampling and the Dermatopharmacokinetic Method. *Journal of Investigative Dermatology, 127*(1), 170-178. doi:10.1038/sj.jid.5700495

Bhide, A., Chandraharan, E., & Acharya, G. (2016). Fetal monitoring in labor: Implications of evidence generated by new systematic review. *Acta Obstet Gynecol Scand, 95*(1), 5-8. doi.org/10.1111/aogs.12830

Birgisdottir, B. T., Holzmann, M., Varli, I. H., Graner, S., Saltvedt, S., & Nordström, L. (2017). Reference values for Lactate Pro 2™ in fetal blood sampling during labor: a cross-sectional study. *45*(3), 321. doi.org/10.1515/jpm-2016-0027

Björkman, S. T., Foster, K. A., O'Driscoll S, M., Healy, G. N., Lingwood, B. E., Burke, C., & Colditz, P. B. (2006). Hypoxic/Ischemic models in newborn piglet: comparison of constant FiO2 versus variable FiO2 delivery. *Brain Res, 1100*(1), 110-117. doi:10.1016/j.brainres.2006.04.119

Blad, S., Welin, A.-K., Kjellmer, I., Rosén, K. G., & Mallard, C. (2008). ECG and Heart Rate Variability Changes in Preterm and Near-Term Fetal Lamb Following LPS Exposure. *Reproductive Sciences, 15*(6), 572-583. doi:10.1177/1933719107314060

Blair, E., & Stanley, F. J. (1988). Intrapartum asphyxia: A rare cause of cerebral palsy. *The Journal of Pediatrics, 112*(4), 515-519. doi.org/10.1016/S0022-3476(88)80161-6

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet, 327*(8476), 307-310. doi:10.1016/S0140-6736(86)90837-8

Bland, J. M., & Altman, D. G. (1999). Measuring agreement in method comparison studies. *Statistical Methods in Medical Research, 8*(2), 135-160. doi:10.1177/096228029900800204

Blechner, J. N. M. (1993). Maternal-Fetal Acid-Base Physiology. *Clinical Obstetrics & Gynecology, 36*(1), 3-12.

Blix, E., Brurberg, K. G., Reierth, E., Reinar, L. M., & Øian, P. (2016). ST waveform analysis versus cardiotocography alone for intrapartum fetal monitoring: a systematic review and meta-analysis of randomized trials. *Acta Obstet Gynecol Scand, 95*(1), 16-27. doi:10.1111/aogs.12828

Blix, E., & Öhlund, L. S. (2007). Norwegian midwives' perception of the labour admission test. *Midwifery, 23*(1), 48-58. doi.org/10.1016/j.midw.2005.10.003

Block, E. S., & Erskine, L. (2012). Interviewing by Telephone: Specific Considerations, Opportunities, and Challenges. *International Journal of Qualitative Methods, 11*(4), 428-445. doi:10.1177/160940691201100409

Bloom, S. L., Belfort, M., & Saade, G. (2016). What we have learned about intrapartum fetal monitoring trials in the MFMU Network. *Seminars in Perinatology, 40*(5), 307-317. doi.org/10.1053/j.semperi.2016.03.008

Bloom, S. L., Spong, C. Y., Thom, E., Varner, M. W., Rouse, D. J., Weininger, S., ... Anderson, G. (2006). Fetal Pulse Oximetry and Cesarean Delivery. *N Engl J Med, 355*(21), 2195-2202. doi:10.1056/NEJMoa061170

Bollella, P., Sharma, S., Cass, A. E. G., & Antiochia, R. (2019). Microneedle-based biosensor for minimally-invasive lactate detection. *Biosensors and Bioelectronics, 123*, 152-159. doi.org/10.1016/j.bios.2018.08.010

Bollmann, M. D., Revelly, J. P., Tappy, L., Berger, M. M., Schaller, M. D., Cayeux, M. C., ... Chioléro, R. L. (2004). Effect of bicarbonate and lactate buffer on glucose and lactate metabolism during hemodiafiltration in patients with multiple organ failure. *Intensive Care Med, 30*(6), 1103-1110. doi:10.1007/s00134-004-2251-3

Bonaventura, J. M., Sharpe, K., Knight, E., Fuller, K. L., Tanner, R. K., & Gore, C. J. (2015). Reliability and accuracy of six hand-held blood lactate analysers. *Journal of Sports Science & Medicine, 14*(1), 203-214.

Bowen, G. A. (2008). Naturalistic inquiry and the saturation concept: a research note. *Qualitative Research, 8*(1), 137-152. doi:10.1177/1468794107085301

Bowler, T., & Beckmann, M. (2014). Comparing fetal scalp lactate and umbilical cord arterial blood gas values. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 54*(1), 79-83. doi:10.1111/ajo.12144

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology, 3*(2), 77-101. doi:10.1191/1478088706qp063oa

Braun, V., & Clarke, V. (2019). Reflecting on reflexive thematic analysis. *Qualitative Research in Sport, Exercise and Health, 11*(4), 589-597. doi:10.1080/2159676X.2019.1628806

Bretscher, J., & Saling, E. (1967). pH values in the human fetus during labor. *Am J Obstet Gynecol, 97*(7), 906-911. doi.org/10.1016/0002-9378(67)90515-7

Britten, N., Jones, R., Murphy, E., & Stacy, R. (1995). Qualitative research methods in general practice and primary care. *Family Practice, 12*(1), 104-114. doi:10.1093/fampra/12.1.104

Bullens, L. M., Moors, S., van Runnard Heimel, P. J., van der Hout-van der Jagt, M. B., & Oei, S. G. (2016). Practice variation in the management of intrapartum fetal distress in The Netherlands and the Western world. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 205*, 48-53. doi.org/10.1016/j.ejogrb.2016.08.012

Calnan, J. S., Pflug, J. J., Chisholm, G. D., & Taylor, L. M. (1972). Pathophysiology of tissue fluid. *Proceedings of the Royal Society of Medicine, 65*(8), 715-719.

Campanile, M., D'Alessandro, P., Della Corte, L., Saccone, G., Tagliaferri, S., Arduino, B., ...Berghella, V. (2020). Intrapartum cardiotocography with and without computer analysis: a systematic review and meta-analysis of randomized controlled trials. *J Matern Fetal Neonatal Med, 33*(13), 2284-2290. doi:10.1080/14767058.2018.1542676

Carminati, L. (2018). Generalizability in Qualitative Research: A Tale of Two Traditions. *Qualitative Health Research, 28*(13), 2094-2101. doi:10.1177/1049732318788379

Castillo-Melendez, M., Baburamani, A. A., Cabalag, C., Yawno, T., Witjaksono, A., Miller, S. L., & Walker, D. W. (2013). Experimental Modelling of the Consequences of Brief Late Gestation Asphyxia on Newborn Lamb Behaviour and Brain Structure. *PLOS ONE, 8*(11), e77377. doi:10.1371/journal.pone.0077377

Cengiz, E., & Tamborlane, W. V. (2009). A tale of two compartments: interstitial versus blood glucose monitoring. *Diabetes Technol Ther, 11 Suppl 1*, S11-16.

doi:10.1089/dia.2009.0002

Chakkarapani, E., & Thoresen, M. (2015). The Newborn Pig Global Hypoxic-Ischemic Model of Perinatal Brain and Organ Injury. In J. Y. Yager (Ed.), *Animal Models of Neurodevelopmental Disorders* (pp. 171-189). New York, NY: Springer New York.

Chandraharan, E. (2017). Applying Fetal Physiology to Interpret CTG Traces: Predicting the NEXT Change. In E. Chandraharan (Ed.), *Handbook of CTG Interpretation: From Patterns to Physiology* (pp. 32-40). Cambridge: Cambridge University Press.

Chandraharan, E., & Wiberg, N. (2014). Fetal scalp blood sampling during labor: an appraisal of the physiological basis and scientific evidence. *Acta Obstet Gynecol Scand, 93*(6), 544-547. doi:10.1111/aogs.12416

Chandraharan, E. (2014). Fetal scalp blood sampling during labour: is it a useful diagnostic test or a historical test that no longer has a place in modern clinical obstetrics? *BJOG, 121*(9), 1056-1062. doi:10.1111/1471-0528.12614

Chandraharan, E., Lowe, V., Ugwumadu, A., & Arulkumaran, S. (2013). Impact of Fetal ECG (STAN) and competency based training on intrapartum interventions and perinatal outcomes at a Teaching Hospital in London: 5 Year Analysis. *BJOG, 120*, 428-429.

Chatterjee, M. S., Hetzel, F., & Kaminetzky, H. A. (1984). Fetal tissue pH-continuous intrapartum monitoring. *International Journal of Gynecology & Obstetrics, 22*(1), 41-46. doi.org/10.1016/0020-7292(84)90102-4

Cheung, P.-Y., Gill, R. S., & Bigam, D. L. (2011). A Swine Model of Neonatal Asphyxia. *Journal of Visualized Experiments*(56). doi:10.3791/3166

Ching, C. T. S., & Connolly, P. (2008). Reverse iontophoresis: A non-invasive technique for measuring blood lactate level. *Sensors and Actuators B: Chemical, 129*(1), 352-358. doi.org/10.1016/j.snb.2007.08.031

Chou, Y. H., Tsou Yau, K. I., & Wang, P. J. (1998). Clinical application of the measurement of cord plasma lactate and pyruvate in the assessment of high-risk neonates. *Acta Paediatr, 87*(7), 764-768. doi:10.1080/080352598750013851

Chuang, C. K., Wang, T. J., Yeung, C. Y., Hsieh, W. S., Lin, D. S., Ho, S. C., & Lin, S. P. (2006). Interference and blood sample preparation for a pyruvate enzymatic assay. *Clin Biochem, 39*(1), 74-77. doi:10.1016/j.clinbiochem.2005.10.007

Clark, S. L., Hamilton, E. F., Garite, T. J., Timmins, A., Warrick, P. A., & Smith, S. (2017). The limits of electronic fetal heart rate monitoring in the prevention of neonatal metabolic acidemia. *American Journal of Obstetrics and Gynecology, 216*(2), 163.e161-163.e166. doi.org/10.1016/j.ajog.2016.10.009

Clark, S. L., & Paul, R. H. (1985). Intrapartum fetal surveillance: The role of fetal scalp blood sampling. *American Journal of Obstetrics and Gynecology, 153*(7), 717-720. doi.org/10.1016/0002-9378(85)90330-8

Clough, G. F., Boutsiouki, P., Church, M. K., & Michel, C. C. (2002). Effects of blood flow on the in vivo recovery of a small diffusible molecule by microdialysis in human skin. *J Pharmacol Exp Ther, 302*(2), 681-686. doi:10.1124/jpet.102.035634

Cobelli, C., Schiavon, M., Dalla Man, C., Basu, A., & Basu, R. (2016). Interstitial Fluid Glucose Is Not Just a Shifted-in-Time but a Distorted Mirror of Blood Glucose: Insight from an In Silico Study. *Diabetes Technol Ther, 18*(8), 505-511. doi:10.1089/dia.2016.0112

Collins, J.-A., Rudenski, A., Gibson, J., Howard, L., & O'Driscoll, R. (2015). Relating oxygen partial pressure, saturation and content: the haemoglobin–oxygen dissociation curve. *Breathe, 11*(3), 194-201. doi:10.1183/20734735.001415

Confidential Enquiry into Stillbirths and Deaths in Infancy. (1993). *Confidential Enquiry into Stillbirths and Deaths in Infancy: Intrapartum deaths.* London: CESDI.

Counsilman, C. E., Heeger, L. E., Tan, R., Bekker, V., Zwaginga, J. J., Te Pas, A. B., & Lopriore, E. (2021). Iatrogenic blood loss in extreme preterm infants due to frequent laboratory tests and procedures. *J Matern Fetal Neonatal Med*, *34*(16), 2660–2665. doi:10.1080/14767058.2019.1670800

Cummins, G., Kremer, J., Bernassau, A., Brown, A., Bridle, H. L., Schulze, H., ... Desmulliez, M. P. Y. (2018). Sensors for Fetal Hypoxia and Metabolic Acidosis: A Review. *Sensors (Basel), 18*(8). doi:10.3390/s18082648

Davies, M. I., Cooper, J. D., Desmond, S. S., Lunte, C. E., & Lunte, S. M. (2000). Analytical considerations for microdialysis sampling. *Adv Drug Deliv Rev, 45*(2-3), 169-188.

de Boer, J., Plijter-Groendijk, H., Visser, K. R., Mook, G. A., & Korf, J. (1994). Continuous monitoring of lactate during exercise in humans using subcutaneous and transcutaneous microdialysis. *Eur J Appl Physiol Occup Physiol, 69*(4), 281-286.

de Lange, E. C. M. (2013). Recovery and Calibration Techniques: Toward Quantitative Microdialysis. In M. Müller (Ed.), *Microdialysis in Drug Development* (pp. 13-33). New York, NY: Springer New York.

de Viragh, P. A., & Meuli, M. (1995). Human scalp hair follicle development from birth to adulthood: statistical study with special regard to putative stem cells in the bulge and proliferating cells in the matrix. *Arch Dermatol Res, 287*(3-4), 279-

284. doi:10.1007/bf01105079

Debeer, S., Le Luduec, J. B., Kaiserlian, D., Laurent, P., Nicolas, J. F., Dubois, B., & Kanitakis, J. (2013). Comparative histology and immunohistochemistry of porcine versus human skin. *Eur J Dermatol, 23*(4), 456-466. doi:10.1684/ejd.2013.2060

Department of Health. (2017). *Safer Maternity Care: The National Maternity Safety Strategy – Progress and Next steps.* London: Department of Health

Department of Health and Social Care. (2020). *Ockenden Report: Emerging Findings and Recommendations from the Independent Review of Maternity Services at the Shrewsbury and Telford Hospital NHS Trust*. DHSC

Derks, J. B., Oudijk, M. A., Torrance, H. L., Rademaker, C. M. A., Benders, M. J., Rosen, K. G., ... Giussani, D. A. (2010). Allopurinol Reduces Oxidative Stress in the Ovine Fetal Cardiovascular System After Repeated Episodes of Ischemia-Reperfusion. *Pediatric Research, 68*(5), 374-380. doi:10.1203/PDR.0b013e3181ef7780

de-Souza, I. M. F., Vitral, G. L. N., & Reis, Z. S. N. (2019). Skin thickness dimensions in histological section measurement during late-fetal and neonatal developmental period: A systematic review. *Skin Research and Technology, 25*(6), 793-800. doi.org/10.1111/srt.12719

Diehl, K.-H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., ... Vorstenbosch, C. V. D. (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of applied toxicology, 21*(1), 15-23. doi:10.1002/jat.727

Dimopoulou, I., Nikitas, N., Orfanos, S. E., Theodorakopoulou, M., Vassiliadi, D., Ilias, I., ... Ungerstedt, U. (2011). Kinetics of adipose tissue microdialysis-derived metabolites in critically ill septic patients: associations with sepsis severity and clinical outcome. *Shock, 35*(4), 343-348. doi:10.1097/SHK.0b013e318206aafa

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Hum Dev, 3*(1), 79-83. doi:10.1016/0378-3782(79)90022-7

Domoki, F., Zimmermann, A., Cserni, G., Bori, R., Temesvári, P., & Bari, F. (2006). Reventilation with room air or 100% oxygen after asphyxia differentially affects cerebral neuropathology in newborn pigs. *Acta Paediatrica, 95*(9), 1109-1115. doi.org/10.1080/08035250600717139

Doria, V., Papageorghiou, A. T., Gustafsson, A., Ugwumadu, A., Farrer, K., & Arulkumaran, S. (2007). Review of the first 1502 cases of ECG-ST waveform analysis during labour in a teaching hospital. *BJOG, 114*(10), 1202-1207. doi:10.1111/j.1471-0528.2007.01480.x

Drabble, L., Trocki, K. F., Salcedo, B., Walker, P. C., & Korcha, R. A. (2016). Conducting qualitative interviews by telephone: Lessons learned from a study of alcohol use among sexual minority and heterosexual women. *Qualitative social work : QSW : research and practice, 15*(1), 118-133. doi:10.1177/1473325015585613

Draper, E. S., Kurinczuk, J. J., & Kenyon, S. (Eds) on behalf of MBRRACE-UK (2017). *MBRRACE-UK 2017 Perinatal Confidential Enquiry: Term, singleton, intrapartum stillbirth and intrapartum-related neonatal death*. Oxford: National Perinatal Epidemiology Unit. Retrieved from https://www.npeu.ox.ac.uk/mbrrace-uk/reports/perinatal-mortality-and-morbidity-confidential-enquiries

Duburcq, T., Favory, R., Mathieu, D., Hubert, T., Mangalaboyi, J., Gmyr, V., ... Jourdain, M. (2014). Hypertonic sodium lactate improves fluid balance and hemodynamics in porcine endotoxic shock. *Critical Care, 18*(4), 467. doi:10.1186/s13054-014-0467-3

Dunn, J.-O., Mythen, M., & Grocott, M. (2016). Physiology of oxygen transport. *BJA Education, 16*(10), 341-348. doi:10.1093/bjaed/mkw012

East, C. E., Davey, M.-A., Kamlin, C. O. F., Davis, P. G., Sheehan, P. M., Kane, S. C., ... Group, T. F. S. (2021). The addition of fetal scalp blood lactate measurement as an adjunct to cardiotocography to reduce caesarean sections during labour: The Flamingo randomised controlled trial. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 61*(5), 684-692. doi.org/10.1111/ajo.13327

East, C. E., Leader, L. R., Sheehan, P., Henshall, N. E., Colditz, P. B., & Lau, R. (2015). Intrapartum fetal scalp lactate sampling for fetal assessment in the presence of a non-reassuring fetal heart rate trace. *Cochrane Database of Systematic Reviews*(5). doi:10.1002/14651858.CD006174.pub3

East, C. E., Brennecke, S. P., King, J. F., Chan, F. Y., & Colditz, P. B. (2006). The effect of intrapartum fetal pulse oximetry, in the presence of a nonreassuring fetal heart rate pattern, on operative delivery rates: A multicenter, randomized, controlled trial (the FOREMOST trial). *American Journal of Obstetrics and Gynecology, 194*(3), 606.e601-606.e616. doi.org/10.1016/j.ajog.2005.08.051

East, C. E., Begg, L., Colditz, P. B., & Lau, R. (2014). Fetal pulse oximetry for fetal assessment in labour. *Cochrane Database of Systematic Reviews*(10). doi:10.1002/14651858.CD004075.pub4

Egeli, A. K., Framstad, T., & Morberg, H. (1998). Clinical Biochemistry, Haematology and Body Weight in Piglets. *Acta Veterinaria Scandinavica, 39*(3), 381-393. doi:10.1186/BF03547786

Eiby, Y. A., Wright, L. L., Kalanjati, V. P., Miller, S. M., Bjorkman, S. T., Keates, H. L., ... Lingwood, B. E. (2013). A Pig Model of the Preterm Neonate: Anthropometric

and Physiological Characteristics. *PLOS ONE, 8*(7), e68763. doi:10.1371/journal.pone.0068763

Ellmerer, M., Schaupp, L., Trajanoski, Z., Jobst, G., Moser, I., Urban, G., ... Wach, P. (1998). Continuous measurement of subcutaneous lactate concentration during exercise by combining open-flow microperfusion and thin-film lactate sensors. *Biosens Bioelectron, 13*(9), 1007-1013. doi:10.1016/s0956-5663(98)00002-5

Ellmerer, M., Haluzik, M., Blaha, J., Kremen, J., Svacina, S., Plasnik, A., ... Pieber, T. R. (2009). Clinical evaluation of subcutaneous lactate measurement in patients after major cardiac surgery. *International Journal of Endocrinology, 2009*, 390975. doi:10.1155/2009/390975

Engidawork, E., Chen, Y., Dell'Anna, E., Goiny, M., Lubec, G., Ungerstedt, U., ... Herrera-Marschitz, M. (1997). Effect of perinatal asphyxia on systemic and intracerebral pH and glycolysis metabolism in the rat. *Exp Neurol, 145*(2 Pt 1), 390-396. doi:10.1006/exnr.1997.6482

Engle, W. D., Laptook, A. R., & Perlman, J. M. (1999). Acute changes in arterial carbon dioxide tension and acid–base status and early neurologic characteristics in term infants following perinatal asphyxia. *Resuscitation, 42*(1), 11-17. doi.org/10.1016/S0300-9572(99)00081-7

Ennis, M., Clark, A., & Grudzinskas, J. G. (1991). Change in obstetric practice in response to fear of litigation in the British Isles. *The Lancet, 338*(8767), 616-618. doi:https://doi.org/10.1016/0140-6736(91)90616-W

Evans, L., Rhodes, A., Alhazzani, W., Antonelli, M., Coopersmith, C. M., French, C., ... Levy, M. (2021). Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. *Critical Care Medicine, 49*(11), e1063-e1143. doi:10.1097/ccm.0000000000005337

Faubert, B., Li, K. Y., Cai, L., Hensley, C. T., Kim, J., Zacharias, L. G., ... De Berardinis, R. J. (2017). Lactate Metabolism in Human Lung Tumors. *Cell, 171*(2), 358-371.e359. doi:10.1016/j.cell.2017.09.019

Fogh-Andersen, N., Altura, B. M., Altura, B. T., & Siggaard-Andersen, O. (1995). Composition of interstitial fluid. *Clinical Chemistry, 41*(10), 1522-1525. doi:10.1093/clinchem/41.10.1522

Friis-Hansen, B. (1961). Body water compartments in children: changes during growth and related changes in body composition. *Pediatrics, 28*(2), 169.

Fritz, K. I., Zubrow, A., Mishra, O. P., & Delivoria-Papadopoulos, M. (2005). Hypercapnia-induced modifications of neuronal function in the cerebral cortex of newborn piglets. *Pediatr Res, 57*(2), 299-304. doi:10.1203/01.Pdr.0000148718.47137.9b

Garberg, H. T., Huun, M. U., Escobar, J., Martinez-Orgado, J., Loberg, E. M., Solberg, R., & Didrik Saugstad, O. (2016). Short-term effects of cannabidiol after global hypoxia-ischemia in newborn piglets. *Pediatr Res, 80*(5), 710-718. doi:10.1038/pr.2016.149

Gardner, D. S., Fletcher, A. J. W., Bloomfield, M. R., Fowden, A. L., & Giussani, D. A. (2002). Effects of prevailing hypoxaemia, acidaemia or hypoglycaemia upon the cardiovascular, endocrine and metabolic responses to acute hypoxaemia in the ovine fetus. *J Physiol, 540*(Pt 1), 351-366. doi:10.1113/jphysiol.2001.013434

Garite, T. J., Dildy, G. A., McNamara, H., Nageotte, M. P., Boehm, F. H., Dellinger, E. H., ... Swedlow, D. B. (2000). A multicenter controlled trial of fetal pulse oximetry in the intrapartum management of nonreassuring fetal heart rate patterns. *Am J Obstet Gynecol, 183*(5), 1049-1058. doi.org/10.1067/mob.2000.110632

Georgieva, A., Moulden, M., & Redman, C. W. G. (2013). Umbilical cord gases in relation to the neonatal condition: the EveREst plot. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 168*(2), 155-160. doi.org/10.1016/j.ejogrb.2013.01.003

Georgieva, A., Abry, P., Chudáček, V., Djurić, P. M., Frasch, M. G., Kok, R., ... Vullings, R. (2019). Computer-based intrapartum fetal monitoring and beyond: A review of the 2nd Workshop on Signal Processing and Monitoring in Labor (October 2017, Oxford, UK). *Acta Obstet Gynecol Scand, 98*(9), 1207-1217. doi.org/10.1111/aogs.13639

German Society of Gynecology and Obstetrics, Maternal Fetal Medicine Study Group, German Society of Prenatal Medicine and Obstetrics, & German Society of Perinatal Medicine. (2014). S1-Guideline on the Use of CTG During Pregnancy and Labor: Long version - AWMF Registry No. 015/036. *Geburtshilfe und Frauenheilkunde, 74*(8), 721-732. doi:10.1055/s-0034-1382874

Gittelsohn, J., Shankar, A. V., West, K. P., Ram, R. M., & Gnywali, T. (1997). Estimating Reactivity in Direct Observation Studies of Health Behaviors. *Human Organization, 56*(2), 182-189.

Go, S., Kramer, T. T., Verhoeven, A. J., Oude Elferink, R. P. J., & Chang, J.-C. (2021). The extracellular lactate-to-pyruvate ratio modulates the sensitivity to oxidative stress-induced apoptosis via the cytosolic NADH/NAD+ redox state. *Apoptosis, 26*(1), 38-51. doi:10.1007/s10495-020-01648-8

Goodwin, M., Milner-Masterson, L., & Paul, R. (1994). Elimination of Fetal Scalp Blood Sampling on a Large Clinical Service. *Obstet Gynecol, 83*(6), 971.

Gorman, J. M., Battista, D., Goetz, R. R., Dillon, D. J., Liebowitz, M. R., Fyer, A. J., ... Klein, D. F. (1989). A comparison of sodium bicarbonate and sodium lactate infusion in the induction of panic attacks. *Archives of General Psychiatry, 46*,

145-150. doi:10.1001/archpsyc.1989.01810020047008

Goyert, G. L., Bottoms, S. F., Treadwell, M. C., & Nehra, P. C. (1989). The Physician Factor in Cesarean Birth Rates. *N Engl J Med, 320*(11), 706-709. doi:10.1056/nejm198903163201106

Gracia-Perez-Bonfils, A., & Chandraharan, E. (2017). Physiology of Fetal Heart Rate Control and Types of Intrapartum Hypoxia. In *Handbook of CTG Interpretation: From Patterns to Physiology* (pp. 13-25). Cambridge: Cambridge University Press.

Graham, E. M., Ruis, K. A., Hartman, A. L., Northington, F. J., & Fox, H. E. (2008). A systematic review of the role of intrapartum hypoxia-ischemia in the causation of neonatal encephalopathy. *Am J Obstet Gynecol, 199*(6), 587-595. doi.org/10.1016/j.ajog.2008.06.094

Graham, C. A., Leung, L. Y., Lo, R. S., Lee, K. H., Yeung, C. Y., Chan, S. Y., ... Hung, K. K. (2019). Agreement between capillary and venous lactate in emergency department patients: prospective observational study. *BMJ Open, 9*(4), e026109. doi:10.1136/bmjopen-2018-026109

Greenhalgh, T. (2005). *Diffusion of innovations in health service organisations : a systematic literature review*. Malden, MA: Blackwell.

Greenhouse, S. W., & Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika, 24*(2), 95-112. doi:10.1007/BF02289823

Gunn, A. J., Parer, J. T., Mallard, E. C., Williams, C. E., & Gluckman, P. D. (1992). Cerebral Histologic and Electrocorticographic Changes after Asphyxia in Fetal Sheep. *Pediatric Research, 31*(5), 486-491. doi:10.1203/00006450-199205000-00016

Hall, B., Wong, D. D., Rawlinson, W. D., Tracy, M. B., & Tracy, S. K. (2014). A validation study: assessing the reliability of the hand held StatStripXPress lactate meter to test lactate in amniotic fluid. *BMC Res Notes, 7*, 935. doi:10.1186/1756-0500-7-935

Hamdy, N., Eide, S., Sun, H.-S., & Feng, Z.-P. (2020). Animal models for neonatal brain injury induced by hypoxic ischemic conditions in rodents. *Experimental Neurology, 334*, 113457. doi.org/10.1016/j.expneurol.2020.113457

Hammarlund-Udenaes, M. (2017). Microdialysis as an Important Technique in Systems Pharmacology – a Historical and Methodological Review. *AAPS J*, *19*, 1294–1303. https://doi.org/10.1208/s12248-017-0108-2

Hannon, J. P., Bossone, C. A., & Wade, C. E. (1990). Normal physiological values for conscious pigs used in biomedical research. *Lab Anim Sci, 40*(3), 293-298.

Hasenour, C. M., Rahim, M., & Young, J. D. (2020). In Vivo Estimates of Liver Metabolic Flux Assessed by 13C-Propionate and 13C-Lactate Are Impacted by Tracer Recycling and Equilibrium Assumptions. *Cell Reports, 32*(5), 107986. doi.org/10.1016/j.celrep.2020.107986

Hatherill, M., McIntyre, A. G., Wattie, M., & Murdoch, I. A. (2000). Early hyperlactataemia in critically ill children. *Intensive Care Med, 26*(3), 314-318. doi:10.1007/s001340051155

Haverkamp, A. D., Orleans, M., Langendoerfer, S., McFee, J., Murphy, J., & Thompson, H. E. (1979). A controlled trial of the differential effects of intrapartum fetal monitoring. *Am J Obstet Gynecol, 134*(4), 399-412. doi:10.1016/S0002-9378(16)33082-4

Healthcare Safety Investigation Branch. (2020). *Delays to intrapartum intervention once fetal compromise is suspected: Independent report by the Healthcare Safety Investigation Branch 2019-2020*. London: HSIB. Retrieved from https://www.hsib.org.uk/investigations-and-reports/delays-to-intrapartum-intervention-once-fetal-compromise-is-suspected

Heazell, A. E. P., Riches, J., Hopkins, L., & Myers, J. E. (2011). Fetal blood sampling in early labour: is there an increased risk of operative delivery and fetal morbidity? *BJOG, 118*(7), 849-855. doi:10.1111/j.1471-0528.2011.02922.x

Heinis, A., van Dillen, J., Oosting, J., Rhose, S., Vandenbussche, F., & Van Drongelen, J. (2017). Clinical evaluation of Statstrip® Lactate for use in fetal scalp blood sampling. *Acta Obstet Gynecol Scand, 96*(3), 334-341. doi:10.1111/aogs.13078

Heltne, J. K., Husby, P., Koller, M.-E., & Lund, T. (1998). Sampling of interstitial fluid and measurement of colloid osmotic pressure in pigs: evaluation of the wick method. *Laboratory Animals, 32*(4), 439-445. doi:10.1258/002367798780599848

Herpin, P., Le Dividich, J., Hulin, J. C., Fillaut, M., De Marco, F., & Bertin, R. (1996). Effects of the level of asphyxia during delivery on viability at birth and early postnatal vitality of newborn pigs. *J Anim Sci, 74*(9), 2067-2075. doi:10.2527/1996.7492067x

Herrero, P., Dence, C. S., Coggan, A. R., Kisrieva-Ware, Z., Eisenbeis, P., & Gropler, R. J. (2007). L-3-11C-Lactate as a PET Tracer of Myocardial Lactate Metabolism: A Feasibility Study. *Journal of Nuclear Medicine, 48*(12), 2046-2055. doi:10.2967/jnumed.107.044503

Himmelmann, K., & Uvebrant, P. (2018). The panorama of cerebral palsy in Sweden part XII shows that patterns changed in the birth years 2007–2010. *Acta Paediatrica, 107*(3), 462-468. doi:10.1111/apa.14147

Hindley, C., & Thomson, A. M. (2007). Intrapartum fetal monitoring and the spectre of litigation: A qualitative study of midwives' views. *Clinical Governance: An International Journal, 12*(4), 233-243. doi:10.1108/14777270710828900

Hofmeyer, A. T., & Scott, C. M. (2007). Moral Geography of Focus Groups with Participants Who Have Pre-existing Relationships in the Workplace. *International Journal of Qualitative Methods, 6*(2), 69-79. doi:10.1177/160940690700600207

Hollnagel, E., Wears, R. L., & Braithwaite, J. (2015). *From Safety-I to Safety-II: A White Paper*. The Resilient Health Care Net: Published simultaneously by the University of Southern Denmark, University of Florida, USA, and Macquarie University, Australia. Retrieved from https://www.england.nhs.uk/signuptosafety/wp-content/uploads/sites/16/2015/10/safety-1-safety-2-whte-papr.pdf

Holmes, F. L. (1986). Claude Bernard, The "Milieu Intérieur", and Regulatory Physiology. *History and Philosophy of the Life Sciences, 8*(1), 3-25.

Holmes, D. T., & Buhr, K. A. (2007). Error propagation in calculated ratios. *Clinical Biochemistry, 40*(9), 728-734. doi.org/10.1016/j.clinbiochem.2006.12.014

Holzinger, A., Bonfig, W., Kusser, B., Eggermann, T., Müller, H., & Munch, H. G. (2006). Use of long-term microdialysis subcutaneous glucose monitoring in the management of neonatal diabetes. A first case report. *Biol Neonate, 89*(2), 88-91. doi:10.1159/000088349

Holzmann, M., Wretler, S., Cnattingius, S., & Nordström, L. (2015). Neonatal outcome and delivery mode in labors with repetitive fetal scalp blood sampling. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 184*, 97-102. doi.org/10.1016/j.ejogrb.2014.11.012

Holzmann, M., & Nordström, L. (2010). Follow-up national survey (Sweden) of routines for intrapartum fetal surveillance. *Acta Obstet Gynecol Scand, 89*(5), 712-714. doi:10.3109/00016340903545009

Housseine, N., Punt, M. C., Browne, J. L., Meguid, T., Klipstein-Grobusch, K., Kwast, B. E., ... Rijken, M. J. (2018). Strategies for intrapartum foetal surveillance in low- and middle-income countries: A systematic review. *PLOS ONE, 13*(10), e0206295. doi:10.1371/journal.pone.0206295

Huang, L., Zhao, F., Qu, Y., Zhang, L., Wang, Y., & Mu, D. (2017). Animal models of hypoxic-ischemic encephalopathy: optimal choices for the best outcomes. *Rev Neurosci, 28*(1), 31-43. doi:10.1515/revneuro-2016-0022

Hutchinson, P. J., Jalloh, I., Helmy, A., Carpenter, K. L., Rostami, E., Bellander, B. M., ... Ungerstedt, U. (2015). Consensus statement from the 2014 International Microdialysis Forum. *Intensive Care Med, 41*(9), 1517-1528.

doi:10.1007/s00134-015-3930-y

Ignatov, P. N., & Lutomski, J. E. (2016). Quantitative cardiotocography to improve fetal assessment during labor: a preliminary randomized controlled trial. *Eur J Obstet Gynecol Reprod Biol, 205*, 91-97. doi:10.1016/j.ejogrb.2016.08.023

Ilias, I., Apollonatou, S., Vassiliadi, D.-A., Nikitas, N., Theodorakopoulou, M., Diamantakis, A., ... Dimopoulou, I. (2018). Adipose tissue lactate clearance but not blood lactate clearance is associated with clinical outcome in sepsis or septic shock during the post-resuscitation period. *Metabolites, 8*(2), 28. doi:10.3390/metabo8020028

Inder, T., Neil, J., Yoder, B., & Rees, S. (2004). Non-human primate models of neonatal brain injury. *Seminars in Perinatology, 28*(6), 396-404. doi.org/10.1053/j.semperi.2004.10.002

INFANT Collaborative Group. (2017). Computerised interpretation of fetal heart rate during labour (INFANT): a randomised controlled trial. *Lancet, 389*(10080), 1719-1729. doi:10.1016/s0140-6736(17)30568-8

Physiological CTG Interpretation (2018). *Intrapartum Fetal Monitoring Guidelines*. Retrieved from https://physiological-ctg.com/guideline.html:

Iorizzo, L., Klausen, T. W., Wiberg-Itzel, E., Ovin, F., & Wiberg, N. (2019a). Use of Lactate ProTM2 for measurement of fetal scalp blood lactate during labor – proposing new cutoffs for normality, preacidemia and acidemia: a cross-sectional study. *J Matern Fetal Neonatal Med, 32*(11), 1762-1768. doi:10.1080/14767058.2017.1416603

Iorizzo, L., Persson, K. E. M., Kristensen, K. H., & Wiberg, N. (2019b). Reliability of the point-of care analyzer "StatStrip® Xpress™" for measurement of fetal blood lactate. *Clin Chim Acta, 495*, 88-93. doi:10.1016/j.cca.2019.04.003

Jakacka, N., Snarski, E., & Mekuria, S. (2016) Prevention of Iatrogenic Anemia in Critical and Neonatal Care. *Adv Clin Exp Med, 25*(1), 191-197. doi:10.17219/acem/32065

Jansson, P. A., Smith, U., & Lönnroth, P. (1990). Evidence for lactate production by human adipose tissue in vivo. *Diabetologia, 33*(4), 253-256. doi:10.1007/BF00404805

Jansson, P. A., Krogstad, A. L., & Lönnroth, P. (1996). Microdialysis measurements in skin: evidence for significant lactate release in healthy humans. *American Journal of Physiology - Endocrinology And Metabolism, 271*(1), 138-142. doi:10.1152/ajpendo.1996.271.1.e138

Jin, G., Cheng, Q., Feng, J., & Li, F. (2008). On-Line Microdialysis Coupled to Analytical Systems. *Journal of Chromatographic Science, 46*(3), 276-287.

doi:10.1093/chromsci/46.3.276

Johnson, P. D., & Besselsen, D. G. (2002). Practical Aspects of Experimental Design in Animal Research. *ILAR Journal, 43*(4), 202-206. doi:10.1093/ilar.43.4.202

Jonsson, M., Nordén-Lindeberg, S., Ostlund, I., & Hanson, U. (2009). Metabolic acidosis at birth and suboptimal care: illustration of the gap between knowledge and clinical practice. *BJOG, 116*(11), 1453-1460. doi:10.1111/j.1471-0528.2009.02269.x

Jørgensen, J. S., & Weber, T. (2014). Fetal scalp blood sampling in labor – a review. *Acta Obstet Gynecol Scand, 93*(6), 548-555. doi:10.1111/aogs.12421

Kaasen, A., Aanstad, K. J., Pay, A. S. D., Økland, I., & Blix, E. (2019). National survey of routines for intrapartum fetal monitoring in Norway. *Acta Obstet Gynecol Scand, 98*(3), 390-395. doi:10.1111/aogs.13500

Kastellorizios, M., & Burgess, D. J. (2015). Continuous metabolic monitoring based on multi-analyte biomarkers to predict exhaustion. *Sci Rep, 5*, 10603. doi:10.1038/srep10603

Kawakita, T., Reddy, U. M., Landy, H. J., Iqbal, S. N., Huang, C. C., & Grantz, K. L. (2016). Neonatal complications associated with use of fetal scalp electrode: a retrospective study. *BJOG, 123*(11), 1797-1803. doi:10.1111/1471-0528.13817

Kelly, S., Redmond, P., King, S., Oliver-Williams, C., Lamé, G., Liberati, E., ... Burt, J. (2021). Training in the use of intrapartum electronic fetal monitoring with cardiotocography: systematic review and meta-analysis. *BJOG, 128*(9), 1408-1419. doi.org/10.1111/1471-0528.16619

King, N., & Brooks, J. M. (2017). Template Analysis for Business and Management Students. London: SAGE Publications Ltd. Retrieved from https://dx.doi.org/10.4135/9781473983304

King's Fund. (2008). *Safe Births: Everybody's Business - An independent inquiry into the safety of maternity services in England*. London: King's Fund. Retrieved from https://www.kingsfund.org.uk/projects/safer-births

Kitlinski, M. L. M. D., Kallen, K. P., Marsal, K. M. D. P., & Olofsson, P. M. D. P. (2003). Gestational Age-Dependent Reference Values for pH in Umbilical Cord Arterial Blood at Term. *Obstet Gynecol, 102*(2), 338-345.

Klaus, S., Heringlake, M., Gliemroth, J., Pagel, H., Staubach, K., & Bahlmann, L. (2003). Biochemical tissue monitoring during hypoxia and reoxygenation. *Resuscitation, 56*(3), 299-305. doi:10.1016/S0300-9572(02)00342-8

Koehler, R. C., Yang, Z. J., Lee, J. K., & Martin, L. J. (2018). Perinatal hypoxic-ischemic brain injury in large animal models: Relevance to human neonatal

encephalopathy. *J Cereb Blood Flow Metab, 38*(12), 2092-2111. doi:10.1177/0271678x18797328

Kopterides, P., Theodorakopoulou, M., Ilias, I., Nikitas, N., Frantzeskaki, F., Vassiliadi, D. A., ... Dimopoulou, I. (2012). Interrelationship between blood and tissue lactate in a general intensive care unit: a subcutaneous adipose tissue microdialysis study on 162 critically ill patients. *J Crit Care, 27*(6), 742.e749-718. doi:10.1016/j.jcrc.2012.08.003

Kraut, J. A., & Madias, N. E. (2014). Lactic Acidosis. *N Engl J Med, 371*(24), 2309-2319. doi:10.1056/NEJMra1309483

Krogstad, A. L., Jansson, P. A., Gissien, P., & Lönnroth, P. (1996). Microdialysis methodology for the measurement of dermal interstitial fluid in humans. *British Journal of Dermatology (1951), 134*(6), 1005-1012. doi:10.1046/j.1365-2133.1996.d01-893.x

Kruger, K., Kublickas, M., & Westgren, M. (1998). Lactate in scalp and cord Blood from fetuses with ominous fetal heart rate patterns. *Obstet Gynecol, 92*(6), 918-922. doi.org/10.1016/s0029-7844(98)00347-0

Kruger, K., Hallberg, B., Blennow, M., Kublickas, M., & Westgren, M. (1999). Predictive value of fetal scalp blood lactate concentration and pH as markers of neurologic disability. *Am J Obstet Gynecol, 181*(5), 1072-1078. doi.org/10.1016/S0002-9378(99)70083-9

Kulcu, E., Tamada, J. A., Reach, G., Potts, R. O., & Lesho, M. J. (2003). Physiological Differences Between Interstitial Glucose and Blood Glucose Measured in Human Subjects. *Diabetes Care, 26*(8), 2405-2409. doi:10.2337/diacare.26.8.2405

Kurinczuk, J. J., White-Koning, M., & Badawi, N. (2010). Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. *Early Human Development, 86*(6), 329-338. doi.org/10.1016/j.earlhumdev.2010.05.010

Kusaka, T., Matsuura, S., Fujikawa, Y., Okubo, K., Kawada, K., Namba, M., ... Itoh, S. (2004). Relationship between cerebral interstitial levels of amino acids and phosphorylation potential during secondary energy failure in hypoxic-ischemic newborn piglets. *Pediatr Res, 55*(2), 273-279. doi:10.1203/01.Pdr.0000102702.39608.82

Kyng, K. J., Skajaa, T., Kerrn-Jespersen, S., Andreassen, C. S., Bennedsgaard, K., & Henriksen, T. B. (2015). A Piglet Model of Neonatal Hypoxic-Ischemic Encephalopathy. *J Vis Exp*(99), e52454. doi:10.3791/52454

Lame, G., Liberati, E., Burt, J., Draycott, T., Winter, C., Ward, J., & Dixon-Woods, M. (2019). IMproving the practice of intrapartum electronic fetal heart rate

MOnitoring with cardiotocography for safer childbirth (the IMMO programme): protocol for a qualitative study. *BMJ Open, 9*(6), e030271. doi:10.1136/bmjopen-2019-030271

Landman, A., Immink-Duijker, S. T., Mulder, E. J. H., Koster, M. P. H., Xodo, S., Visser, G. H. A., ... Kwee, A. (2019). Significant reduction in umbilical artery metabolic acidosis after implementation of intrapartum ST waveform analysis of the fetal electrocardiogram. *Am J Obstet Gynecol, 221*(1), 63.e61-63.e13. doi:10.1016/j.ajog.2019.02.049

Langhan, M. L., Riera, A., Kurtz, J. C., Schaeffer, P., & Asnes, A. G. (2015). Implementation of newly adopted technology in acute care settings: a qualitative analysis of clinical staff. *Journal of Medical Engineering & Technology, 39*(1), 44-53. doi:10.3109/03091902.2014.973618

Lawton, J., Kirkham, J., Rankin, D., White, D. A., Elliott, J., Jaap, A., ... Heller, S., on behalf of the REPOSE group. (2016). Who gains clinical benefit from using insulin pump therapy? A qualitative study of the perceptions and views of health professionals involved in the Relative Effectiveness of Pumps over MDI and Structured Education (REPOSE) trial. *Diabetic Medicine, 33*(2), 243-251. doi.org/10.1111/dme.12879

Lawton, J., Kimbell, B., Rankin, D., Ashcroft, N. L., Varghese, L., Allen, J. M., ... Hovorka, R., on behalf of the CLOuD Consortium (2020). Health professionals' views about who would benefit from using a closed-loop system: a qualitative study. *Diabetic Medicine, 37*(6), 1030-1037. doi.org/10.1111/dme.14252

Lear, C. A., Wassink, G., Westgate, J. A., Nijhuis, J. G., Ugwumadu, A., Galinsky, R., ... Gunn, A. J. (2018). The peripheral chemoreflex: indefatigable guardian of fetal physiological adaptation to labour. *J Physiol, 596*(23), 5611-5623. doi:10.1113/JP274937

Lee, A. C. C., Kozuki, N., Blencowe, H., Vos, T., Bahalim, A., Darmstadt, G. L., ... Lawn, J. E. (2013). Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. *Pediatric Research, 74*(1), 50-72. doi:10.1038/pr.2013.206

Leegsma-Vogt, G., Janle, E., Ash, S. R., Venema, K., & Korf, J. (2003). Utilization of in vivo ultrafiltration in biomedical research and clinical applications. *Life Sciences, 73*(16), 2005-2018. doi.org/10.1016/S0024-3205(03)00569-1

Levick, J. R., & Michel, C. C. (2010). Microvascular fluid exchange and the revised Starling principle. *Cardiovascular Research, 87*(2), 198-210. doi:10.1093/cvr/cvq062

Liberati, E. G., Tarrant, C., Willars, J., Draycott, T., Winter, C., Kuberska, K., ... Dixon-Woods, M. (2021). Seven features of safety in maternity units: a framework

based on multisite ethnography and stakeholder consultation. *BMJ Quality & Safety, 30*(6), 444-456. doi:10.1136/bmjqs-2020-010988

Liberati, E. G., Tarrant, C., Willars, J., Draycott, T., Winter, C., Chew, S., & Dixon-Woods, M. (2019). How to be a very safe maternity unit: An ethnographic study. *Soc Sci Med, 223*, 64–72. doi.org/10.1016/j.socscimed.2019.01.035

Liljeström, L., Wikström, A.-K., Skalkidou, A., Åkerud, H., & Jonsson, M. (2014). Experience of fetal scalp blood sampling during labor. *Acta Obstet Gynecol Scand, 93*(1), 113-117. doi:10.1111/aogs.12271

Lincoln, Y. S., & Guba, E. G. (1985). *Naturalistic inquiry*. London: Sage Publications Ltd.

Lindecrantz, K. G., Lilja, H., Widmark, C., & Rosén, K. G. (1988). Fetal ECG during labour: a suggested standard. *J Biomed Eng, 10*(4), 351-353. doi:10.1016/0141-5425(88)90067-2

Linderkamp, O., Betke, K., Güntner, M., Jap, G. H., Riegel, K. P., & Walser, K. (1981). Blood volume in newborn piglets: effects of time of natural cord rupture, intra-uterine growth retardation, asphyxia, and prostaglandin-induced prematurity. *Pediatr Res, 15*(1), 53-57. doi:10.1203/00006450-198101000-00013

Liston, R., Sawchuck, D., & Young, D. (2018). No. 197b-Fetal Health Surveillance: Intrapartum Consensus Guideline. *Journal of Obstetrics and Gynaecology Canada, 40*(4), e298-e322. doi:10.1016/j.jogc.2018.02.011

Lopes-Pereira, J., Costa, A., Ayres-De-Campos, D., Costa-Santos, C., Amaral, J., & Bernardes, J. (2019). Computerized analysis of cardiotocograms and ST signals is associated with significant reductions in hypoxic-ischemic encephalopathy and cesarean delivery: an observational study in 38,466 deliveries. *Am J Obstet Gynecol, 220*(3), 269.e261-269.e268. doi:10.1016/j.ajog.2018.12.037

Low, J. A., Lindsay, B. G., & Derrick, E. J. (1997). Threshold of metabolic acidosis associated with newborn complications. *Am J Obstet Gynecol, 177*(6), 1391-1394. doi.org/10.1016/S0002-9378(97)70080-2

Lowe, B., & Beckmann, M. (2016). Involving the consultant before fetal blood sampling. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 56*(4), 387-390. doi:10.1111/ajo.12480

Magro, M. (2017). *Five years of cerebral palsy claims: A thematic review of NHS Resolution data*. London: NHS Resolution. Retrieved from https://resolution.nhs.uk/resources/five-years-of-cerebral-palsy-claims/

Mahendru, A. A., & Lees, C. C. (2011). Is intrapartum fetal blood sampling a gold standard diagnostic tool for fetal distress? *European Journal of Obstetrics & Gynecology and Reproductive Biology, 156*(2), 137-139.

doi.org/10.1016/j.ejogrb.2010.12.044

Mallonee, S., Fowler, C., & Istre, G. R. (2006). Bridging the gap between research and practice: a continuing challenge. *Inj Prev, 12*(6), 357-359. doi:10.1136/ip.2006.014159

Martin, L. J., Brambrink, A., Koehler, R. C., & Traystman, R. J. (1997). Primary sensory and forebrain motor systems in the newborn brain are preferentially damaged by hypoxia-ischemia. *J Comp Neurol, 377*(2), 262-285.

Martin, G. P., Kocman, D., Stephens, T., Peden, C. J., & Pearse, R. M. (2017). Pathways to professionalism? Quality improvement, care pathways, and the interplay of standardisation and clinical autonomy. *Sociol Health Illn, 39*(8), 1314-1329. doi:10.1111/1467-9566.12585

Martinez, A., Chiolero, R., Bollman, M., Revelly, J.-P., Berger, M., Cayeux, C., & Tappy, L. (2003). Assessment of adipose tissue metabolism by means of subcutaneous microdialysis in patients with sepsis or circulatory failure: Adipose tissue metabolism in shock. *Clinical physiology and functional imaging, 23*(5), 286-292. doi:10.1046/j.1475-097X.2003.00512.x

Martínez-Rodríguez, R., Mota-Rojas, D., Trujillo-Ortega, M. E., Orozco-Gregorio, H., Hernández-González, R., Roldan-Santiago, P., ... Ramírez-Necoechea, R. (2011). Physiological response to hypoxia in piglets of different birth weight. *Italian Journal of Animal Science, 10*(4), e56. doi:10.4081/ijas.2011.e56

Martini, W. Z., Cortez, D. S., Dubick, M. A., Park, M. S., & Holcomb, J. B. (2008). Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *J Trauma, 65*(3), 535-543. doi:10.1097/TA.0b013e31818379a6

Mason, J. (2002). *Qualitative Researching* (Second Ed. ed.). London: SAGE Publications Ltd.

Maxwell, S. E., & Delaney, H. D. (2004). *Designing experiments and analyzing data: A model comparison perspective, 2nd ed*. Mahwah, NJ: Lawrence Erlbaum Associates Publishers.

Mayes, M. E., Wilkinson, C., Kuah, S., Matthews, G., & Turnbull, D. (2018). Change in practice: a qualitative exploration of midwives' and doctors' views about the introduction of STan monitoring in an Australian hospital. *BMC Health Services Research, 18*. doi.org/10.1186/s12913-018-2920-5

McCormack, J. P., & Holmes, D. T. (2020). Your results may vary: the imprecision of medical measurements. *BMJ, 368*, m149. doi:10.1136/bmj.m149

McCormick, C., Heath, D., & Connolly, P. (2012). Towards blood free measurement of

glucose and potassium in humans using reverse iontophoresis. *Sensors and Actuators B: Chemical, 166-167*, 593-600. doi.org/10.1016/j.snb.2012.03.016

McKevitt, S., Gillen, P., & Sinclair, M. (2011). Midwives' and doctors' attitudes towards the use of the cardiotocograph machine. *Midwifery, 27*(6), e279-285. doi:10.1016/j.midw.2010.11.003

Miller, P. R., Taylor, R. M., Tran, B. Q., Boyd, G., Glaros, T., Chavez, V. H., ... Polsky, R. (2018). Extraction and biomolecular analysis of dermal interstitial fluid collected with hollow microneedles. *Communications Biology, 1*(1), 173. doi:10.1038/s42003-018-0170-z

Miller, P. R., Skoog, S. A., Edwards, T. L., Lopez, D. M., Wheeler, D. R., Arango, D. C., ... Narayan, R. J. (2012). Multiplexed microneedle-based biosensor array for characterization of metabolic acidosis. *Talanta, 88*, 739-742. doi:10.1016/j.talanta.2011.11.046

Miller, C. (1995). In-depth interviewing by telephone: Some practical considerations. *Evaluation & Research in Education, 9*(1), 29-38. doi:10.1080/09500799509533370

Ming, D. K., Jangam, S., Gowers, S. A. N., Wilson, R., Freeman, D. M. E., Boutelle, M. G., ... Holmes, A. H. (2022). Real-time continuous measurement of lactate through a minimally invasive microneedle patch: a phase I clinical study. *BMJ Innovations, 8*(2), 87-94. doi:10.1136/bmjinnov-2021-000864

Mokarami, P., Wiberg, N., & Olofsson, P. (2012). An overlooked aspect on metabolic acidosis at birth: blood gas analyzers calculate base deficit differently. *Acta Obstet Gynecol Scand, 91*(5), 574-579. doi.org/10.1111/j.1600-0412.2011.01364.x

Munro, C. L., & Savel, R. H. (2016). Narrowing the 17-Year Research to Practice Gap. *American Journal of Critical Care, 25*(3), 194-196. doi:10.4037/ajcc2016449

Murphy, K. W., & Macdonald, D. (1990). Fetal blood sampling in Dublin. A year's review. *Journal of Obstetrics and Gynaecology, 10*(3), 194-198. doi:10.3109/01443619009151156

Nandi, P., & Lunte, S. M. (2009). Recent trends in microdialysis sampling integrated with conventional and microanalytical systems for monitoring biological events: a review. *Anal Chim Acta, 651*(1), 1-14. doi:10.1016/j.aca.2009.07.064

National Institute for Health and Care Excellence. (2017). *NICE Clinical Guideline 190. Intrapartum Care: Care of healthy women and their babies during childbirth*. London: NICE. Retrieved from https://www.nice.org.uk/guidance/cg190

Nederlandse Vereniging voor Obstetrie en Gynaecologie. (2019). Richtlijn - Foetale bewaking [Fetal monitoring during labour]. Version 3.0 [[Dutch]]. Retrieved from

https://www.nvog.nl

Neilson, J. P. (2015). Fetal electrocardiogram (ECG) for fetal monitoring during labour. *Cochrane Database of Systematic Reviews*(12). doi:10.1002/14651858.CD000116.pub5

Nelson, K. B., & Leviton, A. (1991). How Much of Neonatal Encephalopathy is due to Birth Asphyxia? *American Journal of Diseases of Children, 145*(11), 1325-1331. doi:10.1001/archpedi.1991.02160110117034

NHS England. (2019). *Saving Babies' Lives Care Bundle Version 2: A care bundle for reducing perinatal mortality*. Leeds: Maternity Transformation Programme. Retrieved from https://www.england.nhs.uk/wp-content/uploads/2019/03/Saving-Babies-Lives-Care-Bundle-Version-Two-Updated-Final-Version.pdf

NHS Litigation Authority. (2012). *Ten Years of Maternity Claims: An Analysis of NHS Litigation Authority Data*. London: NHS Litigation Authority. Retrieved from https://resolution.nhs.uk/resources/ten-years-of-maternity-claims-an-analysis-of-nhs-litigation-authority-data/

NHS Resolution. (2019). *The Early Notification scheme progress report: collaboration and improved experience for families*. London: NHS Resolution. Retrieved from https://resolution.nhs.uk/resources/early-notification-scheme-progress-report/

Nijland, R., Jongsma, H. W., Crevels, J., Menssen, J. J. M., Nijhuis, J. G., & Oeseburg, B. (1996). Transmission Pulse Oximetry in the Fetal Lamb: Is There a Universal Calibration? *Pediatric Research, 39*(3), 464-469. doi:10.1203/00006450-199603000-00014

Nikitas, N., Kopterides, P., Ilias, I., Theodorakopoulou, M., Vassiliadi, D. A., Armaganidis, A., & Dimopoulou, I. (2013). Elevated adipose tissue lactate to pyruvate (L/P) ratio predicts poor outcome in critically ill patients with septic shock: a microdialysis study. *Minerva Anestesiol, 79*(11), 1229-1237.

Nixon, S., Sieg, A., Delgado-Charro, M. B., & Guy, R. H. (2007). Reverse iontophoresis of L-lactate: In vitro and in vivo studies. *Journal of Pharmaceutical Sciences, 96*(12), 3457-3465. doi:10.1002/jps.20989

Nordström, L., Chua, S., Roy, A., Naka, K., Persson, B., & Arulkumaran, S. (1998). Lactate, lactate/pyruvate ratio and catecholamine interrelations in cord blood at delivery in complicated pregnancies. *Early Hum Dev, 52*(1), 87-94. doi:10.1016/s0378-3782(98)00014-0

Nowell, L. S., Norris, J. M., White, D. E., & Moules, N. J. (2017). Thematic Analysis: Striving to Meet the Trustworthiness Criteria. *International Journal of Qualitative Methods, 16*(1), 1609406917733847. doi:10.1177/1609406917733847

Nunes, I., Ayres-de-Campos, D., Ugwumadu, A., Amin, P., Banfield, P., Nicoll, A., ... Bernardes, J. (2017). Central Fetal Monitoring With and Without Computer Analysis: A Randomized Controlled Trial. *Obstet Gynecol, 129*(1), 83-90. doi:10.1097/aog.0000000000001799

Oakley, A., Strange, V., Bonell, C., Allen, E., Stephenson, J., & Team, R. S. (2006). Process evaluation in randomised controlled trials of complex interventions. *BMJ, 332*(7538), 413-416. doi:10.1136/bmj.332.7538.413

O'Brien, Y. M., & Murphy, D. J. (2013). The reliability of foetal blood sampling as a test of foetal acidosis in labour. *Eur J Obstet Gynecol Reprod Biol, 167*(2), 142-145. doi:10.1016/j.ejogrb.2012.11.016

Odendaal, H. (1974). Factors influencing the pH value of foetal scalp blood with special reference to caput succedaenum. *South African Medical Journal, 48*(1), 59-62.

Ogasawara, J., Ikenoue, S., Yamamoto, H., Sato, M., Kasuga, Y., Mitsukura, Y., ... Ochiai, D. (2021). Deep neural network-based classification of cardiotocograms outperformed conventional algorithms. *Sci Rep, 11*(1), 13367. doi:10.1038/s41598-021-92805-9

Ohashi, H., Kawasaki, N., Fujitani, S., Kobayashi, K., Ohashi, M., Hosoyama, A., ... Taira, Y. (2009). Utility of microdialysis to detect the lactate/pyruvate ratio in subcutaneous tissue for the reliable monitoring of hemorrhagic shock. *J Smooth Muscle Res, 45*(6), 269-278. doi:10.1540/jsmr.45.269

Ohashi, H., Kawasaki, N., Komatsu, H., Wada, T., Hosoyama, A., Hanyu, N., ... Taira, Y. (2011). Microdialysis detection of lactate in subcutaneous tissue as a reliable indicator of tissue metabolic disorders in an animal sepsis model. *J Smooth Muscle Res, 47*(1), 37-46. doi:10.1540/jsmr.47.37

Ojala, K., Vääräsmäki, M., Mäkikallio, K., Valkama, M., & Tekay, A. (2006). A comparison of intrapartum automated fetal electrocardiography and conventional cardiotocography – a randomised controlled study. *BJOG, 113*(4), 419-423. doi.org/10.1111/j.1471-0528.2006.00886.x

Olofsson, P. (2015). Determination of base excess in umbilical cord blood at birth: accessory or excess? *Am J Obstet Gynecol, 213*(3), 259-261. doi:10.1016/j.ajog.2015.06.037

Olsson, M., Ho, H.-P., Annerbrink, K., Thylefors, J., & Eriksson, E. (2002). Respiratory Responses to Intravenous Infusion of Sodium Lactate in Male and Female Wistar Rats. *Neuropsychopharmacology, 27*(1), 85-91. doi:10.1016/S0893-133X(02)00296-8

Panda, S., Begley, C., & Daly, D. (2018). Clinicians' views of factors influencing

decision-making for caesarean section: A systematic review and metasynthesis of qualitative, quantitative and mixed methods studies. *PLOS ONE, 13*(7), e0200941. doi:10.1371/journal.pone.0200941

Parer, J. T. (2003). Obstetric technologies: What determines clinical acceptance or rejection of results of randomized controlled trials? *Am J Obstet Gynecol, 188*(6), 1622-1628. doi.org/10.1067/mob.2003.394

Payne, V., Hall, M., Prieto, J., & Johnson, M. (2018). Care bundles to reduce central line-associated bloodstream infections in the neonatal unit: a systematic review and meta-analysis. *Archives of Disease in Childhood - Fetal and Neonatal Edition, 103*(5), F422-F429.

Pehböck, D., Wenzel, V., Voelckel, W., Jonsson, K., Herff, H., Mittlböck, M., & Nagele, P. (2010). Effects of preoxygenation on desaturation time during hemorrhagic shock in pigs. *Anesthesiology, 113*(3), 593-599. doi:10.1097/ALN.0b013e3181e73f07

Pehrson, C., Sorensen, J. L., & Amer-Wåhlin, I. (2011). Evaluation and impact of cardiotocography training programmes: a systematic review. *BJOG, 118*(8), 926-935. doi:10.1111/j.1471-0528.2011.03021.x

Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., ... Würbel, H. (2020). Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLOS Biology, 18*(7), e3000411. doi:10.1371/journal.pbio.3000411

Peskind, E. R., Jensen, C. F., Pascualy, M., Tsuang, D., Cowley, D., Martin, D. C., ... Raskind, M. A. (1998). Sodium lactate and hypertonic sodium chloride induce equivalent panic incidence, panic symptoms, and hypernatremia in panic disorder. *Biological Psychiatry, 44*(10), 1007-1016. doi.org/10.1016/S0006-3223(98)00053-5

Petersen, L. J. (1999). Interstitial lactate levels in human skin at rest and during an oral glucose load: a microdialysis study. *Clinical physiology (Oxford), 19*(3), 246-250. doi:10.1046/j.1365-2281.1999.00174.x

Petticrew, M. (2011). When are complex interventions 'complex'? When are simple interventions 'simple'? *European Journal of Public Health, 21*(4), 397-398. doi:10.1093/eurpub/ckr084

Pinas, A. & Chandraharan, E. (2016). Continuous cardiotocography during labour: Analysis, classification and management. *Best Pract Res Clin Obstet Gynaecol, 30,* 33-47. doi:10.1016/j.bpobgyn.2015.03.022

Pittman, R. N. (2011). Integrated Systems Physiology: From Molecule to Function to Disease. In *Regulation of Tissue Oxygenation*. San Rafael, CA: Morgan &

Claypool Life Sciences.

Pitts, F. N., Jr., & McClure, J. N., Jr. (1967). Lactate metabolism in anxiety neurosis. *N Engl J Med, 277*(25), 1329-1336. doi:10.1056/nejm196712212772502

Plock, N., & Kloft, C. (2005). Microdialysis: theoretical background and recent implementation in applied life-sciences. *Eur J Pharm Sci, 25*(1), 1-24. doi:10.1016/j.ejps.2005.01.017

Pond, W. G., Boleman, S. L., Fiorotto, M. L., Ho, H., Knabe, D. A., Mersmann, H. J., ... Su, D. R. (2000). Perinatal ontogeny of brain growth in the domestic pig. *Proc Soc Exp Biol Med, 223*(1), 102-108. doi:10.1046/j.1525-1373.2000.22314.x

Pope, C., & Mays, N. (1995). Qualitative Research: Reaching the parts other methods cannot reach: an introduction to qualitative methods in health and health services research. *BMJ, 311*(6996), 42-45. doi:10.1136/bmj.311.6996.42

Poscia, A., Messeri, D., Moscone, D., Ricci, F., & Valgimigli, F. (2005). A novel continuous subcutaneous lactate monitoring system. *Biosensors and Bioelectronics, 20*(11), 2244-2250. doi.org/10.1016/j.bios.2004.10.031

Potts, R. O., A. Tamada, J., & J. Tierney, M. (2002). Glucose monitoring by reverse iontophoresis. *Diabetes/Metabolism Research and Reviews, 18*(S1), S49-S53. doi:10.1002/dmrr.210

Ramanah, R., Martin, A., Clement, M. C., Maillet, R., & Riethmuller, D. (2010). Fetal Scalp Lactate Microsampling for Non-Reassuring Fetal Status during Labor: A Prospective Observational Study. *Fetal Diagnosis and Therapy, 27*(1), 14-19. doi:10.1159/000262281

Ranamukhaarachchi, S. A., Lehnert, S., Ranamukhaarachchi, S. L., Sprenger, L., Schneider, T., Mansoor, I., ... Stoeber, B. (2016). A micromechanical comparison of human and porcine skin before and after preservation by freezing for medical device development. *Sci Rep, 6*, 32074. doi:10.1038/srep32074

Redshaw, M., & Henderson, J. (2015). *Safely delivered: a national survey of women's experience of maternity care 2014*. Oxford: National Perinatal Epidemiology Unit. Retrieved from https://www.npeu.ox.ac.uk/

Remzső, G., Németh, J., Varga, V., Kovács, V., Tóth-Szűki, V., Kaila, K., ... Domoki, F. (2020). Brain interstitial pH changes in the subacute phase of hypoxic-ischemic encephalopathy in newborn pigs. *PLOS ONE, 15*(5), e0233851. doi:10.1371/journal.pone.0233851

Reynolds, A. J., Murray, M. L., Geary, M. P., Ater, S. B., & Hayes, B. C. (2022). Fetal heart rate patterns in labor and the risk of neonatal encephalopathy: A case control study. *European Journal of Obstetrics & Gynecology and Reproductive*

Biology, *273*, 69–74. doi:10.1016/j.ejogrb.2022.04.021

Rhöse, S., Heinis, A. M. F., Vandenbussche, F., van Drongelen, J., & van Dillen, J. (2014). Inter- and intra-observer agreement of non-reassuring cardiotocography analysis and subsequent clinical management. *Acta Obstet Gynecol Scand, 93*(6), 596-602. doi:10.1111/aogs.12371

Rice, J. E., Vannucci, R. C., & Brierley, J. B. (1981). The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Annals of Neurology, 9*(2), 131-141. doi.org/10.1002/ana.410090206

Richards, J. G., Todd, K. G., Emara, M., Haase, E., Cooper, S. L., Bigam, D. L., & Cheung, P.-Y. (2006). A dose-response study of graded reoxygenation on the carotid haemodynamics, matrix metalloproteinase-2 activities and amino acid concentrations in the brain of asphyxiated newborn piglets. *Resuscitation, 69*(2), 319-327. doi:10.1016/j.resuscitation.2005.08.012

Rifkin, A., & Siris, S. (1984). Sodium lactate response as a model for panic disorders. *Trends in Neurosciences, 7*(6), 188. doi:10.1016/S0166-2236(84)80007-7

Rimachi, R., Bruzzi de Carvahlo, F., Orellano-Jimenez, C., Cotton, F., Vincent, J. L., & De Backer, D. (2012). Lactate/pyruvate ratio as a marker of tissue hypoxia in circulatory and septic shock. *Anaesth Intensive Care, 40*(3), 427-432. doi:10.1177/0310057x1204000307

Rogers, M. L., Feuerstein, D., Leong, C. L., Takagaki, M., Niu, X., Graf, R., & Boutelle, M. G. (2013). Continuous online microdialysis using microfluidic sensors: dynamic neurometabolic changes during spreading depolarization. *ACS Chem Neurosci, 4*(5), 799-807. doi:10.1021/cn400047x

Rogers, M. L., Leong, C. L., Gowers, S. A. N., Samper, I. C., Jewell, S. L., Khan, A., ... Boutelle, M. G. (2017). Simultaneous monitoring of potassium, glucose and lactate during spreading depolarization in the injured human brain – Proof of principle of a novel real-time neurochemical analysis system, continuous online microdialysis. *Journal of Cerebral Blood Flow & Metabolism, 37*(5), 1883-1895. doi:10.1177/0271678X16674486

Røraas, T., Støve, B., Petersen, P. H., & Sandberg, S. (2016). Biological Variation: The Effect of Different Distributions on Estimated Within-Person Variation and Reference Change Values. *Clinical Chemistry, 62*(5), 725-736. doi:10.1373/clinchem.2015.252296

Rosdahl, H., Ungerstedt, U., Jorfeldt, L., & Henriksson, J. (1993). Interstitial glucose and lactate balance in human skeletal muscle and adipose tissue studied by microdialysis. *J Physiol, 471*, 637-657. doi:10.1113/jphysiol.1993.sp019920

Rose, A., Raja, E. A., Bhattacharya, S., & Black, M. (2018). Intervention thresholds and

cesarean section rates: A time-trends analysis. *Acta Obstet Gynecol Scand, 97*(10), 1257-1266. doi:10.1111/aogs.13409

Rosén, K. G., Dagbjartsson, A., Henriksson, B. A., Lagercrantz, H., & Kjellmer, I. (1984). The relationship between circulating catecholamines and ST waveform in the fetal lamb electrocardiogram during hypoxia. *Am J Obstet Gynecol, 149*(2), 190-195. doi:10.1016/0002-9378(84)90197-2

Rosén, K. G., & Kjellmer, I. (1975). Changes in the fetal heart rate and ECG during hypoxia. *Acta Physiol Scand, 93*(1), 59-66. doi:10.1111/j.1748-1716.1975.tb05790.x

Royal Australian and New Zealand College of Obstetricians and Gynaecologists. (2019). *Intrapartum Fetal Surveillance Clinical Guideline - Fourth Edition*. Melbourne, Australia: RANZCOG. Retrieved from https://ranzcog.edu.au/wp-content/uploads/2022/05/Intrapartum-Fetal-Surveillance.pdf

Royal College of Midwives. (2017). *RCM/RCOG consensus statement on Electronic Fetal Monitoring (EFM)*. London: RCM. Retrieved from https://www.rcm.org.uk/media/2305/fetal-monitoring-consensus-statement.pdf

Royal College of Midwives. (2021). *Making Maternity Services Safer: Nurturing a Positive Culture*. London: RCM. Retrieved from https://www.rcm.org.uk/promoting/professional-practice/safety-in-services/solution-series/

Royal College of Obstetricians and Gynaecologists. (2018). *Each Baby Counts: 2018 Progress Report*. London: RCOG. Retrieved from https://www.rcog.org.uk/eachbabycounts

Royal College of Obstetricians and Gynaecologists. (2017). *Each Baby Counts: 2015 Full Report*. London: RCOG. Retrieved from https://www.rcog.org.uk/eachbabycounts

Royal College of Obstetricians and Gynaecologists. (2020). *Each Baby Counts: 2019 Progress Report*. London: ROCG. Retrieved from https://www.rcog.org.uk/eachbabycounts

Royal College of Obstetricians and Gynaecologists. (2015). *Scientific Impact Paper No. 47 - Is it Time for UK Obstetricians to Accept Fetal Scalp Lactate as an Alternative to Scalp pH?* London: RCOG. Retrieved from https://www.rcog.org.uk/guidance/browse-all-guidance/scientific-impact-papers/

Royal College of Obstetricians and Gynaecologists. (2007). *Safer Childbirth: Minimum Standards for the Organisation and Delivery of Care in Labour*. London: RCOG. Retrieved from https://www.rcog.org.uk

Royal College of Obstetricians and Gynaecologists. (2022). *RCOG Workforce Report*

2022. London: RCOG. Retrieved from https://www.rcog.org.uk/media/fdtlufuh/workforce-report-july-2022-update.pdf

Rumajogee, P., Bregman, T., Miller, S. P., Yager, J. Y., & Fehlings, M. G. (2016). Rodent Hypoxia–Ischemia Models for Cerebral Palsy Research: A Systematic Review. *Frontiers in Neurology, 7*(57). doi:10.3389/fneur.2016.00057

Ryan, R. M., & Deci, E. L. (2000). Self-determination theory and the facilitation of intrinsic motivation, social development, and well-being. *American Psychologist, 55*, 68-78. doi:10.1037/0003-066X.55.1.68

Sacco, A., Muglu, J., Navaratnarajah, R., & Hogg, M. (2015). ST analysis for intrapartum fetal monitoring. *The Obstetrician & Gynaecologist, 17*(1), 5-12. doi:10.1111/tog.12154

Saccone, G., Schuit, E., Amer-Wåhlin, I., Xodo, S., & Berghella, V. (2016). Electrocardiogram ST Analysis During Labor: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Obstet Gynecol, 127*(1), 127-135. doi:10.1097/aog.0000000000001198

Saling, E. (1962). [A new method for examination of the child during labor. Introduction, technic and principles]. *Arch Gynakol, 197*, 108-122. doi:10.1007/bf02590014

Salles, A., Wright, R. C., Milam, L., Panni, R. Z., Liebert, C. A., Lau, J. N., ... Mueller, C. M. (2019). Social Belonging as a Predictor of Surgical Resident Well-being and Attrition. *J Surg Educ, 76*(2), 370-377. doi:10.1016/j.jsurg.2018.08.022

Salvatore, D., Numerato, D., & Fattore, G. (2018). Physicians' professional autonomy and their organizational identification with their hospital. *BMC Health Services Research, 18*(1), 775. doi:10.1186/s12913-018-3582-z

Sanders, J. (2019). The POOL Study - Establishing the safety of waterbirth for mothers and babies: A cohort study with nested qualitative component. Protocol 26 Feb 2019 (HTA - 16/149/01). Retrieved from https://www.journalslibrary.nihr.ac.uk/programmes/hta/1614901/

Santo, S., Ayres-de-Campos, D., Costa-Santos, C., Schnettler, W., Ugwumadu, A., Da Graça, L. M., & the, F. M. C. C. (2017). Agreement and accuracy using the FIGO, ACOG and NICE cardiotocography interpretation guidelines. *Acta Obstet Gynecol Scand, 96*(2), 166-175. doi:10.1111/aogs.13064

Sawatsky, A. P., O'Brien, B. C., & Hafferty, F. W. (2022). Autonomy and developing physicians: Reimagining supervision using self-determination theory. *Medical Education, 56*(1), 56-63. doi:https://doi.org/10.1111/medu.14580

Saylor, R. A., & Lunte, S. M. (2015). A review of microdialysis coupled to microchip electrophoresis for monitoring biological events. *J Chromatogr A, 1382*, 48-64.

doi:10.1016/j.chroma.2014.12.086

Schiavon, M., Dalla Man, C., Dube, S., Slama, M., Kudva, Y. C., Peyser, T., ... Cobelli, C. (2015). Modeling Plasma-to-Interstitium Glucose Kinetics from Multitracer Plasma and Microdialysis Data. *Diabetes Technology & Therapeutics, 17*(11), 825-831. doi:10.1089/dia.2015.0119

Schierenbeck, F., Nijsten, M. W. N., Franco-Cereceda, A., & Liska, J. (2014). Introducing intravascular microdialysis for continuous lactate monitoring in patients undergoing cardiac surgery: a prospective observational study. *Critical Care, 18*(2), R56. doi:10.1186/cc13808

Seifman, MA., Fuzzard, S. K., To, H., & Nestel, D. (2022). COVID-19 impact on junior doctor education and training: a scoping review. *Postgraduate Medical Journal, 98*(1160), 466-476.

Seymour, C. W., McCreary, E. K., & Stegenga, J. (2020). Sensible Medicine – Balancing Intervention and Inaction During the COVID-19 Pandemic. *JAMA, 324*(18), 1827-1828. doi:10.1001/jama.2020.20271

Shim, J. K., Russ, A. J., & Kaufman, S. R. (2008). Late-life cardiac interventions and the treatment imperative. *PLOS Medicine, 5*(3), 0344-0346. doi:10.1371/journal.pmed.0050007

Shippenberg, T. S., & Thompson, A. C. (2001). Overview of microdialysis. *Curr Protoc Neurosci, Chapter 7*. doi:10.1002/0471142301.ns0701s00

Shoushtarian, M., Barnett, M., McMahon, F., & Ferris, J. (2014). Impact of introducing Practical Obstetric Multi-Professional Training (PROMPT) into maternity units in Victoria, Australia. *BJOG, 121*(13), 1710-1718. doi.org/10.1111/1471-0528.12767

Siggaard-Andersen, O. (1971). An acid-base chart for arterial blood with normal and pathophysiological reference areas. *Scand J Clin Lab Invest, 27*(3), 239-245. doi:10.3109/00365517109080214

Sim, J., & Waterfield, J. (2019). Focus group methodology: some ethical challenges. *Quality & Quantity, 53*(6), 3003-3022. doi:10.1007/s11135-019-00914-5

Simpson, J., & Stephenson, T. (1993). Regulation of extracellular fluid volume in neonates. *Early Human Development, 34*(3), 179-190. doi.org/10.1016/0378-3782(93)90175-T

Singh, R., Kirtley, J., Minhas, J. S., Lakhani, D., & Carr, S. (2019). Exploring Junior Doctor Morale in a UK Hospital. *Journal of the Royal College of Physicians of Edinburgh, 49*(4), 312-316. doi:10.4997/jrcpe.2019.414

Sinha, P. (2017). Don't Just Do Something, Stand There! *JAMA Internal Medicine, 177*(10), 1420-1421. doi:10.1001/jamainternmed.2017.3628

Sirian, R., & Wills, J. (2009). Physiology of apnoea and the benefits of preoxygenation. *Continuing Education in Anaesthesia Critical Care & Pain, 9*(4), 105-108. doi:10.1093/bjaceaccp/mkp018

Skår, R. (2010). The meaning of autonomy in nursing practice. *J Clin Nurs, 19*(15-16), 2226-2234. doi:10.1111/j.1365-2702.2009.02804.x

Skiöld, B., Petersson, G., Ahlberg, M., Stephansson, O., & Johansson, S. (2017). Population-based reference curve for umbilical cord arterial pH in infants born at 28 to 42 weeks. *Journal of Perinatology, 37*(3), 254-259. doi:10.1038/jp.2016.207

Smith, A., Yang, D., Delcher, H., Eppstein, J., Williams, D., & Wilkes, S. (1999). Fluorescein Kinetics in Interstitial Fluid Harvested from Diabetic Skin during Fluorescein Angiography: Implications for Glucose Monitoring. *Diabetes Technology & Therapeutics, 1*(1), 21-27. doi:10.1089/152091599317530

Smith, A. J., Clutton, R. E., Lilley, E., Hansen, K. E. A., & Brattelid, T. (2018). PREPARE: guidelines for planning animal research and testing. *Lab Anim, 52*(2), 135-141. doi:10.1177/0023677217724823

Smith, V., Begley, C. M., Clarke, M., & Devane, D. (2012). Professionals' views of fetal monitoring during labour: a systematic review and thematic analysis. *BMC Pregnancy and Childbirth, 12*(1), 166. doi:10.1186/1471-2393-12-166

Sørensen, L. B., Gazerani, P., Wåhlén, K., Ghafouri, N., Gerdle, B., & Ghafouri, B. (2018). Investigation of biomarkers alterations after an acute tissue trauma in human trapezius muscle, using microdialysis. *Sci Rep, 8*(1), 3034. doi:10.1038/s41598-018-21185-4

Stahle, L., Segersvard, S., & Ungerstedt, U. (1991). A comparison between three methods for estimation of extracellular concentrations of exogenous and endogenous compounds by microdialysis. *J Pharmacol Methods, 25*(1), 41-52. doi:10.1016/0160-5402(91)90021-v

Standring, S., Anand, N., & Tunstall, R. (2021). *Gray's anatomy : the anatomical basis of clinical practice* (Forty-second edition. ed.). New York: Elsevier.

Steer, P. J. (1987). Is fetal blood sampling and pH estimation helpful or harmful? *Archives of Disease in Childhood, 62*(11), 1097. doi:10.1136/adc.62.11.1097

Steer, P. J., Kovar, I., McKenzie, C., Griffin, M., & Linsell, L. (2019). Computerised analysis of intrapartum fetal heart rate patterns and adverse outcomes in the INFANT trial. *BJOG, 126*(11), 1354-1361. doi:10.1111/1471-0528.15535

Sternberg, F., Meyerhoff, C., Mennel, F. J., Mayer, H., Bischof, F., & Pfeiffer, E. F. (1996). Does fall in tissue glucose precede fall in blood glucose? *Diabetologia, 39*(5), 609-612. doi:10.1007/BF00403309

Sturges, J. E., & Hanrahan, K. J. (2004). Comparing Telephone and Face-to-Face Qualitative Interviewing: a Research Note. *Qualitative Research, 4*(1), 107-118. doi:10.1177/1468794104041110

Sullivan, T. P., Eaglstein, W. H., Davis, S. C., & Mertz, P. (2001). The pig as a model for human wound healing. *Wound Repair Regen, 9*(2), 66-76. doi:10.1046/j.1524-475x.2001.00066.x

Summerfield, A., Meurens, F., & Ricklin, M. E. (2015). The immunology of the porcine skin and its value as a model for human skin. *Mol Immunol, 66*(1), 14-21. doi:10.1016/j.molimm.2014.10.023

Symonds, E. M., & Senior, O. E. (1991). The anatomy of obstetric litigation. *Current Obstetrics & Gynaecology, 1*(4), 241-243. doi.org/10.1016/0957-5847(91)90055-3

Takada, S. H., Sampaio, C. A. G., Allemandi, W., Ito, P. H., Takase, L. F., & Nogueira, M. I. (2011). A modified rat model of neonatal anoxia: Development and evaluation by pulseoximetry, arterial gasometry and Fos immunoreactivity. *Journal of Neuroscience Methods, 198*(1), 62-69. doi.org/10.1016/j.jneumeth.2011.03.009

Taleb, N. N. (2013). *Antifragile : things that gain from disorder*. London: Penguin Books.

Ten Cate, T. J., Kusurkar, R. A., & Williams, G. C. (2011). How self-determination theory can assist our understanding of the teaching and learning processes in medical education. AMEE guide No. 59. *Med Teach, 33*(12), 961-973. doi:10.3109/0142159x.2011.595435

Teramo, K. (1969). The Validity of Foetal Capillary Blood Samples during Labour. *Gynecologic and Obstetric Investigation, 167*(6), 511-521. doi:10.1159/000302269

Tholance, Y., Barcelos, G., Quadrio, I., Renaud, B., Dailler, F., & Perret-Liaudet, A. (2011). Analytical validation of microdialysis analyzer for monitoring glucose, lactate and pyruvate in cerebral microdialysates. *Clin Chim Acta, 412*(7-8), 647-654. doi:10.1016/j.cca.2010.12.025

Thoresen, M., Penrice, J., Lorek, A., Cady, E. B., Wylezinska, M., Kirkbride, V., ... et al. (1995). Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatr Res, 37*(5), 667-670. doi:10.1203/00006450-199505000-00019

Thorp, J. A., Dildy, G. A., Yeomans, E. R., Meyer, B. A., & Parisi, V. M. (1996). Umbilical cord blood gas analysis at delivery. *Am J Obstet Gynecol, 175*(3 Pt 1), 517-522. doi:10.1053/ob.1996.v175.a74401

Tiessen, R. G., Kaptein, W. A., Venema, K., & Korf, J. (1999). Slow ultrafiltration for continuous in vivo sampling: application for glucose and lactate in man. *Anal Chim Acta, 379*(3), 327-335. doi.org/10.1016/S0003-2670(98)00601-1

Tigchelaar, F., Groen, H., Westgren, M., Huinink, K. D., Cremers, T., & van den Berg, P. P. (2020). A new microdialysis probe for continuous lactate measurement during fetal monitoring: Proof of concept in an animal model. *Acta Obstet Gynecol Scand, 99*(10), 1411-1416. doi:10.1111/aogs.13865

Timonen, S., & Holmberg, K. (2018). The importance of the learning process in ST analysis interpretation and its impact in improving clinical and neonatal outcomes. *Am J Obstet Gynecol, 218*(6), 620.e1-620.e7. doi.org/10.1016/j.ajog.2018.03.017

Tong, A., Sainsbury, P., & Craig, J. (2007). Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *International Journal for Quality in Health Care, 19*(6), 349-357. doi:10.1093/intqhc/mzm042

Toro-Ramos, T., Paley, C., Pi-Sunyer, F. X., & Gallagher, D. (2015). Body composition during fetal development and infancy through the age of 5 years. *Eur J Clin Nutr, 69*(12), 1279-1289. doi:10.1038/ejcn.2015.117

Trujillo-Ortega, M. E., Mota-Rojas, D., Olmos-Hernández, A., Alonso-Spilsbury, M., González, M., Orozco, H., ... Nava-Ocampo, A. A. (2007). A study of piglets born by spontaneous parturition under uncontrolled conditions: could this be a naturalistic model for the study of intrapartum asphyxia? *Acta Biomed, 78*(1), 29-35.

Tsikouras, P., Koukouli, Z., Niesigk, B., Manav, B., Farmakides, G., Csorba, R., ... Teichmann, A. T. (2018). Predictive value of fetal scalp pH and base excess for fetal acidosis and poor neonatal outcome. *J Matern Fetal Neonatal Med, 31*(23), 3166-3171. doi:10.1080/14767058.2017.1365132

Tuffnell, D., Haw, W. L., & Wilkinson, K. (2006). How long does a fetal scalp blood sample take? *BJOG, 113*(3), 332-334. doi:10.1111/j.1471-0528.2006.00859.x

Tufford, L., & Newman, P. (2010). Bracketing in Qualitative Research. *Qualitative Social Work, 11*(1), 80-96. doi:10.1177/1473325010368316

Turner, J. M., Mitchell, M. D., & Kumar, S. S. (2020). The physiology of intrapartum fetal compromise at term. *Am J Obstet Gynecol, 222*(1), 17-26. doi.org/10.1016/j.ajog.2019.07.032

Tuuli, M., Stout, M., Shanks, A., Odibo, A., Macones, G., & Cahill, A. (2014). Umbilical Cord Arterial Lactate Compared With pH for Predicting Neonatal Morbidity at Term. *Obstet Gynecol, 124*(4), 756-761.

Ugwumadu, A. (2014). Are we (mis)guided by current guidelines on intrapartum fetal heart rate monitoring? Case for a more physiological approach to interpretation. *BJOG, 121*(9), 1063-1070. doi:10.1111/1471-0528.12900

Ungerstedt, U., & Pycock, C. (1974). Functional correlates of dopamine neurotransmission. *Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften, 30*, 44. doi:10.5169/seals-307978

Vaddiraju, S., Burgess, D. J., Tomazos, I., Jain, F. C., & Papadimitrakopoulos, F. (2010). Technologies for Continuous Glucose Monitoring: Current Problems and Future Promises. *Journal of Diabetes Science and Technology, 4*(6), 1540-1562. doi:10.1177/193229681000400632

van Braak, M., de Groot, E., Veen, M., Welink, L., & Giroldi, E. (2018). Eliciting tacit knowledge: The potential of a reflective approach to video-stimulated interviewing. *Perspectives on medical education, 7*(6), 386-393. doi:10.1007/s40037-018-0487-9

van den Bosch, M., van de Linde, I. B., Kemp, B., & van den Brand, H. (2022). Disentangling Litter Size and Farrowing Duration Effects on Piglet Stillbirth, Acid–Base Blood Parameters and Pre-Weaning Mortality. *Frontiers in Veterinary Science, 9*. doi:10.3389/fvets.2022.836202

van den Heuvel, I., Vlasselaers, D., Wouters, P. J., Milants, I., Ellger, B., Vanhorebeek, I., & Van den Berghe, G. (2009). Serial lactate measurements using microdialysis of interstitial fluid do not correlate with plasma lactate in children after cardiac surgery. *Pediatr Crit Care Med, 10*(1), 66-70. doi:10.1097/PCC.0b013e31819374b0

van Dijk, A. J., van Loon, J. P., Taverne, M. A., & Jonker, F. H. (2008). Umbilical cord clamping in term piglets: a useful model to study perinatal asphyxia? *Theriogenology, 70*(4), 662-674. doi:10.1016/j.theriogenology.2008.04.044

Vannucci, R. C., Towfighi, J., Heitjan, D. F., & Brucklacher, R. M. (1995). Carbon dioxide protects the perinatal brain from hypoxic-ischemic damage: an experimental study in the immature rat. *Pediatrics, 95*(6), 868-874.

Vayssière, C., David, E., Meyer, N., Haberstich, R., Sebahoun, V., Roth, E., ... Langer, B. (2007). A French randomized controlled trial of ST-segment analysis in a population with abnormal cardiotocograms during labor. *Am J Obstet Gynecol, 197*(3), 299.e291-296. doi:10.1016/j.ajog.2007.07.007

Victory, R., Penava, D., Da Silva, O., Natale, R., & Richardson, B. (2004). Umbilical cord pH and base excess values in relation to adverse outcome events for infants delivering at term. *Am J Obstet Gynecol, 191*(6), 2021-2028. doi:10.1016/j.ajog.2004.04.026

Visser, G. H., Ayres-de-Campos, D., & Panel, F. I. F. M. E. C. (2015). FIGO consensus guidelines on intrapartum fetal monitoring: Adjunctive technologies. *International Journal of Gynecology & Obstetrics, 131*(1), 25-29. doi:10.1016/j.ijgo.2015.06.021

Vitral, G. L. N., Aguiar, R. A. P. L., de Souza, I. M. F., Rego, M. A. S., Guimarães, R. N., & Reis, Z. S. N. (2018). Skin thickness as a potential marker of gestational age at birth despite different fetal growth profiles: A feasibility study. *PLOS ONE, 13*(4), e0196542. doi:10.1371/journal.pone.0196542

Vogel, J. P., Betrán, A. P., Vindevoghel, N., Souza, J. P., Torloni, M. R., Zhang, J., ... Temmerman, M. (2015). Use of the Robson classification to assess caesarean section trends in 21 countries: a secondary analysis of two WHO multicountry surveys. *The Lancet Global Health, 3*(5), e260-e270. doi:10.1016/S2214-109X(15)70094-X

Wang, M., Chua, S. C., Bouhadir, L., Treadwell, E. L., Gibbs, E., & McGee, T. M. (2018). Point-of-care measurement of fetal blood lactate – Time to trust a new device. *Aust N Z J Obstet Gynaecol, 58*(1), 72-78. doi:10.1111/ajo.12671

Weber, T. (1982). Cardiotocography supplemented with continuous fetal pH monitoring during labor. Effect on rate of obstetrical interventions and neonatal condition. *Acta Obstet Gynecol Scand, 61*(4), 351-355. Retrieved from http://europepmc.org/abstract/MED/7148410

Weiner, C. P., Collins, L., Bentley, S., Dong, Y., & Satterwhite, C. L. (2016). Multi-professional training for obstetric emergencies in a US hospital over a 7-year interval: An observational study. *Journal of Perinatology, 36*(1), 19-24. doi:10.1038/jp.2015.136

Weir, G., Ramage, L. E., Akyol, M., Rhodes, J. K., Kyle, C. J., Fletcher, A. M., ... Stimson, R. H. (2018). Substantial Metabolic Activity of Human Brown Adipose Tissue during Warm Conditions and Cold-Induced Lipolysis of Local Triglycerides. *Cell Metabolism, 27*(6), 1348-1355.e1344. doi:10.1016/j.cmet.2018.04.020

Wentholt, I. M., Hart, A. A., Hoekstra, J. B., & Devries, J. H. (2007). Relationship between interstitial and blood glucose in type 1 diabetes patients: delay and the push-pull phenomenon revisited. *Diabetes Technol Ther, 9*(2), 169-175. doi:10.1089/dia.2006.0007

Westerhuis, M., Visser, G. H. A., Moons, K. G. M., van Beek, E., Benders, M. J., Bijvoet, S. M., ... Kwee, A. (2010). Cardiotocography plus ST analysis of fetal electrocardiogram compared with cardiotocography only for intrapartum monitoring: a randomized controlled trial. *Obstet Gynecol, 115*(6), 1173-1180. doi:10.1097/AOG.0b013e3181dfffd6

Westgate, J., Harris, M., Curnow, J. S. H., & Greene, K. R. (1993). Plymouth randomized trial of cardiotocogram only versus ST waveform plus cardiotocogram for intrapartum monitoring in 2400 cases. *Am J Obstet Gynecol, 169*(5), 1151-1160. doi.org/10.1016/0002-9378(93)90273-L

Westgren, M., Kruger, K., Ek, S., Grunevald, C., Kublickas, M., Naka, K., ... Persson, B. (1998). Lactate compared with pH analysis at fetal scalp blood sampling: a prospective randomised study. *BJOG, 105*(1), 29-33. doi:10.1111/j.1471-0528.1998.tb09346.x

Wheble, A. M., Gillmer, M. D. G., Spencer, J. A. D., & Sykes, G. S. (1989). Changes in fetal monitoring practice in the UK: 1977–1984. *BJOG, 96*(10), 1140-1147. doi:10.1111/j.1471-0528.1989.tb03188.x

Wiberg, N., Källén, K., Herbst, A., & Olofsson, P. (2010). Relation between umbilical cord blood pH, base deficit, lactate, 5-minute Apgar score and development of hypoxic ischemic encephalopathy. *Acta Obstet Gynecol Scand, 89*(10), 1263-1269. doi:10.3109/00016349.2010.513426

Wiberg, N., Källén, K., & Olofsson, P. (2006a). Physiological development of a mixed metabolic and respiratory umbilical cord blood acidemia with advancing gestational age. *Early Human Development, 82*(9), 583-589. doi.org/10.1016/j.earlhumdev.2005.12.005

Wiberg, N., Källén, K., Herbst, A., Åberg, A., & Olofsson, P. (2008). Lactate concentration in umbilical cord blood is gestational age-dependent: a population-based study of 17867 newborns. *BJOG, 115*(6), 704-709. doi:10.1111/j.1471-0528.2008.01707.x

Wiberg, N., Källén, K., & Olofsson, P. (2006b). Base deficit estimation in umbilical cord blood is influenced by gestational age, choice of fetal fluid compartment, and algorithm for calculation. *Am J Obstet Gynecol, 195*(6), 1651-1656. doi.org/10.1016/j.ajog.2006.05.043

Wiberg-Itzel, E., Lipponer, C., Norman, M., Herbst, A., Prebensen, D., Hansson, A., ... Nordström, L. (2008). Determination of pH or lactate in fetal scalp blood in management of intrapartum fetal distress: randomised controlled multicentre trial. *BMJ, 336*(7656), 1284. doi:10.1136/bmj.39553.406991.25

Wiig, H., & Swartz, M. A. (2012). Interstitial Fluid and Lymph Formation and Transport: Physiological Regulation and Roles in Inflammation and Cancer. *Physiological Reviews, 92*(3), 1005-1060. doi:10.1152/physrev.00037.2011

Wikander, I., Roos, T., Stakkestad, A., & Eriksson, E. (1995). Sodium lactate elicits a rapid increase in blood pressure in Wistar rats and spontaneously hypertensive rats. Effect of pretreatment with the antipanic drugs clomipramine and alprazolam. *Neuropsychopharmacology, 12*(3), 245-250. doi:10.1016/0893-

133x(94)00082-b

Wolf, A., Renehan, K., Ho, K. K. Y., Carr, B. D., Chen, C. V., Cornell, M. S., ... Chen, H. (2018). Evaluation of Continuous Lactate Monitoring Systems within a Heparinized In Vivo Porcine Model Intravenously and Subcutaneously. *Biosensors, 8*(4). doi:10.3390/bios8040122

Yang, W., Zhang, X., Wang, N., Tan, J., Fang, X., Wang, Q., ... Li, W. (2016). Effects of Acute Systemic Hypoxia and Hypercapnia on Brain Damage in a Rat Model of Hypoxia-Ischemia. *PLOS ONE, 11*(12), e0167359. doi:10.1371/journal.pone.0167359

Yeh, P., Emary, K., & Impey, L. (2012). The relationship between umbilical cord arterial pH and serious adverse neonatal outcome: analysis of 51 519 consecutive validated samples. *BJOG, 119*(7), 824-831. doi:10.1111/j.1471-0528.2012.03335.x

Yli, B. M., & Kjellmer, I. (2016). Pathophysiology of foetal oxygenation and cell damage during labour. *Best Practice & Research Clinical Obstetrics & Gynaecology, 30*, 9-21. doi.org/10.1016/j.bpobgyn.2015.05.004

Zhang, Q., Ding, Y., Yao, Y., Yu, Y., Yang, L., & Cui, H. (2013). Creating Rat Model for Hypoxic Brain Damage in Neonates by Oxygen Deprivation. *PLOS ONE, 8*(12), e83589. doi:10.1371/journal.pone.0083589

Zomer, H. D., & Trentin, A. G. (2018). Skin wound healing in humans and mice: Challenges in translational research. *Journal of Dermatological Science, 90*(1), 3-12. doi:10.1016/j.jdermsci.2017.12.009

Zoremba, N., Homola, A., Rossaint, R., & Sykova, E. (2014). Interstitial lactate, lactate/pyruvate and glucose in rat muscle before, during and in the recovery from global hypoxia. *Acta Vet Scand, 56*, 72. doi:10.1186/s13028-014-0072-0